An Investigation on the Potential of Mortalin as a Therapeutic Target for Oral Cancer

A thesis submitted for the degree of

Doctor of Philosophy

To

INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI

By

SOSMITHA GIRISA

Department of Biosciences and Bioengineering

Indian Institute of Technology Guwahati

Guwahati, Assam-781039, India

May, 2024.

Dedicated to

My beloved grandparents, parents and siblings,
whose unwavering love, support and encouragement
have been the foundation
and guiding light of my journey

DEPARTMENT OF BIOSCIENCES AND BIOENGINEERING
INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
GUWAHATI-781039

DECLARATION

I hereby declare that the contents of the research work described in this thesis titled **"An Investigation on the Potential of Mortalin as a Therapeutic Target for Oral Cancer"**, is a presentation of my original research work carried out in the Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, India, under the supervision of Prof. Ajaikumar B. Kunnumakkara.

Sincere efforts have been made to duly acknowledge the contributions from others for their ideas, technical help, references or any other help which may be involved in the completion of this thesis work.

08 May, 2024

Sosmitha Girisa
Roll No. 176106101
Department of Biosciences and Bioengineering
Indian Institute of Technology Guwahati
Guwahati, Assam-781039, India

DEPARTMENT OF BIOSCIENCES AND BIOENGINEERING
INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
GUWAHATI-781039

CERTIFICATE

This is to certify that the work described in the thesis titled **"An Investigation on the Potential of Mortalin as a Therapeutic Target for Oral Cancer"**, submitted by Sosmitha Girisa (Roll no: 176106101) to Indian Institute of Technology Guwahati, India, for the award of the degree of Doctor of Philosophy is an authentic record of the research work carried out under my supervision in the Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati, India.

This thesis or any part thereof has not been submitted elsewhere for award of any other degree or diploma.

08 May, 2024

Prof. Ajaikumar B. Kunnumakkara
Cancer Biology Laboratory
Department of Biosciences and Bioengineering
Indian Institute of Technology Guwahati
Guwahati, Assam-781039, India

ACKNOWLEDGEMENTS

The completion of my Ph.D. journey would have lacked its spirit without the invaluable presence of individuals who served as wellsprings of inspiration, support, motivation, guidance, encouragement, and blessings. I am deeply indebted to them for their integral role in this remarkable journey.

First and foremost, I express my sincere gratitude to my supervisor, Prof. Ajaikumar B. Kunnumakkara, for the privilege of being a part of the 'Cancer Biology Laboratory'. I am immensely grateful for his unwavering support, guidance, and motivation throughout my doctoral pursuit. His patience and scientific insights have been influential in shaping my academic and professional career. Working under his mentorship has been a great journey of learning, and I extend my heartfelt thanks for his pivotal contribution to my academic growth and the completion of my PhD.

I am also thankful to my doctoral committee (DC) members, Prof. Ranjan Tamuli (Chairman, DC), Prof. Manish Kumar, and Prof. Chandan Kumar Jana, for their invaluable insights and constructive feedback, that has helped in improving my PhD thesis.

Moreover, I extend my sincere appreciation to Prof. Renu Wadhwa and Prof. Sunil Kaul of National Institute of Advanced Industrial Science and Technology (AIST), Japan for their guidance, suggestions and support in our collaborative project. I would also like to acknowledge collaborators from North East Cancer Hospital and Research Institute (NECHRI), for providing oral tissue samples for the experimental study in this project.

I also extend my gratitude to the Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, for providing the necessary infrastructure and facilities, as well as to the past and present Head of the Department for their facilitation.

I express my thanks to the dedicated staff members of IIT Guwahati, including security personnel and janitorial staff, whose assistance has been invaluable.

Additionally, I am deeply thankful to the Ministry of Human Resource and Development, New Delhi, Government of India, and the Equal Opportunity Cum Special Reservation Cell, Indian Institute of Technology Guwahati, for their financial assistance during my Ph.D. journey.

My heartfelt appreciation also extends to my esteemed seniors and past members of the Cancer Biology Laboratory family: Dr. Monisha, Dr. Nand Kishor Roy, Dr. Anuj, Dr. Devivasha, Dr. Padmavathi, Dr. Bethsebie, Dr. Amrita, Dr. Kishore, Dr. Harsha, Dr. Krishan, Dr. Elina, and Dr. Babita, for imparting invaluable knowledge and guiding me with experimental techniques, and fostering a conducive laboratory environment.

My gratitude also goes to other past members including Shabnam, Jyothsna, Rajesh, Varsha, Nitesh, Nikunj, Rooplekha, Pragya, Asha, Srilakshmi, Srinidhi, Ramya, Aswin, Shailendra, Sharad, Swathi, Queen, Madhuri, Henamayee, Bidisha, Yachna, Semim, Neha, Kabya, Gayathri, Shubhrajyoti, Asho, Nabojit, Joydeep, Gudia, Monikongkona, Elika, Soham, Poonam, Sandra, Suravi, Tushar, Kashish, Mantra, and Ayesha for their invaluable support and cooperation.

Furthermore, I am grateful to my present fellow laboratory members, Dr. Mangala, Dr. Anamika, Parama, Gourav, Aviral, Uzini, Anjana, Mukesh, Vishwa, Aswini, Anuska, Niharika, Mayuk, Bharathwaj, Shweta, Pampitha, Pranav, Sujitha, Nafiseh, Bintee, Ruchira, Hrishikesh, and Shristy, for their continuous support and cooperation.

My journey would have been incomplete without the unwavering support of my family. I express deep gratitude to my parents who has been source of my inspiration, strength, and encouragement throughout. Additionally, I am lucky and thankful for the blessings, prayers, and motivational guidance from my maternal and paternal grandparents, as well as the constant support and cheers from my uncles and aunts. I also extend my heartfelt gratitude to my younger siblings, acknowledging my sister Haimaidi for her unwavering support akin to a constant shadow, my sister Monsina for being inspirational, and my brothers Thomas and Grikham for consistently providing moral support and uplifting spirits.

I also extend my thanks to cousins and family friends especially Maiham, Soham, Maiphrang, Suraj Chauhan, Deepamoni, Bandita, Babita, and Janet for their encouragement and support.

Moreover, I appreciate the well-wishes, encouragement, and support from friends on campus and elsewhere, including Shweta, Adeline, Rafi, Noor, Aman, John, Alka, Payal, Raj, Dechen, Pooja, Shyam, Anu, Sweta, Sanjeev, Pratap, Sandesh, Megha, Tolivi, Nizwma, Phirose, Rajendra, Jonjyoti, Pulak, Rahul, Dharithri, Monika, Lairing, Bunty, Emti, Neha, Anjali, Hemant, Parichita, Lalita, Gayathri, Diganta, Lwmchao, Isha, Tarun, and Jeeva.

My special thanks are also due to Dr. Raj, Dr. Jaspeet, and Dr. Santosh for their invaluable help and encouragement.

I am also grateful to the members of Aditya mess caterers, especially Dhoneswar, Kempraj, Rajiv, Piru, and Dhan Sharma, for their exceptional service and providing healthy and nourishing meals during my Ph.D. journey.

Further, my heartfelt gratitude is extended to all my teachers, from schooling to the present, for their unwavering inspiration. Their dedication to nurturing curiosity and critical thinking has played a pivotal role in shaping my academic journey and instilling in me the passion and perseverance to pursue this career.

I would also like to acknowledge the invaluable contributions of funding agencies, particularly DAICENTER, Department of Biotechnology, Government of India, without whom this work would not have been possible.

I also thank for the regards and blessings from well-wishers, whose support, whether direct or indirect, has been invaluable throughout this journey.

Above all, I am deeply grateful to the "**ALMIGHTY GOD**" for providing me with strength, hope, inspiration, and good health throughout this journey, and for His continued blessings.

In conclusion, I extend my sincere thanks to all who have directly or indirectly contributed to my journey and wish everyone a healthy and fulfilling life. Thank you.

Sosmitha Girisa

TABLE OF CONTENTS

Chapter 1

Introduction and Review of Literature

1.1 Introduction

Oral cancer (OC) or malignancy of oral cavity presents a significant health challenge due to its high incidence and mortality worldwide [Ghantous and Abu Elnaaj, 2017]. According to GLOBOCAN, approximately 377,000 new cases of lip and oral cavity cancers were diagnosed globally in 2020, with around 177,000 related deaths [Sung et al., 2021].

However, it is more prevalent in South Asian and Southeast Asian countries including India, Sri Lanka, Bangladesh, etc. [Sun et al., 2023]. It has been observed that approximately two-third of OC cases occur in developing nations and the economic status of a region markedly affects the disease burden, resulting in a more pronounced impact on developing countries compared to developed ones [Mohamad et al., 2023; Conway et al., 2008]. This cancer may affect different parts of the oral cavity and includes different subtypes. Squamous cell carcinoma of the oral cavity (OSCC) represents the predominant subtype, accounting for 90% of the OC [Rivera and Venegas, 2014]. Other subtypes of this cancer are salivary gland cancers, sarcomas, oral cavity lymphomas, and oral mucosal melanomas [Scully and Porter, 2001]. It also includes a small proportion of the malignancies that arise due to metastasis from carcinomas of the breast, lung, kidney and prostate [Kumar and Manjunatha, 2014; Aksoy et al., 2014].

Oral carcinogenesis is an intricate and multifaceted process that develops through complex epigenetic and genetic changes in epithelial cells, resulting in the transition from normal cells to invasive carcinoma [Rivera, 2015]. These alterations, including mutations and chromosomal abnormalities, disrupt essential cellular functions, such as cell cycle control and DNA repair, driving the transformation of normal keratinocytes into hyperplasia, followed by dysplasia, in situ carcinoma, and ultimately invasive carcinoma [Rivera, 2015; Georgaki et al., 2021]. These processes leading to invasive carcinoma defines the initiation, promotion, and progression of tumor formation that are induced by endogenous and exogenous risk factors [Georgaki et al., 2021; Roy et al., 2019]. Several risk factors contributing to oral carcinogenesis include tobacco consumption, excessive alcohol intake, chewing of areca nut-based products, infection with human papillomavirus (HPV), poor dietary habits, inadequate oral hygiene, aging, exposure to ultra-violet (UV) radiation, as well as other factors such as fungal infections, occupational hazards, and syphilis [Warnakulasuriya, 2009, Ram et al., 2011; Kumar et al., 2016; Dhanuthai et al., 2018]. Among various risk factors concurrent consumption of tobacco and alcohol has emerged as a prominent factor to the rising incidences of OC [D'Souza and Saranath, 2015; Liu et al., 2023].

At present, the main therapeutic modalities used for the treatment of OC are surgery, radiation, chemotherapy and immunotherapy, where the treatment methods are done individually or in combination, with the choice of treatment being influenced by factors such as tumor stage, grade, and the patient's overall condition [Omura, 2014, Silva et al., 2023]. Surgery remains the main treatment for OC, with minimally invasive approaches preferred for early-stage disease. In addition, adjuvant therapies,

radiation or chemoradiation is utilized for high-risk patients, serving as a primary therapy for those unfit for surgery. Moreover, systemic therapy is an option for locoregional recurrence or distant metastases, with potential neoadjuvant application for improved outcomes in advanced cases [Mohamad et al., 2023]. Immunotherapy has also recently emerged as a promising approach for treating OC, using patient's own immune system to combat neoplasm [Li et al., 2018; Liu et al., 2022]. Examples of immunotherapy approaches inlcude targeted monoclonal antibodies, immune checkpoint modulators, hematopoietic stem cell (HSC) transplants, chimeric antigen receptor-T-cell therapies, etc. [Patil and Nagaraju, 2021]. Recently, the Food and Drug Administration (FDA) has authorized the use of monoclonal antibodies like nivolumab for treating recurrent or metastatic squamous cell carcinoma of head and neck (HNSCC), and pembrolizumab for metastatic and unresectable recurrent HNSCC (www.fda.gov/drugs) [Algazi and Grandis, 2016]. In addition, the immunotherapy studies have also paved the way for targeted and personalized precision therapy for OC [Patil and Nagaraju, 2021; Liu et al., 2022].

Despite advances in treatment, mortality rates remain high due to limited efficacy and adverse side effects, and late-stage diagnosis, especially in rural areas, leading to lower survival rates [Li et al., 2018; Bordoloi et al., 2019; Mira et al., 2024]. In addition, radiotherapy and chemotherapy damages the healthy cells, tissues and organs, especially where the cells are rapidly growing and dividing [Shapira et al., 2011]. Moreover, a significant challenge with chemotherapy is the occurrence of chemoresistance, where cancer cells become unresponsive to the chemotherapeutic agent. This is mainly due to the decreased influx and uptake of the drugs by the cancer cells and the activation of different survival mechanisms [Debela et al., 2021; Khatoon et al., 2022]. The 5-year survival rate for OC patients remains low ranging approximately 50-60% [Mira et al., 2024; Justesen et al., 2024].

Therefore, it is an imperative need to identify novel molecular targets, and develop safe, efficacious, and affordable drugs for this cancer. Hence, this study explored the role of Mortalin as a therapeutic target and investigated the potential of WiA as Mortalin inhibitor by various pre-clinical studies.

1.2 Review of literature

OC is classified within the broader spectrum of head and neck cancers, encompassing malignancies affecting the lips, mouth, and posterior throat regions, including various sub-locations such as the buccal cavity lining, tongue, and different areas of the throat at the back of the mouth [Argiris et al., 2008; www.nidcr.nih.gov/health]. The OC is classified on the basis of seven subsites of the oral cavity. These subsites include the alveolar ridge, buccal region, floor of the mouth, hard palate, lip, retromolar trigone, tongue, and soft palate [Wong and Wiesenfeld, 2018]. This cancer has different subtypes with squamous cell carcinoma (SCC) accounting for majority of the cases, and other types such as minor salivary gland malignancies, sarcomas, melanoma, and lymphoma accounting for less than 10% [Wong and Wiesenfeld, 2018]. The initial signs of OC typically involve persistent ulcers, sores, or lumps in the mouth that do not heal, prompting further investigation. Additionally, premalignant lesions like leukoplakia, erythroplakia, or mixed erythroleukoplakia, characterized by white or red patches on the gums, tongue, or oral cavity lining, warrant attention for potential OC

risk. The identification of potential risk and early detection of symptoms might prevent or improve the conditions in the patients of OC [Weaver, 2023].

1.2.1 Types of OC

The oral malignancies can be divided into several categories based on its tissues being affected. They are briefly described below:

1.2.1.1 Squamous Cell Carcinoma

SCC is the predominant form of OC, comprising 90% of the cases, with the floor of the mouth and tongue being the most affected sites [Feller and Lemmer, 2012]. It originates from the stratified squamous epithelial layer of the oral mucosa and is linked to factors like tobacco consumption, alcohol usage, and HPV infection. The occurrence of SCC increases with age, predominantly affecting individuals over 40 years [Rivera and Venegas, 2014]. Emerging either from preexisting potentially malignant lesions or occasionally developing within premalignant epithelium OC, it typically manifests as ulcers or lumps, presenting as red or white lesions, or a combination thereof, and occasionally accompanied by lymph node swelling [Feller and Lemmer, 2012; Markopuolos, 2012]. These conditions are often associated with stiffness of the oral cavity [Markopuolos, 2012]. SCC is typically treated with surgery, radiation, or chemotherapy, alone or in combination [Feller and Lemmer, 2012; Baillie et al., 2017]. The manageable locally advanced OSCC involves excision of the primary tumor and affected neck tissues with negative surgical margins, followed by postoperative radiotherapy or chemoradiotherapy based on the patient's risk [Vishak et al., 2015]. The chemotherapeutic drugs like cisplatin and 5-fluorouracil (5-FU) are used for chemotherapy, and these drugs have shown more efficacy in patients in combination with other therapies [Vishak et al., 2015]. Despite the five-year survival outcome remains low at around 50%, primarily due to the late-stage diagnosis, with about two-thirds of patients presenting with advanced condition [Feller and Lemmer, 2012]. In addition, due to high loco-regional recurrence in SCC, it contributes to elevated mortality and an unfavorable prognosis in patients [Rapidis et al., 2009; Baillie et al., 2017]. Moreover, other treatment-related complications, including xerostomia, mucositis, and trismus, impact the daily quality of life in patients [Rezazadeh et al., 2023].

1.2.1.2 Verrucous Carcinoma

VC, also referred to as Ackermann's tumor, is a rare subtype of SCC predominantly found in the oral cavity, characterized by slow growth and local invasiveness, particularly affecting the buccal mucosa, gingiva, and tongue [Walvekar et al., 2009; Stransky et al., 2011; Hosseinpour et al., 2017]. Histologically, VC exhibits an exophytic growth pattern comprised of well-differentiated keratinizing epithelium with minimal atypia and significant chronic inflammatory infiltration, often featuring locally destructive margins [Oliveira et al., 2006]. While metastasis is infrequent despite local tumor progression, its resemblance to verrucous hyperplasia and occasional presence of SCC foci complicate diagnosis, necessitating thorough examination by pathologists [Shergill et al., 2015; Damm, 2002; Sharma et al., 2016]. Surgical excision with adequate margins remains the preferred treatment approach,

although recurrent cases may require additional interventions such as radiotherapy with or without chemotherapy [Kang et al., 2003; Walvekar et al., 2009].

1.2.1.3 Salivary gland carcinomas

The predominant malignant tumors of the salivary glands are mucoepidermoid carcinoma (MEC), acinic cell carcinoma (ACCa), and adenoid cystic carcinoma (ACC). Due to the similar cytomorphologic features they share with other benign and cancerous lesions, as well as non-neoplastic lesions, only few fractions of these neoplasms are reliably diagnosed [Miller et al., 2019].

1.2.1.3.1 Adenoid Cystic Carcinoma

ACC is a common malignant tumor of the salivary glands, affecting both minor and major glands in the oral as well as maxillofacial region, particularly in the parotid and submandibular glands, with frequent occurrences seen in the palate [Giannini et al., 2006; Xu et al., 2024]. It exhibits slow growth, posing a high risk of locoregional recurrence and delayed distant metastasis, commonly to the lungs followed by bones [Chang et al., 2022]. Patients might experience pain and paresthesia as a part of symptoms due to perineural and intraneural invasion, especially in parotid gland [Ullah et al., 2024]. ACC accounts for 1% of head and neck tumors and approximately 10% of salivary gland tumors, with 10–20% of cases originating from minor salivary glands [Giannini et al., 2006]. The individuals with age group approximately 50 years and above, particularly women, are highly affected by minor salivary gland ACC, with the incidences remaining quite high and prognosis being poor [Giannini et al., 2006]. In case of major salivary gland, the individuals with lower age group around 40 years are affected [Giannini et al., 2006]. Surgical resection is the main treatment for palatal ACC, but its challenging location often results in incomplete tumor removal, leading to frequent local recurrence or dissemination along nerve trunks [Armstrong et al., 2020; Chang et al., 2022]. This necessitates a combination approach involving radiotherapy and/or chemotherapy [Chang et al., 2022]. One of the major problems in conventional surgery is oronasal fistula formation, causing hypernasality and food/liquid leakage into the nasal cavity, significantly affecting quality of life [Chang et al., 2022].

1.2.1.3.2 Mucoepidermoid carcinoma

MEC is the most frequently occurring malignancy in the salivary glands, usually appearing as painless swelling in the parotid glands, though it can also develop in various locations in minor salivary glands [Peraza et al., 2020; Shi et al., 2024]. It arises from the reserve cells present in excretory ducts and comprises mucous, epidermoid, and undifferentiated intermediate cell types. MEC demonstrates varied biological behaviors, with high-grade tumors being aggressive and low-grade variants generally showing a benign nature [Ozawa et al., 2020]. Women are predominantly affected with MEC and it typically starts around 49 years, with a 5-year survival probability of 67% to 90% depending on the grade [Devaraju et al., 2014; Shi et al., 2024]. Low to intermediate grade MEC is typically managed with surgical resection ensuring clear margins, with adjuvant radiotherapy recommended for cases with perineural invasion, lymph node involvement, or advanced high-grade tumors.

Combined chemoradiotherapy enhances regional control, but does not prolong the patient's overall survival (OS) [Peraza et al., 2020; Sama et al., 2021; Shi et al., 2024]. Systemic therapies are mainly used for palliative care to relieve cancer-related symptoms or in cases of rapid disease progression [Sama et al., 2021].

1.2.1.3.3 Acinic cell carcinoma

ACCa is a low-grade malignant epithelial tumor primarily affecting the salivary glands, comprising approximately 6% of salivary gland tumors [Al-Zaher et al., 2009; Cavaliere et al., 2020; Kademani and Bagheri, 2008]. It is defined by the presence of neoplastic cells consisting of serous acinar cell differentiation, often identifiable by cytoplasmic secretory granules containing zymogens [Al-Zaher et al., 2009; Ilayaraja et al., 2014]. ACCa primarily originates in the parotid gland (3-4%), often presenting with painful swelling, although it can also develop in other sites of salivary gland [Al-Zaher et al., 2009]. Notably, it tends to affect a relatively younger age and exhibits a female predilection [Kademani and Bagheri, 2008; Al-Zaher et al., 2009]. Surgery remains as the primary treatment; however, the overall prognosis is influenced by the extent of the lesion and the effectiveness of the initial resection [Kudpaje et al., 2014]. The survival statistics for ACCa indicate a five-year rate of about 76% [Kademani and Bagheri, 2008]. Although metastasis rates are low, regional and distant spread can still occur, with the lungs being the primary site of distant metastasis, followed by bone [Kademani and Bagheri, 2008].

1.2.1.4 Oral melanoma

Oral melanoma is an extremely rare and aggressive malignancy that progresses rapidly and represents a small fraction of all melanomas (0.2% to 8%) and oral malignancies (1% to 2%) [Aguas et al., 2009; Warszawik-Hendzel et al., 2014]. Mucosal melanomas, in particular, have the lowest 5-year survival rate ranging from 25% to 42%, among melanomas, possibly due to its delayed detection [Mihajlovic et al., 2018]. Typically, it appears as a pigmented lesion, either flat or nodular, exhibiting a range of colors, including dark brown, black, gray, purple, red, or regions of lighter pigmentation [Zito et al., 2023]. In addition, amelanotic lesions with absence of pigmentation were also reported, which might delay the diagnosis [Mihajlovic et al., 2018; Zito et al., 2023]. The causes and mechanisms of development are not well understood; however, primary oral mucosal melanomas mostly arise spontaneously and up to 37% of cases may be preceded by pigmented lesions persisting for months to years [Lambertini et al., 2018]. The primary treatment involves radical operative resection, often succeeded by adjuvant radiation and chemotherapy in patients with high-risk features, which can enhance locoregional control but does not increase OS [López et al., 2015; Zito et al., 2023].

1.2.1.5 Oral cavity lymphomas

Oral cavity lymphomas are rare, comprising approximately 3% of all lymphoma cases and around 4% in patients with acquired immunodeficiency syndrome (AIDS) [Silva TD et al., 2016]. Oral cavity lymphomas affect the areas such as the buccal mucosa, gingiva, floor of the mouth, tongue, palate, and lips [Moin et al., 2017]. The diagnosis of this malignancy is challenging due to clinical features resembling other conditions

like periodontal disease and osteomyelitis, often leading to delayed treatment and poor prognosis [Silva et al., 2016]. The treatment typically involves combinations of chemotherapy, radiotherapy, and surgery for non-Hodgkin's lymphoma (NHL) management [Moin et al., 2017].

1.2.1.6 Sarcoma

Sarcoma comprises various malignant tumors originating in mesenchymal soft tissues, occurring rarely and accounting for less than 1% of oral malignancies [Pandey et al., 2000; Yamaguchi et al., 2004]. Rhabdomyosarcoma, fibrous histiocytoma, fibrosarcoma, neurofibrosarcoma, angiosarcoma malignant schwannoma and malignant rhabdoid tumor are examples of different sarcoma that affect the head and neck region [Pandey et al., 2000; Sumida et al., 2015]. Despite diverse origins, they share similarities in clinical presentation, history and outcomes [Pandey et al., 2000]. Sarcomas often spread aggressively with high metastatic potential, and requires early identification for its surgical removal. There is no definite treatment as the cases are very rare, however, approach combining surgery and radiotherapy shows potential to enhance locoregional control and improve quality of life for these patients [Yamaguchi et al., 2004; Sumida et al., 2015].

1.3 Risk Factors and Prevention

OC is influenced by a complex interplay of various risk factors manifesting as environmental, lifestyle and genetic risk factors (**Fig. 1.1**). The various risk factors involved in OC are summarized below:

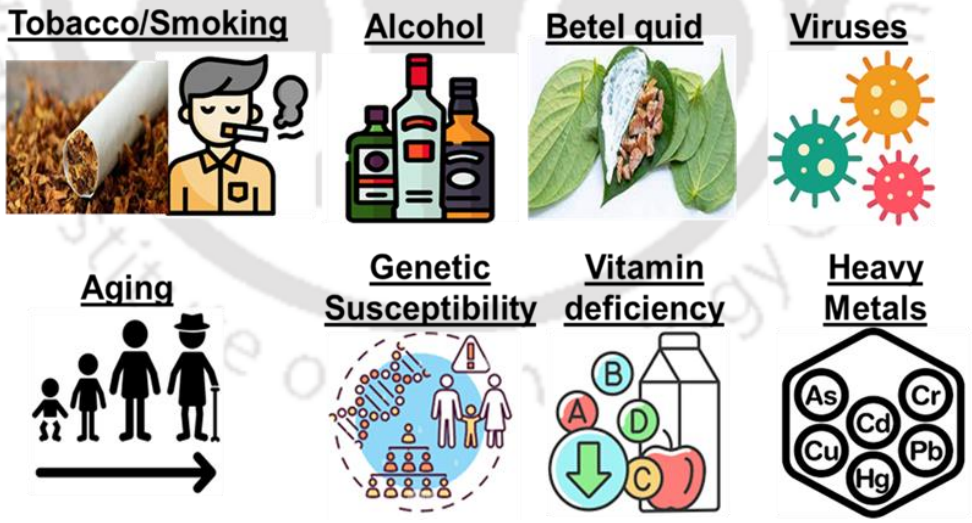

Fig. 1.1 Risk factors of OC (Image credit: BioRender.com, Google images)

1.3.1 Tobacco

Tobacco stands as a prominent carcinogenic factor in OC development across both genders [Johnson, 2001]. Predominantly, smoking, in various forms such as cigarettes, cigars, pipes, bidis, hookahs, or chillums, constitutes the most prevalent form of tobacco misuse. The carcinogenic components in cigarette smoke, including nicotine, exert biological effects on the brain, gastrointestinal tract, and oral cavity

[Irani, 2020]. With over 60 carcinogens identified in cigarette smoke, including notable ones like tobacco-specific nitrosamines (TSNs) such as N'-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), polycyclic aromatic hydrocarbons (e.g., benzo[a]pyrene (BaP)) and aromatic amines (e.g., 4-aminobiphenyl), the risk of OC development is significantly elevated in tobacco users [Zhang et al., 2019]. The epidemiological correlation between smoking and OC is stark, with smokers are at a 7 to 10 times higher risk compared to non-smokers and a threefold increased likelihood of second primary cancer occurrence [Zhang et al., 2019]. Oral snuff and pipe tobacco, also contains nicotine and its metabolites like NNK and NNN, contribute to carcinogenicity through their binding to nicotinic acetylcholine receptors, fostering cell proliferation and providing a conducive environment for tumor growth [Irani, 2020]. In addition, cannabis smoke contains higher concentrations of cancer-causing polyaromatic hydrocarbons compared to tobacco smoke, along with phenols and polycyclic aromatic hydrocarbons, and marijuana tar which contains 50% more BaP than unfiltered tobacco. These carcinogenic molecules in cannabis elevates the risk of oral carcinogenesis [Cretu et al, 2024]. Additionally, the increasing global prevalence of smokeless tobacco contributes to increase risk of cancer, resulting in the delivery of nicotine through mucous membranes. Other carcinogenic substances present in smokeless tobacco includes BaP, nitrosamines, formaldehyde, polonium, cadmium, and lead [Janbaz et al., 2014]. One of the examples of smokeless tobacco known as 'tuibur', or 'hidakphu', consists of tobacco smoke infused with water and is prevalent in the Indian Northeastern states of Mizoram and Manipur. It is typically kept in the mouth for a period before being spat out, and is recognized for its elevated levels of NNN and heavy metals [Sinha et al., 2004; Pachuau et al., 2022]. The carcinogenic substances and their byproducts in tobacco induce oral tumors, forming DNA adducts within keratinocyte stem cells, resulting in significant mutations during DNA replication [Kakabadze et al., 2020]. Carcinogenic metabolism involves enzymes like P450 enzymes and glutathione-S-transferase (GST) to metabolize these carcinogens, while genetic variations in these enzymes increase susceptibility to tobacco-induced cancers [Kumar and Zain, 2004; Ali et al., 2004]. These xenobiotic metabolizing enzymes (XMEs), found in liver and mucosa of upper aerodigestive tract are crucial for activating and detoxifying of carcinogens [Renu et al., 2013; Beyerle et al., 2020]. Specific genetic variations in XME can elevate the risk of cancer by mishandling the carcinogenic metabolism, resulting in increased exposure to these harmful substances [Renu et al., 2013; Buch et al., 2008]. In addition, the head and neck cancer patients often exhibit high degree of genomic instability and reduced repair mechanism in response to the DNA damage caused by carcinogens [Papalouka et al., 2023].

1.3.2 Betel quid

Betel quid chewing, prevalent in Southeast Asia and the Indian subcontinent, involves a blend of betel leaf, slaked lime, areca nut, and occasionally tobacco. Despite its cultural significance, this practice raises concerns due to its association with various oral health issues, including precancerous lesions like leukoplakia, erythroplakia, and oral submucous fibrosis, indicating a potential risk for OC. Studies have linked betel quid and areca nut usage to unfavorable prognoses in individuals with OC, with

regular chewers experiencing chronic oral irritation and inflammation that can damage epithelial cells [Yang et al., 2021]. In addition, arecoline, the key alkaloid constituent of areca nut influences cellular processes as an agonist on muscarinic acetylcholine receptors, causing cholinergic effects on the parasympathetic nervous system and acting as a psychoactive substance [Papke et al., 2015]. Arecoline and other components induces various pro-carcinogenic changes within the oral mucosa, including the accumulation of nitrosamines and the generation of oxygen-reactive species (ROS), upregulation of heat-shock proteins, integrins and inflammatory cytokines (tumor necrosis factor-α, interleukin (IL)-1β, IL-6, and IL-8), as well as modulation of matrix metalloproteinases and suppression of collagenases [Chang et al., 2009]. In addition, arecoline hampers the DNA repair mechanism, preventing both the endogenous and exogenous DNA damage through its ability to inhibit p53 and suppress DNA repair system [Tsai et al., 2008]. Moreover, compromised oral hygiene among betel chewers disrupts the oral cavity microbiome, potentially creating a conducive environment for bacterial-mediated oral lesions and carcinogenesis. Given the genotoxic, mutagenic, and carcinogenic potential of betel quid components, primarily tobacco and areca nut, research efforts have intensified to better understand the underlying mechanisms driving OC development. In vitro studies on oral mucosal fibroblasts have demonstrated genotoxic and cytotoxic effects of essential betel quid components, along with stimulation of cell proliferation. Meta-analyses, such as the one conducted by Guha et al., have confirmed a substantial increase in the risk of OC associated with betel quid consumption, whether consumed separately or mixed with tobacco. Additionally, the risk of OC due to betel quid consumption also relies on the frequency and duration of chewing [Guha et al., 2014].

1.3.3 Alcohol

Alcohol plays a major role in increasing the risk of OC, especially when combined with tobacco use [Irani, 2020]. The risk of developing oral cancer (OC) is known to increase by 10 to 15 times attributable to the combined intake of alcohol and tobacco [Mummudi et al., 2019]. One of the key mechanisms identified for alcohol-induced carcinogenesis involves the conversion of alcohol into acetaldehyde, a known oncogenic compound, by enzymes like alcohol dehydrogenases (ADHs) [Edenberg, 2007]. Acetaldehyde is subsequently broken down into acetate, facilitated by aldehyde dehydrogenases (ALDH) [Edenberg, 2007; Jiang et al., 2020]. Genetic alterations affecting these enzymes can predispose individuals to acetaldehyde accumulation, raising their risk of cancer [Crabb and Liangpunsakul, 2007]. Acetaldehyde accumulation can lead to DNA damage, interfere with mechanisms of DNA repair, and induce of genetic mutations [Singhania and Mishra, 2024]. In addition, long term alcohol consumption damages the liver, reducing its ability to detoxify carcinogens and causing nutrient deficiencies, which can elevate OC risk [Barve et al., 2017; Reidy et al., 2011]. Additionally, alcohol contains harmful substances like mycotoxins, N-nitroso compounds, urethane, and inorganic arsenic, which further increase its carcinogenic potential inducing cancer risk [Pathak et al., 2023]. Moreover, consuming alcohol excessively can have various impacts on the oral mucosa which include the shrinkage or breakdown of the oral epithelial cell membrane by dissolving its lipid components, resulting in epithelial atrophy thus

promoting the penetration and dissolution of carcinogenic metabolites, as well as interfering with DNA synthesis and repair processes [Petersen, 2009; Cardoso et al., 2020]. Furthermore, alcoholism-related suppression of the immune system may increase susceptibility to cancer. This is often attributed to the suppression of natural killer (NK) cell activity by alcohol, which is crucial for detecting and eliminating tumor cells [Pöschl and Seitz, 2004]. Other factors contributing to immune system impairment in individuals with alcoholism, including malnutrition, deficiencies of vitamins, and development of cirrhosis [Pöschl and Seitz, 2004].

1.3.4 Diet and nutrition

Diet and nutrition are recognized to have a function in the prevention of OC [Helen-Ng et al., 2012]. Consumption of a diet high in fruits, vegetables, and whole grains, particularly those rich in antioxidants and phytochemicals, has been linked to a reduced risk of OC [Liu, 2003; Chandra and Sah, 2012]. Conversely, diets comprising processed foods, red meat, and saturated fats have been correlated with an elevated susceptibility to OC [Taghavi and Yazdi, 2007]. Nutritional deficiencies, such as inadequate intake of vitamins A, C, and E, as well as essential minerals like selenium and zinc, may compromise the immune system and contribute to the development of oral precancerous lesions and oral carcinogenesis [Khanna et al., 2013; Strączek et al., 2023]. These nutrients play crucial roles in scavenging free radicals and preventing lipid peroxidation [Jeng et al., 2001]. Furthermore, diet rich in fish and dairy products are associated with diminished risk of OC [Manoharan et al., 2016]. Overall, adopting a well-balanced diet that prioritizes nutrient-rich foods and antioxidants while minimizing the intake of processed foods can significantly mitigate the risk of OC and enhance overall oral health [Mangalath et al., 2014].

1.3.5 Human papilloma virus infection

HPV has emerged as a significant factor in the development of OC, particularly OSCC. Several HPV strains, including HPV-6, -11, -16, -18, -31, -33, and -42 are frequent in causing infection in oral cavity [Miller and Johnstone, 2001; Meurman, 2010]. Research indicates a high prevalence of HPV DNA in cell samples obtained from OSCC lesions compared to healthy oral mucosa, suggesting a possible involvement of HPV in OC development [Miller and Johnstone, 2001]. Notably, patients infected with HPV-16 and -18 shows 2.8 times higher risk of developing malignant lesions within the oral cavity, suggesting their importance as significant risk factors for the development of OC [Miller and Johnstone, 2001]. HPV basically infects basal epithelial cells in the oral cavity, expressing viral proteins like E6 and E7. These proteins disrupt cellular regulation by targeting tumor suppressors p53 and retinoblastoma protein (pRB), leading to uncontrolled cell growth. As infection progresses, viral DNA replicates, and infectious virions are shed into the environment. HPV infection can also lead to latency, with potential reactivation under certain conditions, contributing to malignant transformation in OC [Sand and Jalouli, 2014].

However, HPV-positive OCs often respond better to radiotherapy, attributed to the unique biological characteristics of HPV-driven tumors. Mechanistically, HPV-driven tumors show high radiosensitivity due to viral oncoproteins E6 and E7 interfering

with DNA repair, rendering them more susceptible to radiation-induced DNA damage. Furthermore, the robust immune response in HPV-positive tumors enhances radiotherapy efficacy by fostering tumor cell elimination. This synergistic effect of HPV infection and radiotherapy emphasizes the superior treatment outcomes observed among HPV-positive OC patients compared to those who are HPV-negative [Das and Motghare, 2021].

1.3.6 Aging

Oral health tends to be overlooked among the elderly, yet aging-related oral diseases can significantly impact their quality of life [Guiglia et al., 2010]. OC is particularly prevalent in older individuals and primarily affects older males due to established hazardous factors such as consumption of tobacco and alcohol [Guiglia et al., 2010; Oh et al., 2021]. The likelihood of developing OC escalates with age, with a significant proportion of cases reported in individuals above 40 years. In addition, individuals above 65 years are 7 times more likely to be diagnosed with OC compared to those younger than them [Guiglia et al., 2010; Warnakulasuriya, 2009; Warnakulasuriya, 2010]. Moreover, the younger patients were reported to have better 5-year OS as well as disease-specific survival (DSS) outcomes compared to older patients, with 71% OS and 72% DSS in the young group, and 57% OS and 58% DSS in the older group. Moreover, younger age groups tend to show favored better prognosis and treatment outcomes across all subgroups except those with distant metastases [Chen et al., 2020].

1.3.7 Other factors

Other risk factors such as occupational risk, dental factors, radiation and syphilis are involved in oral carcinogenesis. Dental factors such as poor oral hygiene, compromised dental health, and chronic ulceration from poor-fitting dentures are potential contributors of OC, especially when combined with other risk factors. In addition, occupation with high risk to UVB exposure poses a significant risk for OC [Agrawal et al., 2013]. Moreover, syphilis might also lead to mucosal and gingival lesions that can potentially contribute to development of OC due to chronic inflammation and tissue damage [Jones et al., 2012].

1.4 Premalignant Lesions of the oral cavity

OC typically evolves through a two-step process, with most malignant ulcers arising after the precursor lesions, commonly presented as red or white patches, and are collectively termed ‘potentially malignant disorders (PMDs)’, by the World Health Organization (WHO) [Reibel, 2003; van der Waal, 2009]. The causes of precancerous oral mucosal lesions are not fully understood, but certain factors like tobacco use and alcohol consumption significantly contribute to their development [Yardimci et al., 2014]. The lesions such as oral cavity leukoplakia, oral submucous fibrosis (OSF), oral erythroplakia, and oral lichen planus (OLP) are among the most prevalent PMDs, known for their high rates of transformation into oral malignancy [Yardimci et al., 2014]. The different premalignant lesions of oral cavity are briefly as follows:

Oral leukoplakia is presented as white lesions in the oral cavity and smokers are six times more likely to have leukoplakia than non-smokers [Yardimci et al., 2014; Parlatescu et al., 2014]. Another type of PMDs, erythroplakia is represented as a red patch with a velvety smooth or granular surface, typically found in areas like the floor of the mouth, the ventral surface of the tongue, or the soft palate [Ranganathan and Quail, 2024]. The risk of malignant transformation in erythroplakia exceeds that in leukoplakia by 17-fold [Ranganathan and Quail, 2024]. In addition, OLP represents an autoimmune condition and manifests as reticular white patterns that occur in the tongue, gingiva, etc. and possess more than 1% of malignant transformation [Ismail et al., 2007; Yardimci et al., 2014; Olson et al., 2016; Fitzpatrick et al., 2014]. OSF is an additional precancerous condition that lead to stiffness in the oral mucosa, representing around 4-13% risk of malignant transformation to OC [Arakeri et al., 2013; Yaming et al., 2016; Pandya et al., 2009]. Moreover, oral candidiasis, is observed as white patches and affects the tongue and other parts of oral mucosa, marked by excessive fungal growth [Swain, 2021; Anitha, 2012; Mallya and Mallya, 2019; Vila et al., 2020]. Further, actinic cheilitis is induced by UV radiation and represented as an atrophy of squamous cell epithelium to hyperplasia at the vermilion border [Wood et al., 2011; Shah et al., 2023].

1.5 OC Diagnosis and Staging

The early detection and diagnosis of OC could be done through routine checkup in individuals especially the patients consuming any form of tobacco or alcohol, by primary dental and general practitioners. The routine biopsy of the patients detected with oral potentially malignant lesions could lead to early diagnosis of the associated oral malignancy. For early diagnosis, history of the patients can be looked upon. In addition, proper assessment of the upper airway and digestive system can be done as OC has high risk of developing other cancers in the head and neck area as well as in the lungs [Sankaranarayanan et al., 2015]. Once the disease is diagnosed, proper classification of the severity of the disease is done through tumor-node-metastasis (TNM) staging system defined by the American Joint Committee on Cancer (AJCC) [Hudgins and Beitler, 2013].

In the recent years, to aid up the clinical classification and staging of the oral cavity cancers, various modern advanced technologies such as computed tomography (CT), positron emission tomography (PET), integrated PET-CT, magnetic resonance imaging (MRI), and ultrasonography have been established [Arya et al., 2014; Sarrión Pérez et al., 2015]. Out of these, PET-CT and MRI remain as the primary technique to detect the locoregional recurrence associated with the disease [Kim et al., 2019]. In addition, for the staging of the disease, panendoscopic examination of the OC patients are recommended as approximately 7% of these patients were reported to have associated with inflammation and secondary primary lesion in the upper digestive tract, especially in high risk patients which includes smokers and drinkers [Valentin et al., 2021].

1.6 Tumor-node-metastasis (TNM) staging

The TNM staging is a globally accepted system to describe the anatomic extent of a tumor [Greene and Sobin, 2008]. The prognosis and the choice of treatment strategies

are associated with the size and spread of the primary tumor (T stage) at the regional lymph nodes (N stage) and observation based on the detection or lack of distant metastases (M stage) [Patel and Lydiatt, 2008]. The tumor has to be classified before the treatment and resection and according to this it can classified into two common TNM classification such as clinical staging (cTNM) and pathologic staging (pTNM), respectively. The cTNM is usually based on the clinical assessment and imaging strategies that employs the use of techniques such as CT and MRI while pTNM is based on tumor histopathologic analysis generally applied for determining the course of adjuvant therapy and it can be considered to be more precise and superior than the former. However, if tumor treatment is not involved with surgery and rather involves with neoadjuvant chemo radiation then the pTNM staging is nullified [van der Schroeff and Baatenburg de Jong, 2009]. The details of the TNM staging are given in supplementary table 1.1.

1.7 Molecular mechanism in the development of OC

The process of oral carcinogenesis is a multi-step process affected by various genetic and epigenetic factors [Pérez-Sayáns et al., 2009]. Generally, different processes such as evading growth suppressors, resistance to cell death, enabling replicative immortality, promotion of proliferative signaling, angiogenesis, invasion and metastasis are being known in carcinogenesis [Hanahan and Weinberg, 2011]. Similarly, in head and neck cancers including oral cavity cancer, these processes are linked with alterations in different proteins. Deregulation of different genes and proteins are reported at an early stage, and the frequency of molecular alterations increased as the disease progressed [Shah et al., 2007]. Research have suggested that p16 might be the earliest tumor suppressor that undergo inactivation in this cancer, along with deregulated expression of p53, pRB and cyclin D1 during disease progression. These genes are being investigated for its regulatory mechanism in regulating cell cycle [Shah et al., 2007; Krishna et al., 2015]. Other than cell cycle regulatory proteins, the proteins involved in mitogenic signaling pathway, such as signal transducer and activator of transcription-3 (STAT-3), epidermal growth factor receptor (EGFR), H-Ras, and c-Myc were linked to oral carcinogenesis [Shah et al., 2007]. Moreover, the phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway is highly upregulated in OC [Roy et al., 2019; Harsha et al., 2020]. The mutations in phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), and phosphatase and tensin homolog (PTEN) are prevalent among individuals with head and neck neoplasm [Leemans et al., 2011]. In addition, commonly mutated genes like *CASP8*, Mucin-16 (*MUC16*), heparan sulfate-glucosamine 3-sulfotransferase 6 (*HS3ST6*) and ret finger protein like 4A (RFPL4A) were also observed in OC [Zhou et al., 2024]. Deletions in cyclin-dependent kinase inhibitor 2A (*CDKN2A*) and variations in genes including *CCND1*, *EGFR*, *ERBB2*, and *CCNE1* have been documented in cases of HNSC. Moreover, mutations in *NOTCH* (-1, -2, -3), human Dead-box protein 3 (*DDX3X*), as well as histone methyltransferases, PR/SET domain containing protein 9 (*PRDM9)* and enhancer of zeste homolog 2 (*EZH2)* have been observed in HNSC patients [Stranskyet al., 2011]. Further, sirtuin 1 (SIRT1) is also an important protein associated with the initiation of oral squamous cell carcinoma [Islam et al., 2019].

1.8 Treatment modalities

There are mainly three treatment modalities to treat OC such as surgery, radiation and chemotherapy that is either used alone or in combination (**Fig. 1.2**). Generally, for the early stages- stage I and II, and carcinoma-in situ (CIS), treatment with single modalities can be used meanwhile individuals with the advanced stages (Stages III and IV) are usually subjected to combination of these therapies. In addition, targeted therapy is also available that can inhibit the spread or growth of tumor by disrupting key pathways and molecules involved in cancer development and progression [Carrington C., 2015].

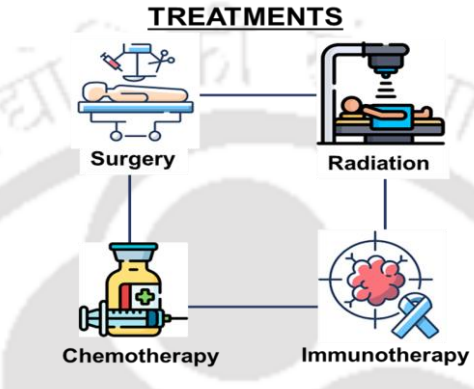

Fig. 1.2 Treatment modalities of OC (Image credit: BioRender.com, Google images)

1.8.1 Surgery

The main approach for treating OC depends on how advanced the disease has progressed, however; surgery typically plays a central role as a mainstay modality for the treatment of this disease [Shah and Gil, 2009]. For an advanced condition, surgery is used in combination with local radiotherapy and/or systemic chemotherapy [Prelec and Laronde, 2014]. The primary focus of surgical resection is to thoroughly remove the tumor tissue without affecting tissue function, while the improper clearance of tumor cells linked to a higher probability of local regional recurrences leads to low survival in patients [Sutton et al., 2003; Prelec and Laronde, 2014]. Generally, three-dimensional margins of 1 cm are considered for resection, and further increased of the surgical margins might lead to esthetic and functional complications in OC patients [Omura, 2014]. In addition, in primary tumor resection, using iodine staining is advisable to identify and outline dysplastic epithelium along with the cancerous lesion [Omura, 2014]. Surgical techniques differ based on the size of lesion and accessibility. Smaller tumors can be removed within the oral cavity, while larger or hard-to-reach tumors might require an external approach, potentially involving the removal of both soft tissue and bone [Prelec and Laronde, 2014].

1.8.2 Radiotherapy

Radiation therapy is generally not a primary treatment for OC, except in cases where tumor excision makes difficult due to its location, like the oropharynx, or if patient does not consent to surgery [Prelec and Laronde, 2014]. There are mainly two types of radiotherapy such as external beam radiotherapy (EBRT) and brachytherapy

[Huang and O'Sullivan, 2013]. EBRT uses linear accelerators outside the patient to deliver radiation to the tumor and surrounding areas. Brachytherapy, also known as interstitial radiation therapy, delivers high radiation doses to the tumor while minimizing exposure to surrounding tissues by placing radiation sources like implants directly into the tumor [Hashibe et al., 2005]. Radiation therapy exhibit very low 5-year survival rates around 15%, while concurrent chemotherapy after radiation might increase the local control of the disease and OS in OC patients [Lang et al., 2021]. However, compared to surgery alone, radiation therapy results in fewer complications, preserves function and aesthetics, and enhances quality of life. The combination of surgery with postoperative radiation therapy is common for large tumors or with positive surgical margins for cancer. It is usually given after surgery to avoid healing issues and infection risks from surgery [Prelec and Laronde, 2014].

1.8.3 Chemotherapy

Chemotherapy utilizes chemotherapeutic drugs for treating OC, often in conjunction with radiation (chemoradiation), post-surgery to eradicate residual cancer cells (adjuvant chemotherapy), and before surgery to reduce the size of large tumor tissues (neoadjuvant or induction chemotherapy) [Huang and O'Sullivan, 2013]. The chemoradiation therapy (CRT) is usually an adjuvant treatment; however, it can be a primary treatment modality when the patients are not fit for surgery [Mohamad et al., 2023]. Neoadjuvant chemotherapy in oral cavity cancer aims to reduce tumor size, potentially enhancing locoregional control and OS, while also facilitating organ preservation in operable cases [Hartner, 2018; Goel et al., 2020]. It can also be employed for borderline resectable or technically unresectable tumors to decrease surgical margins, enhance resectibility, and achieve R0 resection. Additionally, Neoadjuvant chemotherapy may benefit patients with unresectable tumors by improving disease-free survival (DFS) and OS [Goel et al., 2020]. The application of chemotherapy might lead to improved locoregional control, OS, and metastasis-free survival outcomes in OC patients [Dhawan, 2021]

1.8.4 Targeted and Immunotherapy

Immunotherapy is the use of medicines that help to improve patient's immune response and enhance the cytotoxicity towards cancer cells. It can be employed after chemotherapy to treat cancer [www.FDA.gov/drugs]. The use of this approach is expanding due to its propensity to cause minimal damage to the normal cells compared to the chemotherapeutic drugs. Targeted therapies inhibit the tumor growth and spread by modulating the specific pathway proteins and molecules that are integral to the growth and progression of OC [Carrington, 2015]. This therapy specifically targets the overexpressed or mutated proteins present in the cancer. This therapy usually involved the use of small molecule inhibitors and monoclonal antibodies. The former is given orally while the latter is given parenterally, as they are proteins susceptible to degradation in the gastrointestinal tract [Carrington, 2015]. There are different small molecule inhibitors used for this targeted therapy, few of them are inhibitors of EGFR tyrosine kinase, like erlotinib (Tarceva®) and gefitinib (Iressa®), which inhibit EGFR tyrosine kinase by binding to its adenosine triphosphate (ATP) binding site, thereby blocking the phosphorylation [Soulieres et al., 2004;

Kirby et al., 2006; Li et al., 2012]. Sunitinib, pazopanib and sorafenib are additional kinase inhibitors that impact various pathways crucial for cancer cell proliferation, especially by targeting the proteins like vascular endothelial growth factor (VEGF), stem cell factor receptor, platelet-derived growth factor receptor (PDGF), and colony-stimulating factor-1 [Elser et al., 2007; Burstein et al., 2008; Beljanski, 2010; Li et al., 2023]. Moreover, pembrolizumab and nivolumab are immunotherapeutic drugs that target PD-1 and helps killing the recurrent and metastatic cancer cells [www.FDA.gov/drugs].

1.9 Problems Associated with the Conventional Therapies

Though conventional treatments play a pivotal role in cancer treatment, they also come with inherent drawbacks and challenges [Debela et al., 2021]. The major problems associated with the conventional treatment modalities are chemoresistance and tumor recurrence in OC patients [Yang al., 2015; Zhao et al., 2019]. The post-operative recurrence following surgery is frequent, occurring in nearly 50% of cases of OC [Sundberg et al., 2019]. Distant recurrence is also prevalent in OC cases. The likelihood of tumor recurrence is influenced by the diagnosed disease condition and treatment options in OC patients [Zhao et al., 2019]. In addition, radiotherapy induces DNA damage in normal cells, leading to their inability to repair the damage. The conventional radiotherapy employs high-energy photons to target and eliminate cancer cells. Unfortunately, this treatment also affects healthy cells, including those in the hair, salivary glands, and mucosal tissues, causing damage alongside its intended therapeutic effects [Huang, 2013]. Cytotoxic agents like cisplatin, paclitaxel, and vincristine are utilized in the therapeutic management of head and neck carcinomas. Nevertheless, patients often exhibit poor responsiveness to these drugs and eventually develop chemoresistance [Yang et al., 2015; Dai et al., 2020]. Other side effects of chemotherapy include mucositis and myelosuppression along with symptoms like nausea, vomiting, oral ulcers, skin rash, enamel erosion, loss of appetite, neuropathy, and potential pulmonary, renal, ototoxicity, and hematological toxicity [Nandini et al., 2020]. In addition, although targeted therapies are designed to be selective, they can still result in adverse outcomes, spanning from dermatological reactions to severe gut-related complications like perforation. The toxicity profile varies depending on the specific drug and its characteristics; however, most targeted drugs are linked to side effects such as nausea, diarrhea, dermatological issues, hypertension, constipation, and fever [Carrington, 2015].

Therefore, it is crucial to identify novel therapeutic targets for the treatment and management of OC. Hence, in the next part of the literature review, we focused on a protein, Mortalin, for its oncogenic role in different cancers. In addition, we also evaluated the efficacy of WiA in suppressing different hallmarks of cancer. Thus, this literature review might possibly pave the way for the identification and establishment of the potential of Mortalin as therapeutic target in OC.

1.10 Mortalin

Mortalin, classified within the heat shock 70 kDa (HSP70) protein family, is unique as it is not induced by heat, however, due to sequence similarities it has been identified as a member of this family [Yoon et al., 2022]. Mortalin (as Mot-1) was

initially cloned in 1993 by Wadhwa et al., and named for its association with cellular mortality. It was found to be distributed in the cytosolic compartments of mouse embryonic fibroblasts (MEF) as well as in mortal hybrid cells derived from combination of mortal MEF with immortal cells (MN48-1, a derivative of NIH 3T3). Cloning and homology analysis of this protein classified it as a member of HSP70 protein family [Wadhwa et al., 1993a]. In contrast, the immortal mouse cells exhibited Mortalin (as Mot-2) primarily in the perinuclear region, differs by two amino acids compared to Mot-1 [Wadhwa et al., 1993b]. While overexpression of Mot-1 induced cellular senescence, Mot-2 led to malignant transformation. In humans, Mortalin exists as Hmot-2, and does not exist more than one form but their functions are expected to have similar property to Mot-2 protein of the mouse. It has a similar pattern of subcellular distribution to Mot-1 and Mot-2. Its gene, named *HSPA9B*, is located on chromosome 5q31.1. In malignant transformation, Mortalin causes inactivation of p53 and activation of other telomerase and growth promoting proteins [Ryu et al., 2014].

1.10.1 Structure of Mortalin

The human Mortalin gene consists of 2850 base pairs with 17 exons and coding for a protein consisting of 679 amino acids, possessing a molecular weight of 73,913 daltons, and is known to share 98% homology with the mouse Mortalin [Deocaris et al., 2006; Deocaris et al., 2013]. The structural characterization of Mortalin remains to be determined due to challenges in crystallizing the full-length protein [Deocaris et al., 2006]. However, studies have been carried out and have computationally devised its working structure based on conserved domains (**Fig.1.3**) [Grover A et al., 2012]. It was found that Mortalin comprises two principal regions, the C-terminal substrate-binding domain (SBD) and the N-terminal nucleotide-binding domain (NBD). The NBD region is responsible for ATP-binding and regulation of ATPase activity while the SBD part of the structure has "latches" controlling substrate acquisition and retention [Luo et al., 2010; Amick et al., 2014]. Conceptually, Mortalin is depicted as a "kettle pot," structure with the NBD forming the pot's handle and the SBD resembling a molecular basket with "latches." The NBD is 44 kDa and structurally comprises of lobe 1 and lobe 2 which are subdivided with two domains (IA, IB, IIA, and IIB) and stabilized by metal ion interaction creating a cavity for ATP-binding. Upon binding of ATP to the cleft formed by the Lobes IIA and IIB, it induces allosteric changes throughout NBD, where the highly conserved inter-domain segment linking NBD to the SBD is known to transmit these changes [Deocaris et al., 2013]. Conversely, the SBD exhibits greater sequence diversity and may be accountable for interaction of Mortalin with various molecules. The mobile part, i.e., the lid domain of SBD selectively binds with its various partners and releases the substrate being monitored by NBD depending on ATP: adenosine diphosphate (ADP) status [Luo et al., 2010; Deocaris et al., 2013].

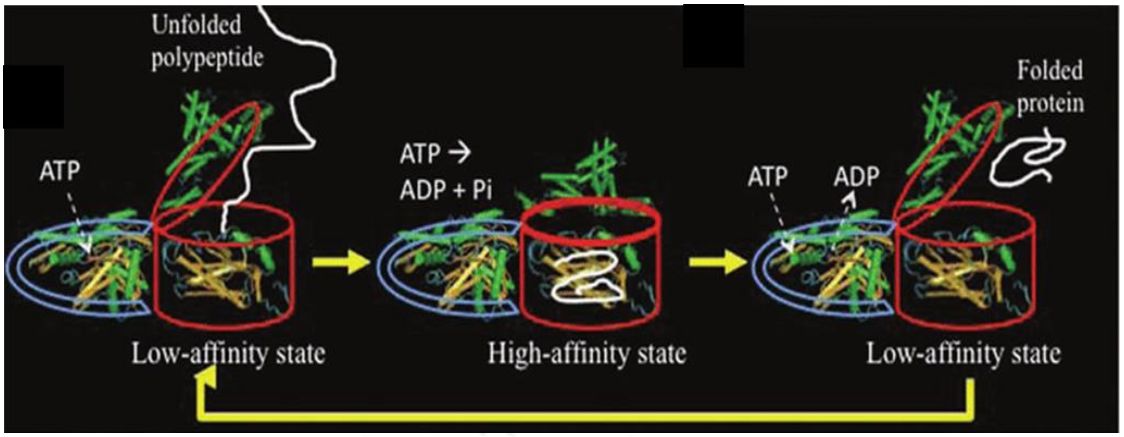

Fig. 1.3 Functional model of Mortalin (Deocaris et al., 2013)

1.10.2 Functions of Mortalin

Members of HSPs acts a molecular chaperone, helping in protein folding and maintaining their native structure and function. They play a significant role under diverse stress response to temperatures, oxygen deprivation, heavy metals, and chemical exposure, ensuring proper protein folding and maintaining cellular stability [Butler et al., 2024]. Similarly, Mortalin plays diverse roles across cellular functions, including stress response, mitochondrial biogenesis, muscle activity, antigen processing, intracellular trafficking, and cell fate regulation [Kaul et al., 2002]. Functionally, Mortalin operates within diverse cellular compartments, with distinct roles dictated by its localization [Baseler et al., 2012]. Within mitochondria, it performs as a chaperone by facilitating folding, stabilization, and translocation of protein through translocase complexes, while also interacting with mitochondrial components [Baseler et al., 2012; Esfahanian et al., 2023]. Moreover, it contributes to biogenesis of iron-sulfur complexes, responding to glucose deprivation, and shields against reactive oxygen species [Esfahanian et al., 2023]. Besides its primary mitochondrial localization, around 30% of Mortalin are present in other subcellular locations such as cytoplasm, endoplasmic reticulum, nucleus, and also circulates in the bloodstream. Outside mitochondria, it interacts with various cellular elements like p53, centrosomes, growth factors, metabolic factors and immune proteins [Baseler et al., 2012]. In addition, the extracellular Mortalin serves a crucial survival function by inhibiting complement-mediated cell death through interaction with components of complement system at the plasma membrane [Esfahanian et al., 2023].

1.10.3 Mortalin in cancers

The overexpression of Mortalin is linked with malignancies (**Fig. 1.4**), impacting the survival rates of cancer patients [Elwakeel, 2022]. In addition, upregulated expression of Mortalin promotes cell proliferation, angiogenesis, and metastasis while downregulating apoptotic signaling across various cancers, through the modulation of different pathways and molecules [Yun et al., 2017]. The overexpression of Mortalin in various cancers influence different cancer hallmarks such as survival, proliferation, epithelial-to-mesenchymal transition (EMT), angiogenesis, invasion, migration, and drug resistance, thereby affecting patient's survival [Yun et al., 2017; Putri et al.,

2019]. It has been established that Mortalin form a complex with tumor suppressor p53, a feature unique to cancer cells, affecting the normal activity of tumor suppressor p53 [Lu et al., 2011]. In hepatocellular carcinoma, expression level of Mortalin was observed to be crucial in carcinogenesis and hence the inactivation of Mortalin by shRNA in hepatocellular carcinoma (HCC) cells (with mutant p53), was found to trigger mutant p53-mediated apoptosis in these cells, suggesting Mortalin-p53 interaction as one of the plausible strategies for its management [Lu et al., 2011]. In addition, elevated Mortalin expression in colon and breast cancers has been linked to unfavorable prognosis and compromised survival among patients. Through the mechanistic studies, it was found that the elevated level of Mortalin increased cell proliferation and induced EMT as well as metastasis in cancer cells, via Wnt/β-catenin cascade [Xu et al., 2020; Zhang et al., 2021]. Additionally, Mortalin was found to induce cancer cell stemness and chemoresistance by overexpressing stemness markers such as octamer-binding transcription factor 4 (Oct-4), ATP-binding cassette super-family G member 2 (ABCG2), prominin-1 or CD133, aldehyde dehydrogenase (ALDH1), cluster of differentiation 9 (CD9), multidrug resistance protein 1 (MRP1) and connexin [Yun et al., 2017]. In line with this, the regulation of cancer cell stemness by Mortalin in breast cancer was known to be associated with the induction of Wnt/GSK3β/β-catenin cascade [Wei et al., 2021]. Extensively, other studies have also reported the associated poor prognosis and poor survival due to overexpressed Mortalin in cancer patients affected with brain tumor, cholangiocarcinoma, gastric, hepatocellular, lung, pancreatic and thyroid cancers [Ando et al., 2014; Starenki et al., 2015; Cui et al., 2017; Sun et al., 2017; Kang et al., 2018; Cheng et al., 2019; Starenki et al., 2019]. Thus, Mortalin functions as a prognostic biomarker in different cancers, as discussed, and this suggests the potential of Mortalin as a therapeutic target for cancer management. However, the involvement of Mortalin has not been evaluated in OC. Therefore, this study aims to investigate Mortalin as a therapeutic target for better management of OC.

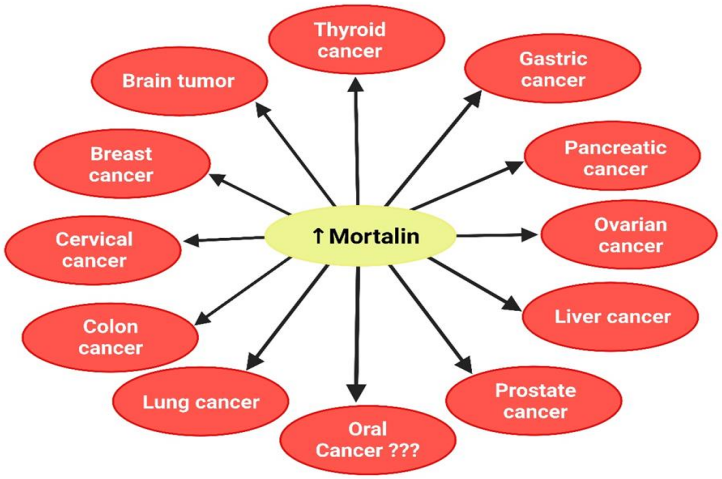

Fig. 1.4. Role of Mortalin in cancer (Image credit: BioRender.com)

1.11 Withaferin A (WiA)

WiA is one of the members of withanolides, mostly present in abundance in *Withania somnifera* (Dunal), also referred to as Ashwagandha [Sultana et al., 2021].

Withanolides constitute a group of steroid-derived lactones, among which WiA is the mostly investigated agent for its cytotoxicity against different neoplasms, such as glioma, leukemia, and carcinomas affecting the breast, cervix, and ovary, etc. [Wijeratne et al., 2018]. Recognized for its anti-cancer property in 1967, it was one of the first withanolides being investigated for its cytotoxicity against cancer, isolated from the leaves of Ashwagandha [Lee and Choi, 2016].

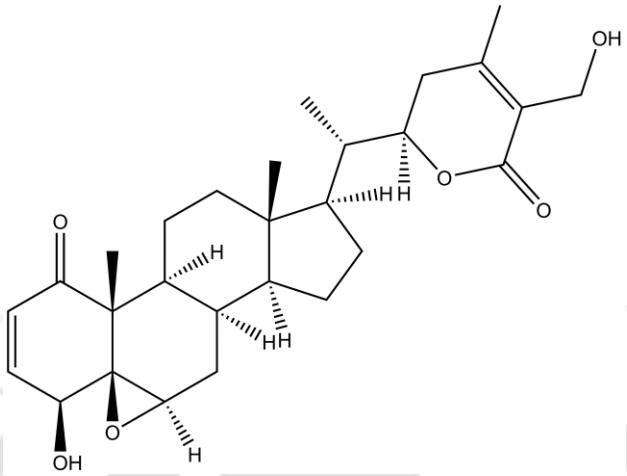

Fig. 1.5. Structure of Withaferin A (WiA) (Image credit: ChemDraw)

1.11.1 Structure and chemistry of WiA

WiA ($C_{28}H_{38}O_6$) (**Fig. 1.4**) is a withanolide compound characterized by hydroxy substitutions at the 4th and 27th positions (specifically the 4β,5β,6β,22R stereoisomer) of its 5,6:22,26-diepoxyergosta-2,24-diene-1,26-dione structure [PubChem CID 265237; https://pubchem.ncbi.nlm.nih.gov/compound/Withaferin-A]. The molecular weight of this compound is 470.6 g/mol, consisting of 28-carbon ergostane backbone and a δ-lactone, comprising of two hydrogen-donating moieties and six hydrogen-accepting groups, making it chemically reactive [Sudeep et al., 2020; Sultana et al., 2021]. It has three reactivity sites: the unsaturated ring A at C2-3 containing a ketone, the epoxide moiety at C5 in B ring, and the unsaturated lactone structure, which serve as targets for nucleophiles such as cysteine sulfhydryl groups of proteins [Sultana et al., 2021].

1.11.2 WiA against cancers

WiA has been explored for its therapeutic potential across diverse cancers. For instance, WiA has been known for its ability to attenuate cell proliferation, invasion, angiogenesis, migration and metastasis, while triggering cell cycle blockade and apoptosis across various neoplasms including lung, gastric, breast, liver, and glioblastoma [Kyakulaga et al., 2018; Kim et al., 2017; Thaiparambil et al., 2011; Wang et al., 2015; Tang et al., 2020]. In addition, the treatment of A549 and H1299 lung carcinoma cells with WiA led to cell cytotoxicity and suppression of cell adhesion, invasion and migration. Additionally, WiA attenuated TGFβ1 and TNF-α-mediated EMT by decreasing the phosphorylation and nuclear transport of mothers against decapentaplegic homolog (Smad)2/3 and nuclear factor kappa B (NF-κB), while also mechanistically elevating E-cadherin and attenuating N-cadherin, fibronectin, vimentin, claudin-1, and Snail expression in lung cancer cells [Kyakulaga

et al., 2018]. Moreover, in gastric cancer, WiA treatment was demonstrated to initiate cell cycle arrest at G2/M phase and inhibit the proliferation and metastatic ability of AGS cells by upregulating the levels of cleaved poly (ADP-ribose) polymerase (PARP) and cleaved caspases (-3, -7, and -9), while downregulating the expression of B-cell lymphoma-2 (Bcl-2) and cyclin B1 [Kim et al., 2017]. Further, WiA treatment inhibited Akt-induced cell proliferation and EMT, while inducing apoptosis in HCT-116 transfectant cells. These effects were associated with the inhibition of the Akt/mTOR/NF-κB signaling axis, decreased expression of β-catenin, Snail, vimentin, and Slug, along with an increase in apoptotic markers such as cleaved caspase-3, cleaved PARP, and Bcl-2-associated protein x (Bax) [Suman et al., 2016]. In the same study, the administration of WiA to pCMV/HCT-116 and Akt/HCT-116 colorectal xenografts led to the inhibition of microvessel formation and tumor growth, with no toxicity side effects to the animal models [Suman et al., 2016]. Besides, WiA was found to bind with mortalin exhibiting affinity of −9.8 Kcal/mol, establishing six hydrogen bonds with the four amino acid residues on Mortalin [Vaishnavi et al., 2012]. These binding ability of WiA with Mortalin leads to impairment of Mortalin-p53 complex triggering cell growth arrest and apoptosis through the suppression of Bcl-2, Bcl-xL, procaspase 3 and pro PARP and induction of Bax and cleaved PARP-1 [Huang et al., 2015; Behl et al., 2020]. Similarly, combination of WiA and CAPE, abrogated Mortalin-p53 interaction and lead to the induction of apoptosis signaling and its associated molecules [Sari et al., 2020]. Thus, suggesting the therapeutic potential of WiA against cancer and its associated hallmarks.

1.12 Importance of the study

OC represents predominantly observed malignancy in males and is ranked fifth among females in India. It is of significant concern as in 2020, more than 1.3 lac incidences and mortality of more than 50 thousand were reported in India (**Fig 1.6**). The survival rates for OC patients are very low, primarily attributable to late stage diagnosis due to negligence of early signs and symptoms. Various molecular alterations have been identified as key contributors to OC development. Challenges associated with conventional therapies, including chemoresistance, tumor recurrence, and adverse side effects on various healthy tissues and organs, contribute to suboptimal management of this disease. Previous literature studies have highlighted the overexpression of Mortalin in diverse cancers and its impact on survival outcomes of patients. Moreover, its upregulated expression induces highly aggressive and proliferative tumors. Unraveling the expression and role of Mortalin could provide insights into its involvement, elucidating the pathways and molecules regulated by this protein in oral tumor development and progression.

Notably, previous studies have established the anti-cancer efficacy of WiA in various malignancies and has shown to modulate different hallmarks of cancer via regulating diverse signaling pathways and proteins. Moreover, recent studies have demonstrated the efficient binding of WiA with Mortalin, and suppressing the oncogenic hallmarks. Thus, exploring the role of Mortalin in OC could offer novel insights for its prognosis, and the potential inhibition of Mortalin by WiA emerges as a promising strategy for the management of OC.

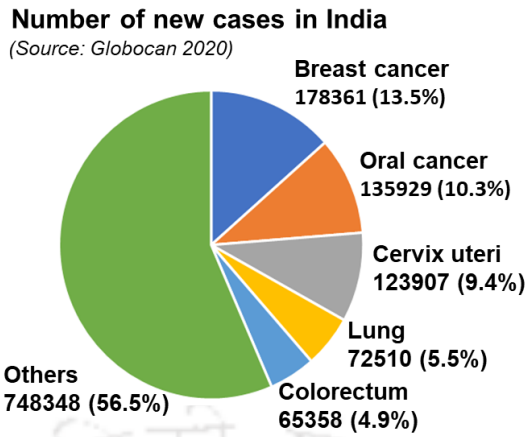

Fig. 1.6 OC statistic in India in 2020

1.14 Objectives

Based on the review of literatures, the given objectives were framed:

- To determine the expression of Mortalin (HSPA9) in different OC cell lines and OC tissues
- To determine the role of Mortalin in regulating different hallmarks of OC
- To determine the effect of WiA on Mortalin expression and on different hallmarks of OC.

Chapter 2

Expression Analyses of Mortalin in OC Cells and Tissues

2.1 Introduction

In the preceding chapter, we have reviewed the multifaceted role of Mortalin across various cancer types, elucidating how its overexpression contributes to the regulation of carcinogenic processes [Yun et al., 2017; Liu et al., 2019]. Through its intricate modulation of various pathways and molecular mechanisms, Mortalin has been implicated in influencing key cancer events, including survival, proliferation, and metastasis, suggesting its significant involvement in carcinogenesis [Yoon et al., 2022; Esfahanian et al., 2023]. Although the research has well documented its association with several cancers like glioblastoma, ovarian cancer, hepatocellular carcinoma, etc., its specific role in OC remains largely unexplored.

Therefore, in the current study we aim to analyze the expression of Mortalin in OC cell lines and tissues compared to their normal counterparts through a combination of computational analysis and in vitro methodologies. To achieve our objectives, we initially assessed the expression of Mortalin in normal and tumor tissues from head and neck cancer patient's data sourced from The Cancer Genome Atlas (TCGA). In addition, we conducted expression profiling of Mortalin through single-cell analysis and subsequently performed correlation analyses to elucidate the association of Mortalin with other genes implicated in OC. These in silico investigations were followed by in vitro expression analyses using commercially obtained tissue microarray (TMA) slide containing OC patient samples, and from the tissue samples collected from North-East Cancer Hospital and Research Institute (NECHRI), Jorabat, Assam, India. Furthermore, employing both computational and experimental methodologies, we examined Mortalin expression across various subcategories of OC patients, including different grades, stages, tumor size, etc.

2.2 Materials and Methods

In this study, we conducted both in silico and in vitro analyses to examine the expression of Mortalin in tumor and normal samples, spanning different categories of OC patients. Further, we investigated the correlation between Mortalin and other genes associated with various hallmarks of OC. The methodologies employed are outlined as follows:

2.2.1 In silico methodologies

The principal objective of our study was to elucidate the expression of Mortalin in HNSCC. To achieve this, we conducted transcriptomic analysis using TCGA database. The analyses were implemented using R programming software. Additionally, we examined the OS rate disparities between patients exhibiting varied expression levels of Mortalin (i.e. high vs low). For this, we investigated the potential implications of Mortalin high vs low expression levels on the OS probabilities of HNSCC patients. This investigation involved the utilization of the KM plotter tool (http://kmplot.com). Moreover, we assessed the datasets sourced

from TCGA for Mortalin's expression across various categories, including stages, grades, tumor sizes, and nodal stages within the HNSCC patient population. Subsequently, the head and neck cancer dataset were retrieved from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) using single-cell RNA-Seq analysis. This dataset includes the expression patterns of diverse single cells obtained from multiple patients afflicted with HNSCC. In addition, the data obtained from TCGA were subjected to correlation studies. The assessment of correlation between Mortalin expression and the gene and protein expression profiles associated with OC hallmarks was conducted using the Corrplot package within the R programming. The bar graphs were plotted using GraphPad Prism version 9.2.1.

2.2.2 In vitro approach

Different in vitro assays were performed for the expression analysis of Mortalin. The techniques are briefed below:

2.2.2.1 Cell culture and chemicals

The OC cell line, SAS, was sourced from the Rajiv Gandhi Centre for Biotechnology (RGCB), Trivandrum, while the normal cell line, HaCaT, was acquired from the National Centre for Cell Science (NCCS), Pune. In addition, the HSC3 OC cell line and anti-Mortalin antibody was generously provided by Dr. Renu Wadhwa from the National Institute of Advanced Industrial Science and Technology (AIST), Japan. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (12100-046, Invitrogen, USA) complemented with 10% fetal bovine serum (FBS) (10270-106, Gibco, USA) and Pen-Strep-1X (15140122, Invitrogen, USA). The cultures grown in 5% CO_2-regulated incubator at 37 °C and maintained humidity 95%.

2.2.2.2 Tissue Microarray

The expression of Mortalin was analyzed in normal and cancerous oral tissues through immunohistochemical analysis. In this assay, we used a TMA slide comprising paraffin-embedded tissues, including normal, malignant, and metastatic oral cavity tissues (OR2081, US Biomax, Inc., USA). This slide comprises a total of 208 tissues from different individuals. The slide consisted of tissues with 35 cases of SCC, 2 basal cell carcinomas, 4 adenocarcinomas, 4 metastatic carcinomas, 10 MEC, 10 adamantinomas, 1 ulcer, 2 cysts, 5 hyperplasia cases, and 11 inflammation cases. It also contained 8 samples of adjacent normal salivary gland tissue, 4 salivary gland tissues, 2 adjacent normal tongue tissues, and 6 tongue tissues. The samples are in duplicate or quadruple cores per case and the details of the TMA slide containing details of tissue samples obtained from OC patients are given in supplementary table 2.1.

2.2.2.3 Immunohischemistry (IHC)

The expression of Mortalin was analyzed in OC tissues compared to normal oral epithelial tissues through IHC. Pierce™ peroxidase IHC detection kit (Cat. No.

36000), Invitrogen, and biotinylated, cross-adsorbed goat anti-mouse IgG (H+L) secondary antibody (Cat. No. # 62-6540), were employed for immunostaining of the TMA. IHC analysis was performed following manufacturer's guidelines, which involves deparaffinization, rehydration, suppressing peroxidase activity, blocking, incubation with primary and secondary antibody, treatment with enzyme conjugate solution, followed by the addition of 3,3'-diaminobenzidine (DAB), then finally counterstained using hematoxylin. Subsequently, the slide was subjected to dehydration and mounted using coverslip and dibutylphthalate polystyrene xylene (DPX) mountant. Next, the slide was examined using the BX43 light microscope from Olympus, Japan. Tissues that were stained brown were considered as positive for Mortalin expression. Scoring was done on the basis of positive staining percentage and its intensity. The scoring details are given in the supplementary table 2.2.

2.2.2.4 Real time PCR

The mRNA levels of Mortalin was determined in OC patient samples collected from NECHRI. The total RNA was extracted from tissue samples employing TRI reagent and cDNA was prepared using reverse transcription kit (high-capacity cDNA kit, Life Technologies). RT-qPCR was performed to analyze the levels of Mortalin mRNA in OC tissue samples compared to normal tissue samples. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was utilized as a control gene to normalize the target gene expression levels. The primers for Mortalin were obtained from Integrated Life Technologies. The details of the tissue obtained from OC patients are listed in supplementary table 2.3.

2.2.2.5 Western blot analysis

The expression of mortalin in OC cell lines (SAS and HSC3) compared to normal cell line, HaCaT, was evaluated using immunoblotting/western blot analysis. For this purpose, the whole-cell lysates were collected using cell lysis buffer (250 mM NaCl, 20 mM HEPES, 2 mM EDTA, 0.1% (v/v) Triton-X100) that contains protease inhibitors (Leupeptin hemisulfate (2 μg/ml), Aprotinin (2 μg/ml), 1 mM DTT, and 1 mM PMSF). Supernatants were collected after centrifugation at 13000 rpm, 4 °C for 15 minutes and quantified using Bradford's reagent (Cat. No. 500-0205, Bio-rad, USA). The protein (25 μg) was combined with 5× laemmli buffer (containing 250 mM TrisHCl, SDS (10%), glycerol (30%), β-mercaptoethanol (5%), and bromophenol blue (0.02%)), subjected to electrophoresis in an SDS-polyacrylamide gel, followed by protein transfer onto nitrocellulose membrane (Bio-Rad, California, USA). Next the membrane was treated with anti-Mortalin primary antibody for overnight, and washed with 1xTBST (contains Tris base (0.2 M), NaCl (1.5 M), and 1% Tween-20), followed secondary antibody (horseradish peroxidase (HRP)-conjugated) incubation. The blots were visualized with Bio-Rad, ChemiDoc™ XRS System (California, USA) using a clarity western ECL substrate solution from Bio-Rad (Cat. No. 1705061; California, USA). GAPDH was used as loading control.

2.3 Statistical analysis

Statistical significance was assessed through Student's t-test. Results were represented in p-values less than 0.001 as '***', 0.01 as '**' and 0.05 represented as '*'. GraphPad prism software was used to prepare the graphs.

2.4 Results

This study basically investigated the expression of Mortalin in OC cell lines and tissue samples. In addition, the expression of Mortalin was studied across tissue samples representing diverse grades and stages of OC. Interestingly, this study showed overexpression of Mortalin in OC cell lines and tissue samples in comparison to their normal counterparts. The results obtained through computational and in vitro analyses are discussed below:

2.4.1 Mortalin was found to be overexpressed in patients with head and neck cancer and is associated with a lower survival rate: An in silico study

The mRNA expression data in the TCGA database for tumor (n=520) and normal (n=44) samples from head and neck tumor patients was downloaded using R programming. The mortalin expression in transcript per million was plotted (**Fig. 2.1A**) for normal and tumor samples. Mortalin was found to be significantly upregulated in tumor samples compared to normal. Additionally, we stratified the samples based on various stages, nodal metastasis conditions, grades, and location of the tumor. We found that Mortalin was significantly overexpressed in stage IV disease (**Fig. 2.1B**) and TNM staging showed that mortalin is consistently overexpressed in all the stages (T1, T2, T3, T4 and Tx) compared to normal (**Fig. 2.1C**). In addition, Mortalin was significantly overexpressed in nodal metastasis positive samples (N2 and N3) compared to non-metastatic samples (**Fig 2.1D**). Moreover, grade-wise stratification of these samples showed that mRNA levels of Mortalin are highly enriched as grade progresses (Grade 2, 3 and 4) compared to normal samples (**Fig 2.1E**). Further, the Mortalin expression in different sites of tumor origin including the anterior floor of mouth (AFM), the base of tongue (BT), cheek mucosa (CM), the floor of mouth (FM), mouth (MU), gum, hard palate (HP), overlapping lesions of lip, oral cavity, pharynx (OP), lip, lower gum (LR), tongue (TG), tonsil (TN) showed increased levels of mRNA expression compared to normal samples (**Fig. 2.1F**). Single cell analysis was also performed for Mortalin expression in various cell types present in tumor microenvironment (TME), however, no significant changes in protein expression was noted among these cells (**Fig.2.1G**).

Survival analysis was conducted to understand the relevance of Mortalin overexpression in head and neck patients. OS analysis among the patients expressing low and high levels of Mortalin mRNA showed that patients who express high levels of Mortalin were found to have a shortened survival period with hazard ratio (HR) of 1.28 (0.96-1.72) and p-value of 0.09. Although it was statistically insignificant, the trend in low survival probability with high expression of Mortalin was observed (**Fig. 2.2A**).

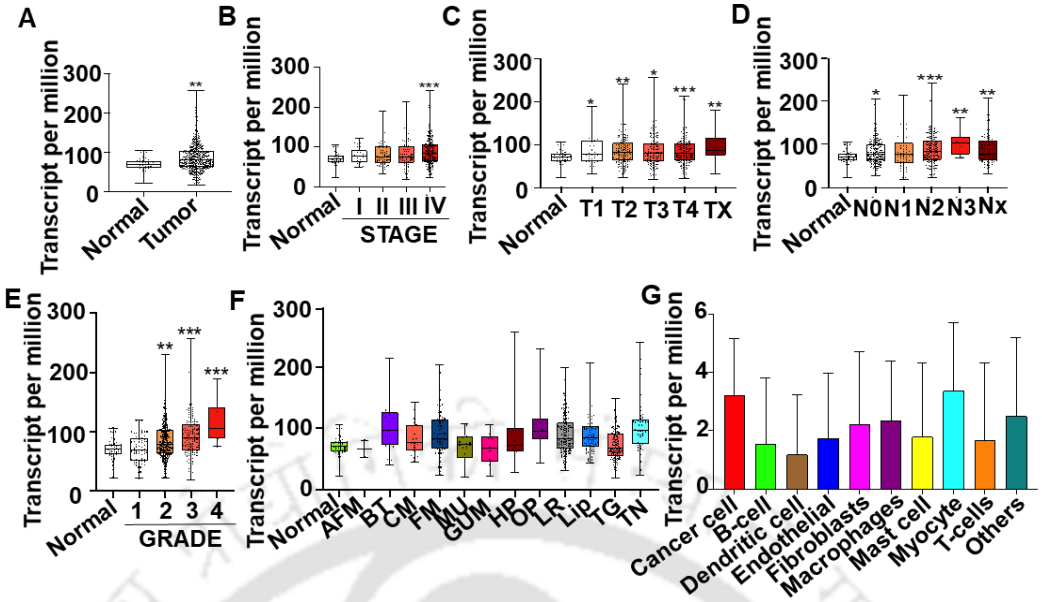

Fig. 2.1 Comprehensive analysis of Mortalin expression in OC, utilizing in silico methodologies. A-F: In silico analysis of Mortalin expression in OC tissue samples obtained from TCGA database. A. Expression of Mortalin in normal vs tumor samples; B, C, D & E represents expression of Mortalin in different categories of OC such as stage, tumor size, nodal stage, and grade, respectively; F. Expression of Mortalin in OC tissue samples obtained from different parts of the oral cavity. G. Mortalin expression through single-cell analysis in different cell types within TME.

On the other hand, the OS probability among the patients who expressed higher levels of mortalin with tissue mesenchymal stem cells (MSC) enriched was shown to have a remarkably lower survival period compared to those who expressed low levels of Mortalin (p-value = 0.031) (**Fig. 2.2B**) whereas this trend was not observed in MSC decreased tissue samples. A similar trend of low survival was observed in patients who expressed higher levels of Mortalin in grade 1 (p-value=0.017) (**Fig. 2.2C**), grade 2 (p-value=0.088) (**Fig. 2.2D**), grade 3 p-value=0.11) (**Fig. 2.2E**), stage I (p-value=0.12) (**Fig. 2.2F**), stage III (p-value=0.042) (**Fig. 2.2G**) and stage IV (p-value=0.065) (**Fig. 2.2H**) diseases.

Further, a significant correlation of mortalin with the expression of mRNA levels of other genes which are involved in the regulation various OC hallmarks have been observed (**Fig. 2.3**)- HSPA9 (Mortalin) vs TP53: R=0.33, p=2.1x10-15; HSPA9 vs PI3KCA: R=0.35, p<2.2x10-16; HSPA9 vs PI3KCB: R=0.38, p<2.2x10-16; HSPA9 vs PTEN: R=0.26, p=8.2x10-10; HSPA9 vs PTGS2: R=0.098, p=0.021; HSPA9 vs RB1: r=0.39, p<2.2x10-16; HSPA9 vs RPS6: R=0.12, p=0.0066; HSPA9 vs SNAI1: R=0.12, p=0.0038; HSPA9 vs SQSTM1: R=0.45, p<2.2x10-16; HSPA9 vs TWIST1: R=-0.012, p=0.78; HSPA9 vs VEGFA: R=0.23, p=3.7x10-8; HSPA9 vs WEE1: R=0.35, p<2.2x10-16; HSPA9 vs XIAP: R=0.37, p<2.2x10-16; HSPA9 vs AKT1: R=0.33, p=8.1x10-16;

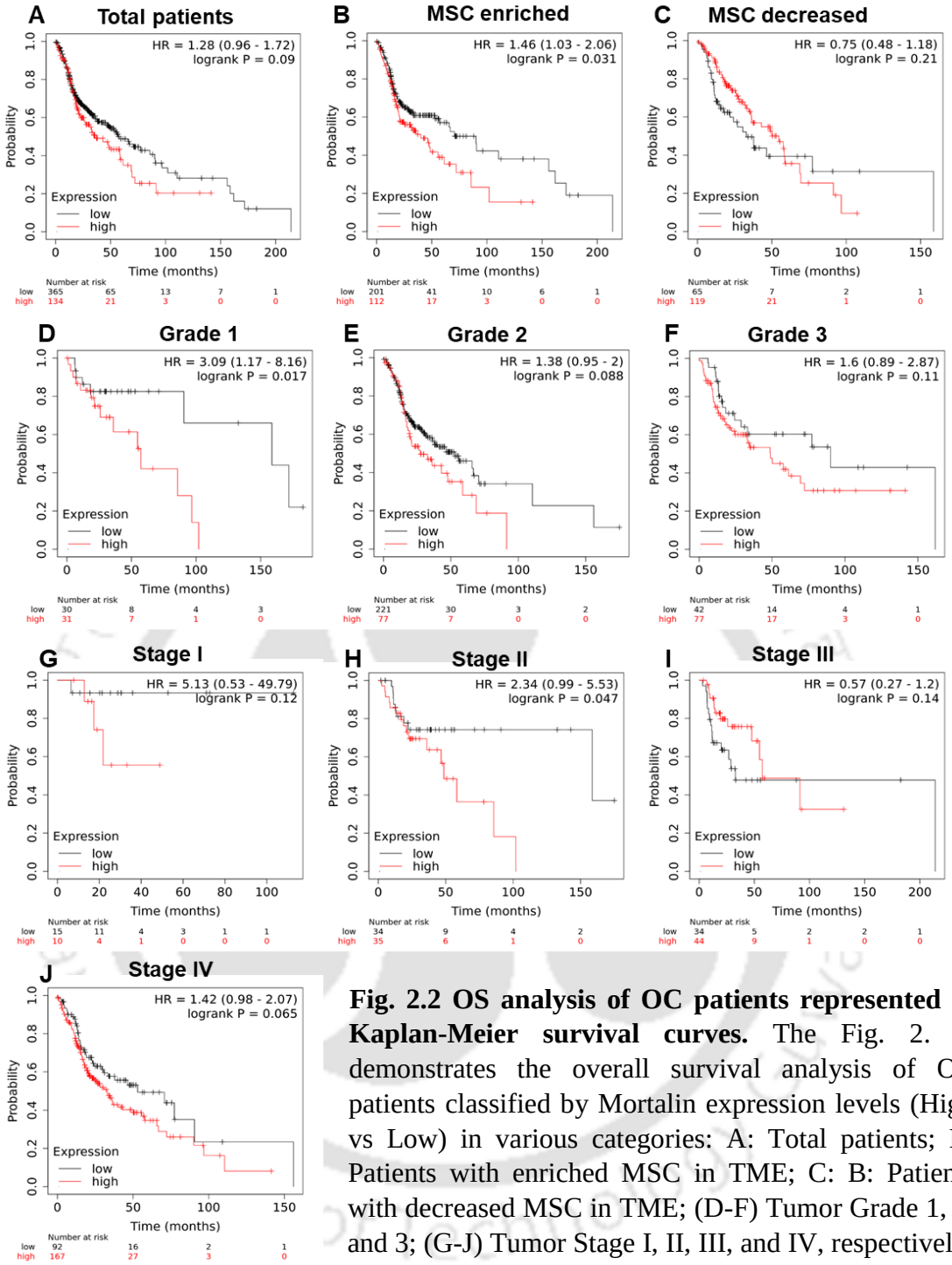

Fig. 2.2 OS analysis of OC patients represented in Kaplan-Meier survival curves. The Fig. 2. 2. demonstrates the overall survival analysis of OC patients classified by Mortalin expression levels (High vs Low) in various categories: A: Total patients; B: Patients with enriched MSC in TME; C: B: Patients with decreased MSC in TME; (D-F) Tumor Grade 1, 2, and 3; (G-J) Tumor Stage I, II, III, and IV, respectively.

HSPA9 vs BCL2: R=0.28, p=8.5x10-12; HSPA9 vs CCNA2: R=0.45, p<2.2x10-16; HSPA9 vs CASP3: R=00.36, p<2.2x10-16; HSPA9 vs CASP9: R=0.31, p=8.9x10-14; HSPA9 vs CCNB1: R=0.49, p<2.2x10-16; HSPA9 vs CCND1: R=0.15, p=0.00046; HSPA9 vs CCNE2: R=0.38, p<2.2x10-16; HSPA9 vs CDH1: R=0.14, p=0.00087; HSPA9 vs CDH2: R=-0.0099, p=0.82; HSPA9 vs CDKN1A: R=-0.11, p=0.01; HSPA9 vs MAP1LC3B: R=0.11, p=0.00074; HSPA9 vs MCL1: R=0.35, p<2.2x10-16; HSPA9 vs MMP-2: R=-0.07, p=0.1; HSPA9 vs MMP-9: R=0.014, p=0.74; HSPA9 vs MTOR: R=0.4, p<2.2x10-16; HSPA9 vs CDKN1B: R=0.36, p<2.2x10-16; HSPA9 vs CXCR4: R=0.19, p=6.2x10-6.

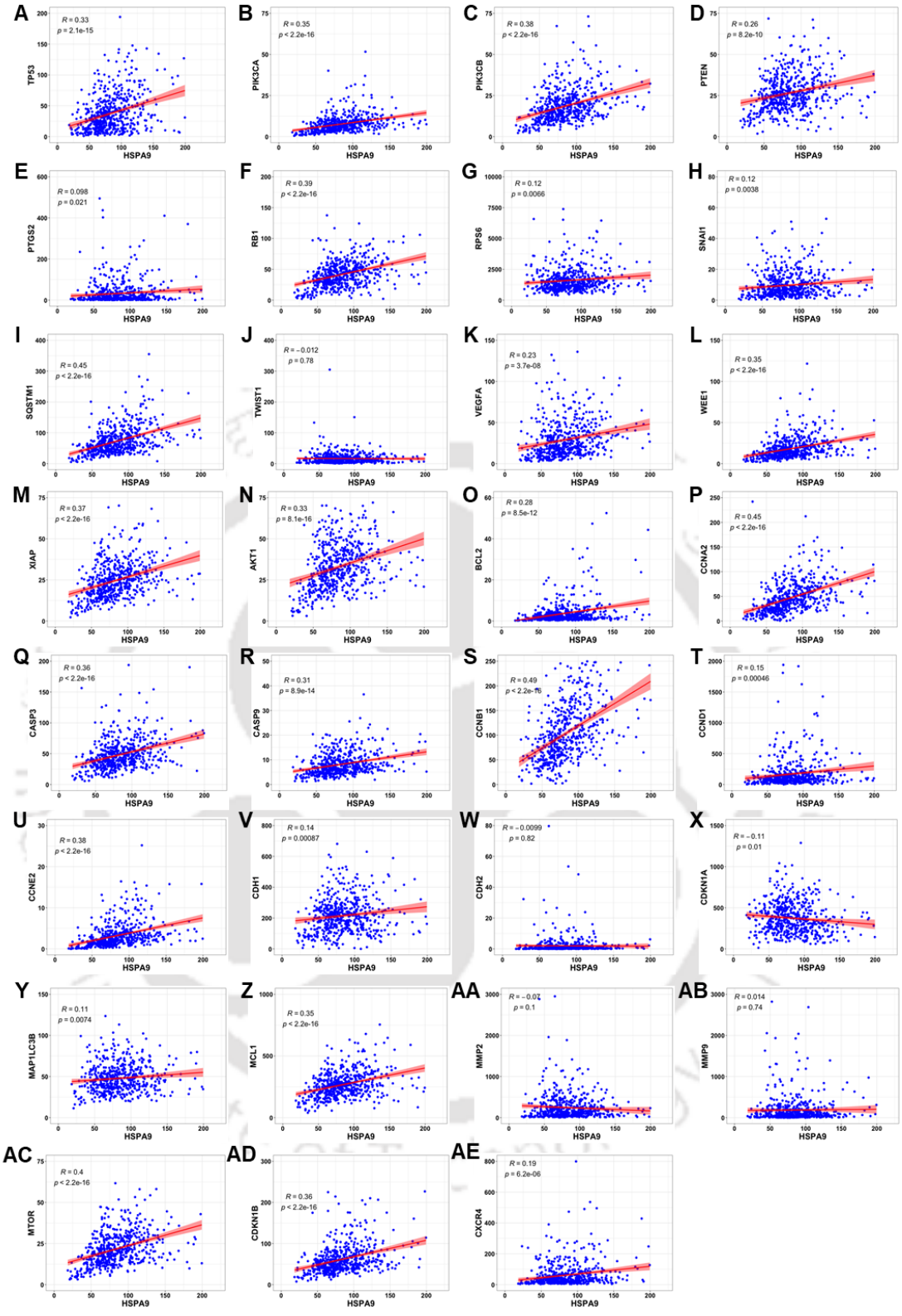

Fig. 2.3 Correlation analysis to determine the association of Mortalin and other genes involved in OC. The overexpression of Mortalin was found to be correlated with different genes pivotal in oral carcinogenesis.

2.4.2 Mortalin was found to be overexpressed in OC cell lines and patients' samples

The IHC analysis on TMA slide was conducted to validate the expression of Mortalin in OC. This experiment showed that Mortalin expression was elevated in OC tissues samples as compared to normal oral tissues. This upregulated expression of Mortalin in normal vs tumor were represented in figure 2.4A and 2.4B, where 2.4A depicts the representative image for normal vs oral tumor tissues and 2.4B represents the expression score of Mortalin in normal vs tumor samples. In addition, the upregulated expression of Mortalin was confirmed in SCC and metastatic SCC tissues with respect to its expression in normal tissues (**Fig. 2.4D**). As OC is a complex disease, understanding its aggressiveness and progression is essential, which could be confirmed through its different grades and stages. Therefore, to understand the involvement of Mortalin in OC severity and progression, we examined the expression Mortalin in the OC tissue samples that represents the patients with different grades and stages. Interestingly, this study showed that expression of Mortalin was elevated in various grades (**Fig. 2.4E**) and stages (**Fig. 2.4F**) of OC patients. This upregulated expression confirms the association of Mortalin in the progression of OC.

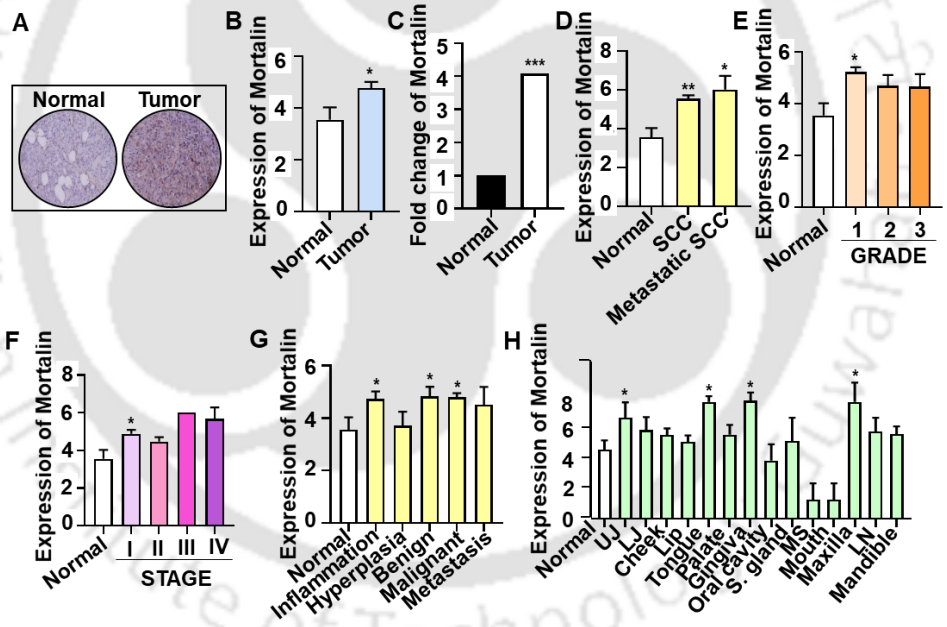

Fig. 2.4. In vitro expression analyses of Mortalin in tissue samples. A, B, D–H: IHC analysis revealing Mortalin expression in OC tissue samples. A and B illustrate representative images and statistical averages, respectively, comparing Mortalin expression in normal vs tumor samples. C: mRNA fold change in Mortalin expression from in OC patient samples obtained from NECHRI; D: Expression of Mortalin in SCC and metastatic SCC OC samples; D & F: Expression of Mortalin in different grades and stage of OC, respectively; G: Mortalin expression in OC samples categorized by pathological stages of oral carcinogenesis; H: Mortalin expression in OC patients across different tissue of origin.

Additionally, the expression of Mortalin was evaluated in different pathological conditions of oral carcinogenesis ranging from inflammation, hyperplasia, benign, malignant and metastatic stages, as compared to control tumor samples (**2.4G**). The expression of Mortalin was found to be upregulated in all these categories except hyperplasic samples, compared to normal tissue samples. Moreover, the overexpression of Mortalin was also determined in different OC tissue samples representing different anatomic sites (**Fig. 2.4H**). It was found that the anatomic sites like upper jaw (UJ), tongue, gingiva and maxilla had higher expression of Mortalin as compared to other sites as well as expression in normal tissue samples.

Next, after confirming the protein expression of Mortalin in OC tissues, we examined the mRNA levels of Mortalin in OC patient samples obtained from NECHRI, through RT-PCR analysis. Correlating to our studies with computational and IHC analyses of the tissue samples, this study showed upregulated Mortalin mRNA expression compared to non-neoplastic tissue samples (**Fig. 2.4C**). This result correlates to the expression of Mortalin found in normal vs tumor samples as confirmed through TCGA and IHC analyses. Hence, this adds up to our previous data, confirming the involvement of Mortalin in OC.

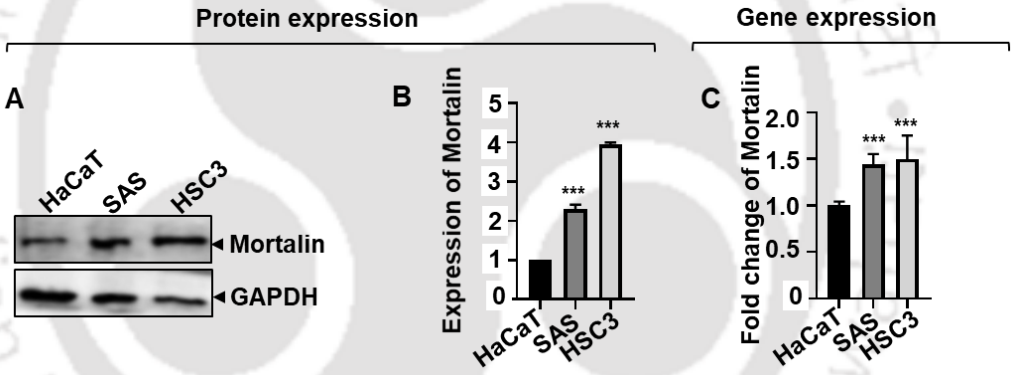

Fig. 2.5 Expression of Mortalin in OC cell lines. A: Immunoblotting images in normal vs OC cell lines determining the expression of Mortalin; B. Quantitative representative of 2.5A; C: mRNA fold change of Mortalin expression in normal vs OC cell lines. HaCaT cell line was used as normal against SAS and HSC3 OC cells.

Further, we evaluated the expression of Mortalin in SAS and HSC3 OC cell lines, compared to normal keratinocyte cell line, HaCaT, through Western blotting and RT-PCR analyses (**Fig. 2.5**). In line with the previous results, the expression of Mortalin was overexpressed in OC cell lines as compared to normal cells (**Fig. 2.5A & Fig. 2.5B**). In addition, the high fold of Mortalin mRNA was confirmed in OC cells compared to HaCaT cells (**Fig. 2.5C**). These observations validate the potential association of Mortalin with OC.

2.5 Conclusion

The studies in the current chapter represents a significant advancement in understanding the role of Mortalin in OC pathogenesis. Through comprehensive in silico analysis, we have observed a consistent pattern of Mortalin overexpression in OC tissue samples when compared to normal tissues. Notably, this upregulation spans across various stages, grades, tumor sizes, and nodal stages, suggesting a pivotal role for Mortalin across in the event of OC progression. The increased expression Mortalin in different grades and nodal stages of OC highly demonstrate the potential involvement of Mortalin in fostering aggressiveness and metastatic features in OC progression. In addition, the overexpression across these sub-categories implies the progressive dysregulation of Mortalin during OC progression and might suggests its involvement in the intricate molecular regulation of signaling networks linked with tumor growth, invasion and metastasis.

Importantly, our findings demonstrate the potential clinical relevance of Mortalin expression levels in OC patients. Through Kaplan-Meier plot, we observed that the oral patients expressing high level of Mortalin expression correlates with diminished overall survival, compared to the group of patients expressing low levels of Mortalin. This decreasing trend of median in OS was consistent across grades and stages of OC in patients with high levels of Mortalin. Hence, suggesting the potential of Mortalin as prognostic marker in OC. These findings also align with earlier observations in colorectal cancer and non-small cell lung carcinoma, where Mortalin overexpression was associated with adverse clinical outcomes, particularly in early-stage disease. In these studies, the overexpression of Mortalin was found to contribute to decreased disease-free survival and overall survival in lung and colorectal patients, compared to the patients having lower expression of Mortalin, suggesting a potential prognostic value of Mortalin in these cancers [Sun et al., 2017]. Thus, Mortalin emerges as a promising candidate for the assessment of its prognostic value in OC patients.

Correspondingly, IHC analysis further validates our in-silico findings, demonstrating overexpression of Mortalin in OC tissues as compared to their normal counterparts. This study has robustly shown the upregulation of Mortalin expression in various OC subtypes, including SCC and metastatic SCC tissues, further emphasizing its association with the aggressiveness of OC. This study correlates with the earlier observations on the role of Mortalin, where its overexpression in breast and lung tumor tissues influence the progression of cancer and was associated with high histological grades, TNM staging, and metastasis [Shin et al., 2003; Sun et al., 2017]. Similar to our in-silico findings, upregulated expression of Mortalin was observed in different sub categories of OC patients such as grade and stage. This upregulation of Mortalin across the stages and grades of OC, might provide the evidence of its essential role in different pathological stages of the disease progression. Hence, it suggests the involvement of Mortalin in OC development and progression.

Moreover, our study with the clinical patient samples obtained from OC patients from NECHRI also confirms the upregulated fold change in Mortalin mRNA

expression. In continuation, the evaluation of Mortalin expression in OC cell lines also revealed its upregulation at both protein and mRNA level in SAS and HSC3 OC cell lines, compared to normal cells, HaCaT. Thus, these studies substantiate the association of Mortalin in oral tumor pathogenesis and progression. Hence, the exploration on the mechanistic insights could potentially reveal the significance of Mortalin in oral carcinogenesis and its potential as a therapeutic target.

Chapter 3

Mechanism of Mortalin in OC

3.1 Introduction

In our preceding chapter, we performed a comprehensive evaluation of Mortalin expression in both OC cell lines and OC tissue samples, demonstrating a consistent upregulation of this protein compared to their respective normal counterparts. This observed upregulation persisted across various clinical parameters including different disease grades, stages, tumor size, and nodal stages of OC, suggesting a significant involvement of Mortalin in the pathogenesis of this malignancy. Notably, this elevated expression of Mortalin was found to be associated with poor OS outcomes among OC patients, delineating its potential as a prognostic indicator and suggesting its putative mechanistic role in driving OC progression. Therefore, in the present chapter, we elucidated the mechanistic role of Mortalin in OC by employing small interfering RNAs (siRNAs)-mediated knockdown to selectively suppress its expression in OC cells, followed by various molecular assays exploring its involvement in different hallmark processes of OC, including cell survival, proliferation, EMT, angiogenesis, migration, and invasion.

3.2 Materials methods

The objective of this current study, was to elucidate the role of Mortalin on various OC hallmarks, including survival, proliferation, EMT, invasion, angiogenesis, and migration. To comprehend this regulatory function, Mortalin was inactivated through the Mortalin-specific siRNA. The confirmation of Mortalin knockdown was achieved through Western blot experiment. Thereafter, other assays were performed to elucidate the mechanistic involvement of Mortalin in OC. The basic materials and methods of the experiments involved are as follows:

3.2.1 Cell culture

The details regarding cell lines and its culture conditions are highlighted in Chapter 2, Section 2.2.1. The maintenance of the cultures was as per the details described in Chapter 2.

3.2.2 siRNA mediated gene inactivation

Specific siRNAs targeting the Mortalin (HSPA9) gene were utilized for gene inactivation, procured from Sigma Merck, USA. OC cells were seeded at $2x10^5$ cells/well concentration in six-well plates, and upon reaching 70% confluency, they were treated with lipid-RNA complexes prepared in incomplete Opti-MEM media. The complexes consisted of either 10 nM siRNA specific to HSPA9 or 10 nM scrambled siRNA, along with the transfection reagent lipofectamine RNAiMAX reagent (Cat No. 13778150, Invitrogen, USA), prepared as per the manufacturer's protocols. After 48 hours of incubation, the cells were collected for further studies. Cells transfected with Mortalin-specific siRNA served as the knockdown group (labelled as siRNA), while those transfected with scrambled siRNA served as the control group (labelled as Control), and Mortalin knockdown expression confirmed in both the groups by Western blot analysis.

3.2.3 Western blot analysis

The experimental procedure described in Chapter 2, Section 2.2.7 were followed to perform this assay using siRNA and scramble-treated samples, thereby confirming the knockdown of Mortalin. The knockdown samples were subsequently employed to investigate the modulation of various hallmark proteins implicated in cellular survival, proliferation, EMT, angiogenesis, migration, and invasion. Additionally, the impact of Mortalin knockdown on the regulation of Akt/mTOR signaling proteins was assessed, adhering to the previously established protocol, with GAPDH used as the loading control. The detailed information regarding the antibodies used for this experiment is provided in supplementary table 3.1.

3.2.4 Proliferation assay

The 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay was conducted to assess the effect of Mortalin knockdown on the proliferation of OC cell lines. Cells were plated at 2000 cells/well density in 96-well plates and incubated for 24 hours at 37°C. The absorbance readings were taken at both 0 and 72 hours. At each specified time point, 10µl MTT-5mg/ml (M6494, Invitrogen, Life Technologies, USA) solution was mixed to the corresponding plate and incubated for 2 hours, subsequently, the culture medium was aspirated and replaced with 100µl DMSO (Code 1.16743.0521, Merck, Darmstadt, Germany) and 1-hour incubation in the dark. The absorbance of the purple formazan product was then measured at 570 nm using Spectra Max iD3 microplate reader from Molecular Devices, USA, and percentage inhibition of proliferation of OC cells was calculated.

3.2.5 Cell cycle analysis

Cell cycle distribution was assessed to evaluate the effect of the siRNA mediated knockdown of Mortalin in regulating cell cycle progression in OC cells. For this assay, the control and siRNA treated cells were collected and rinsed with 1x phosphate buffered saline (PBS) and fixed with ice cold 75% ethanol in PBS for 30 min at −20 °C. The cells were perfused with PBS and stained with propidium iodide (PI)/RNase solution (prepared in 1x PBS-0.1% Triton X) and kept for 20 min under dark condition. The cells were then subjected to flow cytometric analysis employing BD FACS Celesta, BD Biosciences, New Jersey, USA. The acquired data were analyzed utilizing FCS express software to determine the percentages of cells in each phase of the cell cycle.

3.2.6 Colony formation assay

This assay was conducted to determine the impact of Mortalin knockdown on the colony-forming efficiency of OC cells. Cells were seeded at a density of 1 x 10^3 cells/well and incubated for 24 hours. The cells were allowed to form colonies over a period of 7-10 days with routine media changes every two days. Subsequently, the wells were rinsed with PBS and fixed with chilled ethanol for an hour, stained with crystal violet (0.01% w/v; Cat No. 548-6209, SRL Pvt. Ltd., India), and washed again with PBS. Images of the wells were captured and

quantification was performed using Image J software, followed by calculation of the survival fraction.

3.2.7 PI flow cytometric assay

The PI flow cytometry assay was performed to evaluate cell viability. PI is a fluorescent dye that intercalates with nucleic acids, emitting red fluorescence. Intact plasma membranes of live cells restrict PI permeability, resulting in low fluorescence, while damaged membranes of dead cells expose nucleic acids, leading to increased PI binding and higher fluorescence intensity [Crowley et al., 2016; Rosenberg et al., 2019]. Following treatment with either control or Mortalin-specific siRNAs for 48 hours, cells were collected, centrifuged and rinsed with PBS. Subsequently, cells were incubated with 5µl of 1mg/ml PI dye for 10 minutes, and the impact of Mortalin knockdown on cell death was analyzed using FACS Celesta, Becton Dickinson Biosciences, New Jersey, USA.

3.2.8 Annexin V assay

This assay was performed to examine the impact of Mortalin knockdown on apoptosis induction in OC cell lines. Following seeding, the cells were trypsinized, centrifuged and incubated with binding buffer and annexin V. However, for unstained control and PI control samples only binding buffer was used. Succeeding the incubation period, the samples underwent centrifugation again and were supplemented with 195 µl binding buffer. Subsequently, PI dye (5 µl) was added to the PI control sample, while 2.5 µl of PI dye was added to 195 µl of PBS in all other samples, except for the unstained control and annexin V control, prior to analysis. Experimental data were analyzed using a FACS Celesta flow cytometer (BD Biosciences, New Jersey, USA).

3.2.9 JC-1 assay

The assay was conducted to determine the impact of Mortalin knockdown on apoptosis induction, via measuring mitochondrial membrane potential ($\Delta\Psi m$) in OC cells. For this study, cells were plated and incubated for 24 hours, and then treated with either control or siRNA for a duration of 48 hours. Subsequently, the cells underwent trypsinization and centrifugation before being incubated with JC-1 (5,5,6,6'-tetrachloro-1,1',3,3'tetraethylbenzimidazoylcarbocyanine iodide) dye. Additionally, a positive control sample was treated with carbonyl cyanide m-chlorophenyl hydrazone (CCCP). Following another round of centrifugation, the samples were analyzed utilizing a FACS Celesta flow cytometer (Becton Dickinson, New Jersey, USA). The $\Delta\Psi m$ was assessed based on the ratio of JC aggregate to monomer accumulation within the cells, signifying mitochondrial health status. The healthy cells typically exhibit higher levels of JC aggregates within their mitochondria, whereas, damaged cells display reduced formation of JC aggregates, resulting in a predominant presence of JC monomers.

3.2.10 Cancer cell migration assay

In this study, migration assay was conducted to assess the impact of Mortalin knockdown on the directional migration of OC cells. This assay evaluates the

migratory behavior of cells by creating an artificial gap in a confluent monolayer formed by the cells. The migration of cells from the edges towards the gap is subsequently monitored over a specified period of time, and the degree and rate of closure of the gap reflect the migratory efficiency of the cells [Liang et al., 2007]. For this experiment, transfected cells were seeded into a 6-well plate and allowed to form a confluent monolayer. Subsequently, the media were replaced with serum-free media and incubated for 6-8 hours. Following this incubation period, a scratch was created along the midline of each well using a 200 µl microtip. The cells were then washed with PBS to eliminate debris. Then the migration efficiency within the scratched area was regularly monitored. Images were captured at various time points with a Nikon Eclipse TS100 inverted microscope and analyzed for percentage migration using Image J software.

3.2.11 Invasion assay

The invasion of cancer cells into neighboring tissues via the extracellular matrix is a crucial step in tumor metastasis [Fares et al., 2020]. Thus, to elucidate the role of Mortalin in regulating invasion in OC, we evaluated the invasive potential of Mortalin knockdown cells using the Boyden chamber assay [Bordoloi et al., 2020]. The OC knockdown cells were subjected to serum starvation and then seeded at a concentration of $5x10^4$ cells per well onto 8mm pore trans well inserts (Corning, USA) precoated with Matrigel, which were positioned within the wells of a 24-well plate. Cells were seeded using serum-free medium, while the media beneath the trans well inserts contained 30% FBS to act as a chemoattractant. Followed by a 24-hour incubation, the cells were fixed with 4% formaldehyde and exposed to crystal violet (0.01% (w/v) solution. Non-migrating cells were removed from the trans well inserts using cotton swabs. The stained cells were visualized and images were acquired employing inverted Nikon Eclipse TS100 microscope. The percentage invasion of control and siRNA-treated cells was subsequently calculated using Image J software.

3.2.12 Immunocytochemistry (ICC)

To analyze the impact of Mortalin knockdown on autophagy and EMT markers in OC cells, ICC was employed. The control and knockdown cells were seeded at $6x10^4$ cells/well onto cover slips in a 12-well plate and kept at 4°C overnight. After PBS washing, cells were fixed with 4% formaldehyde for 20 minutes, followed by permeabilization with PBST and blocking with 2% bovine serum albumin (BSA). The slides underwent incubation with primary antibodies, anti-LC3B polyclonal antibody (Life technologies, Thermo Fisher Scientific, USA) for autophagy and Snail-NL557 and vimentin-NL493 (from R&D systems, USA) for EMT. For EMT, a single-step staining was performed as the primary antibodies were attached to secondary antibodies. For autophagy, after washing, cells were exposed to goat anti-rabbit IgG secondary antibody conjugated with Alexa Fluor 594 (Invitrogen, USA). Following this, the cells were subjected to 4',6-diamidino-2-phenylindole (DAPI) dye and mounted on a microscopic slide using DPX mountant. The dried mounted slides were then viewed and images were taken using BX43 upright microscope (Olympus, Japan).

3.3 Statistical analysis

Statistical significance was followed as mentioned in Chapter 2, Section 2.3.

3.4 Results and Discussion

In the current chapter, a series of assays were performed with Mortalin knockdown cells to investigate its involvement in the molecular regulation of various hallmarks associated with OC. In addition, the regulatory influence of Mortalin on the Akt/mTOR signaling pathway in OC cells was examined. The results of the study revealed that Mortalin plays a significant role in regulating multiple hallmarks and their associated molecules in OC cells. Additionally, the impact of Mortalin knockdown on the Akt/mTOR signaling pathway further contributes to its regulatory function in OC cells. The detailed findings from the assays of the current chapter are outlined below.

3.4.1 Confirmation of the knockdown of Mortalin in OC cells

The gene expression of Mortalin was selectively inhibited in OC cells (SAS and HSC3) as well as in normal HaCaT cells using Mortalin-specific siRNA, followed by validation via Western blot analysis. The protein expression of the siRNA-treated samples revealed diminished levels of Mortalin, confirming successful knockdown. This effect was observed consistently in both SAS (**Fig. 3.1B & 3.1E**) and HSC3 (**Fig. 3.1C & 3.1F**) OC cells, as well as in normal HaCaT cells (**Fig. 3.1A & 4D**). The knockdown efficiency exceeded up to more than 50% in OC cells. Subsequently, the Mortalin-depleted cells were subjected to different assays, and the findings are discussed in the subsequent sections.

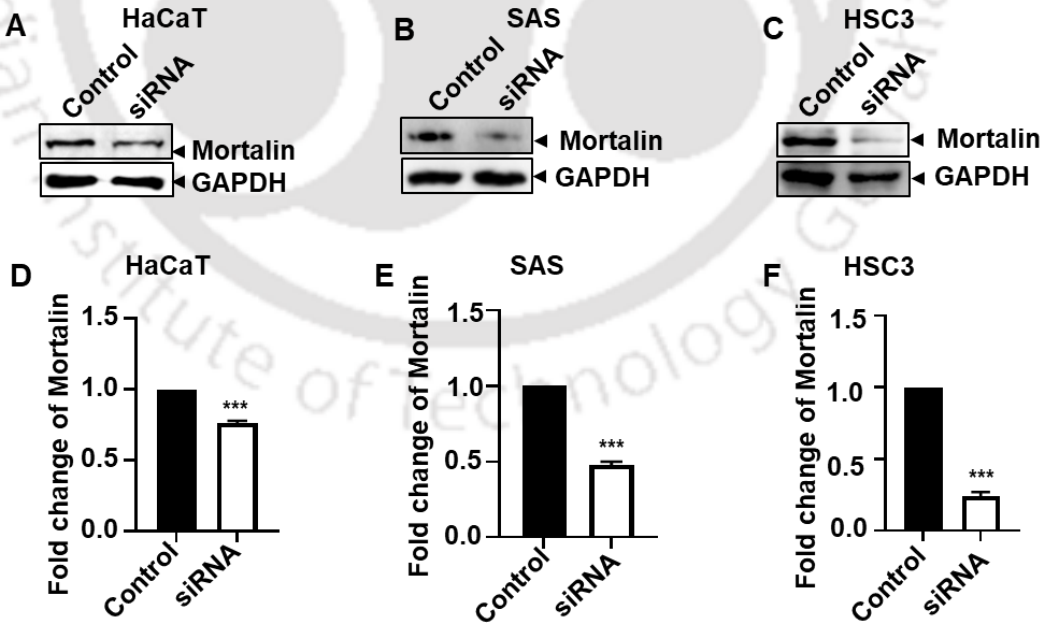

Fig. 3.1 siRNA-mediated knockdown of Mortalin in OC cells. A-C. Confirmation of siRNA-mediated knockdown of Mortalin through immunoblotting in HaCaT, SAS, and HSC3 cell lines, respectively. D, E & F. Quantitative representation of the expression of Mortalin knockdown in HaCaT, SAS and HSC3 cells.

3.4.2 Knockdown of Mortalin suppressed cell survival and proliferation of OC cells

Following the successful knockdown of Mortalin, we thoroughly investigated its impact on the survival and proliferation of OC cells. Intriguingly, our results demonstrated a significant decline in cell proliferation in SAS and HSC3 upon Mortalin inactivation (**Fig. 3.2A & 3.2B**); however, the effect was less pronounced in HaCaT cells (**Fig. 3.2C)**. This differential response suggests a specific regulatory role of Mortalin in promoting the proliferation in OC, potentially through mechanisms distinct from those in the normal cells. As previously reported by Lu WJ et al., Mortalin exhibits distinct subcellular localization patterns and functional roles in normal and cancer cells [Lu et al., 2011]. These differences in response of Mortalin between normal and cancer cells might trigger the observed differential effects following its knockdown in OC and HaCaT cells in our study. Moreover, Lu and group proposed that the variation in the effect of Mortalin knockdown on cellular proliferation between normal and cancer cells could be attributed to the absence of Mortalin-p53 interactions in normal cells [Lu et al., 2011]. However, in tumor cells Mortalin forms complexes with the tumor suppressor p53 protein, thereby impeding its translocation into the nucleus and subsequent transcriptional function which leads to enhanced proliferation of cancer cells [Elwakeel, 2022]. Due to its pivotal role in cell cycle regulation, the inhibition of Mortalin releases p53 from its binding, facilitating its nuclear translocation and subsequently facilitates cell cycle arrest and inhibit proliferation in cancer cells [Nigam et al., 2015]. Correlating to this, we have observed that Mortalin knockdown halted cell cycle at S-phase in OC cells (**Fig. 3.2D-3.2G)**.

Moreover, the propensity of cancer cells to proliferate and form colonies is one of the hallmarks of cancer [Rajendran and Jain, 2018]. As it was reported that Mortalin increased the colony forming ability in breast cancer (MDA-MB-231 and MCF-7 cells) and ovarian cancer (A2780 and A2780/cis ovarian cancer cell lines) cells [Hu et al., 2016; Wei B et al., 2021]. Therefore, in our next experiment, we investigated the colony-forming efficiency of Mortalin-inactivated OC cells, which resulted in the significant suppression of colonies both SAS and HSC3 cells. The corresponding survival fraction graphs represents these findings quantitatively, highlighting the inhibitory effect of Mortalin knockdown on colony formation potential of OC cells (**Fig. 3.2H- 3.2K)**. At the molecular level, our study elucidated the mechanistic regulation of Mortalin-mediated survival and proliferation in OC cells (**Fig. 3.3**). This observation was noted with the elevated levels of p53 and cyclin-dependent kinase (CDK) inhibitors such as p27, p21, and p-wee1 in Mortalin-inactivated SAS and HSC3 cells.

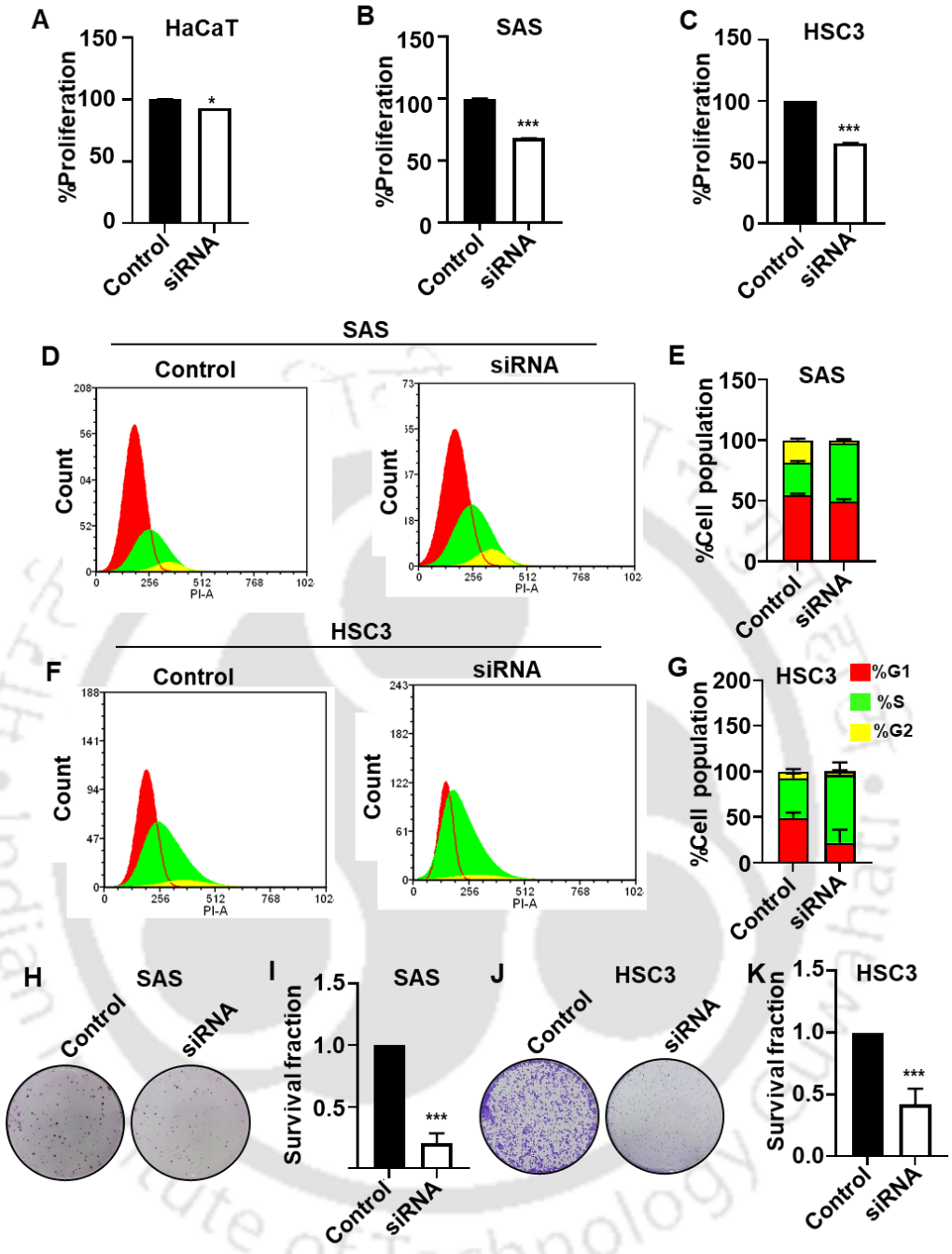

Fig. 3.2 Control and Mortalin knockdown OC cells were subjected to different experiments to determine the role of Mortalin in survival and proliferation. A, B, and C shows the percentage inhibition of proliferation in HaCaT, SAS, and HSC3 cells; D-G. Examined the impact of Mortalin knockdown on the cell cycle of SAS and HSC3 cells. E & G. Knockdown of Mortalin induced S-phase cell cycle arrest in OC cells. H & J: Depiction of colony formation assays conducted in SAS and HSC3 cells, where blue/purple dots show colonies formed in control and siRNA-treated samples; J & L: Quantification of figures in panels J and L, respectively, represented in terms of survival fraction.

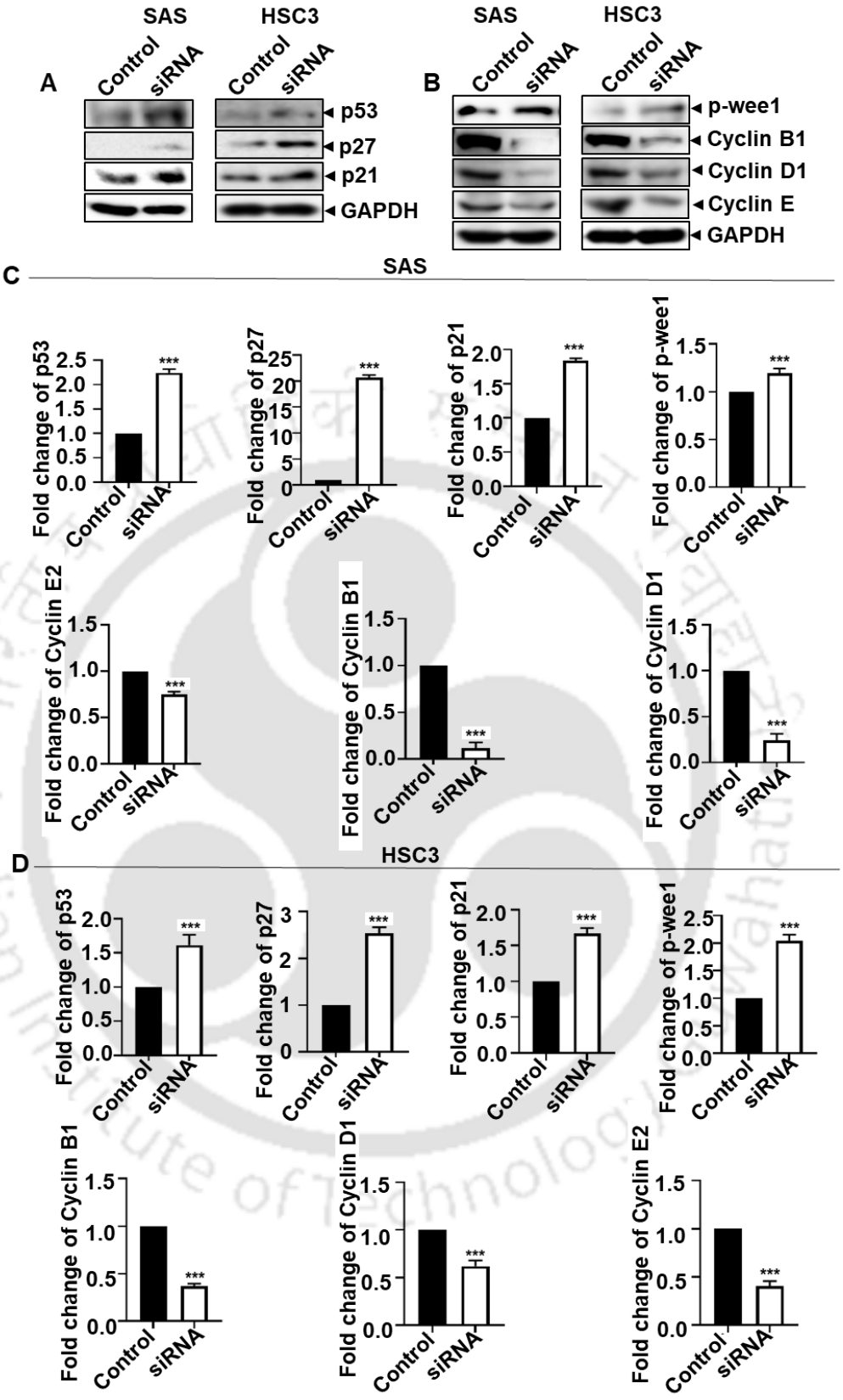

Fig. 3.3. Knockdown of Mortalin modulated different proteins involved in cell survival and proliferation. C & D. Densitometric graphs of various proteins shown in A & B, for SAS and HSC3 cells. Knockdown of Mortalin induced the expression of p53, p-wee1, p27 and p21; while the expression of cyclins (B1, D1 and E2) were downregulated in OC cells.

Conversely, the expression of various cyclins essential for cell cycle progression, including cyclin B1, D1, and E2, was downregulated upon Mortalin knockdown in these OC cells. Cyclins are the important regulators of cell cycle; cyclin D1 induced uncontrolled cell proliferation, cyclin B1 form complex with CDK1 to promote transition from the G2 to the mitotic phase of the cell cycle; cyclin E2 drive cells from G1 to S phase and induce DNA replication [Rogers et al., 2015; Montalto et al., 2020; Aljohani, et al., 2023]. These findings unveiled the multifaceted regulatory role of Mortalin in inducing survival and proliferation pathways in OC, involving the modulation of key proteins associated in these cellular processes. Consistent with the current findings, previous research has also demonstrated the association of Mortalin overexpression with enhanced cell survival and proliferation in various malignancies, including colorectal and thyroid cancers [Starenki et al., 2015; Xu et al., 2020].

3.4.3 Knockdown of Mortalin induced cell death in OC cells

Cancer cells exhibit adaptive mechanisms to evade various forms of cell death, primarily by modulating the expression levels of pro-apoptotic and anti-apoptotic proteins [Sharma et al., 2019]. As we observed from previous experimental results demonstrating the role of Mortalin in regulating the survival and proliferation of OC cells, it becomes pertinent to explore how its involvement in these processes contributes to the cell evasion of cell death pathways. Therefore, our study deciphered the intricate role of Mortalin in cell death regulation through a series of experiments. For this, the Mortalin inactivated cells were subjected to PI flow cytometry assay. This assay revealed the significant induction of cell death in siRNA treated SAS and HSC3 cells as compared to control, with the percentage of cell death exceeding 40% compared to control (**Fig. 3.4A-3.4D)**. This increase in cell death in the siRNA transfected samples signifies the probable participation of Mortalin in the survival of OC cells against cell death pathways, thereby augmenting their proliferative capacity during oral carcinogenesis. Given the crucial role of cell death evasion in OC, we further examined whether suppression of Mortalin via knockdown induced apoptosis. This was evaluated through Annexin V/ FITC assay and JC-1 assay. The Annexin V/FITC assay detects apoptotic cells by binding annexin V to exposed phosphatidylserine on the cell membrane, a characteristic feature of apoptotic cells [Koopman et al., 1994, Vermes et al., 1995]. Conversely, the JC-1 assay assesses mitochondrial health via measuring $\Delta\Psi m$, a critical determinant of apoptosis, by detecting the JC aggregates (red) and monomers (green) formed within the mitochondria in the cells. In the healthy cells with intact mitochondrial membranes, the JC-1 fluorescent dye which is cation in nature enters the mitochondria and undergoes aggregation due to the intact anionic inner mitochondrial membrane. However, since the damaged cells have depolarized membrane potential, the JC dye exists as monomers [Sivandzade et al., 2019]. Interestingly, siRNA-mediated knockdown of Mortalin promoted apoptosis in OC cells, as evidenced by increased apoptotic cell populations in SAS and HSC3 cell lines (**Fig. 3.4E-3.4H)**.

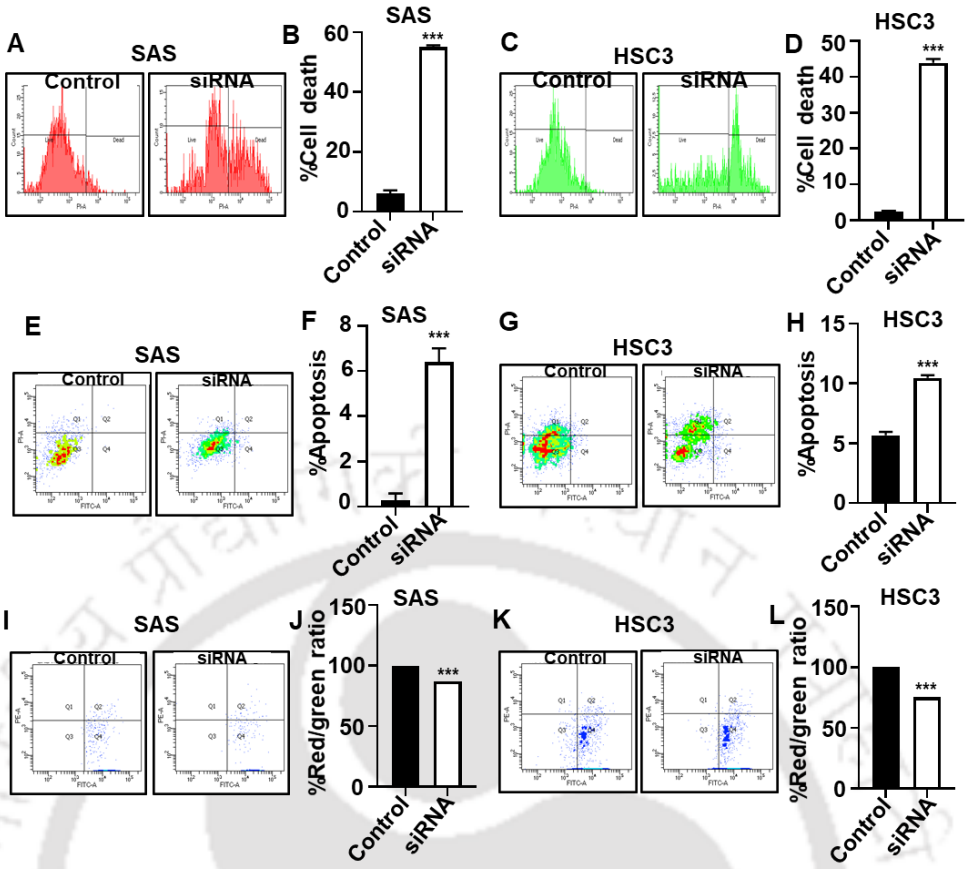

Fig. 3.4 Impact of Mortalin knockdown on cell death in OC. A & C: demonstrate the histogram representations of %Cell death in control and siRNA treated samples for SAS and HSC3 cell lines; B & D: Depict the %Cell death for SAS and HSC3 cells upon Mortalin knockdown, respectively; F & H: Represent %Apoptosis in SAS and HSC3 cells along with contour plots for the given cell lines, represented in fig. 3.4E and 3.4G; J & L: highlights the percentage decrease in mitochondrial potential, represented by the Red/Green ratio: I & K: Representative contour plots for J and L.

Furthermore, JC-1 assay results revealed the compromised ΔΨm in Mortalin knockdown cells relative to the control, signifying the influence of Mortalin in maintaining mitochondrial health status in OC cells (**Fig. 3.4I-3.4L**). This modulatory effect of Mortalin on regulating cell death pathways in OC was further supported by alterations in the expression levels of apoptotic-related proteins. Specifically, the expression of anti-apoptotic proteins such as Bcl-2 and survivin were downregulated, coupled with the upregulation of pro-apoptotic proteins Caspase-3 and -9 in Mortalin knockdown OC cells (**Fig. 3.5**). Bcl-2 and Survivin are anti-apoptotic proteins that prevent apoptosis in cancer cells by inhibiting the activity of caspases [Okuno et al., 1998; Jaiswal et al., 2015]. Conversely, caspases are endoproteases that help in regulation and initiation of apoptosis in cancer cells [Dwivedi et al., 2020]. These findings from the current study aligns with previous investigations, which reported the induction of apoptosis in Mortalin depleted bladder, gastric and thyroid cancer cells through suppression of Bcl-2, and induction of caspase-3 [Dai et al., 2021; Wang et al., 2014; Starenki et al., 2015].

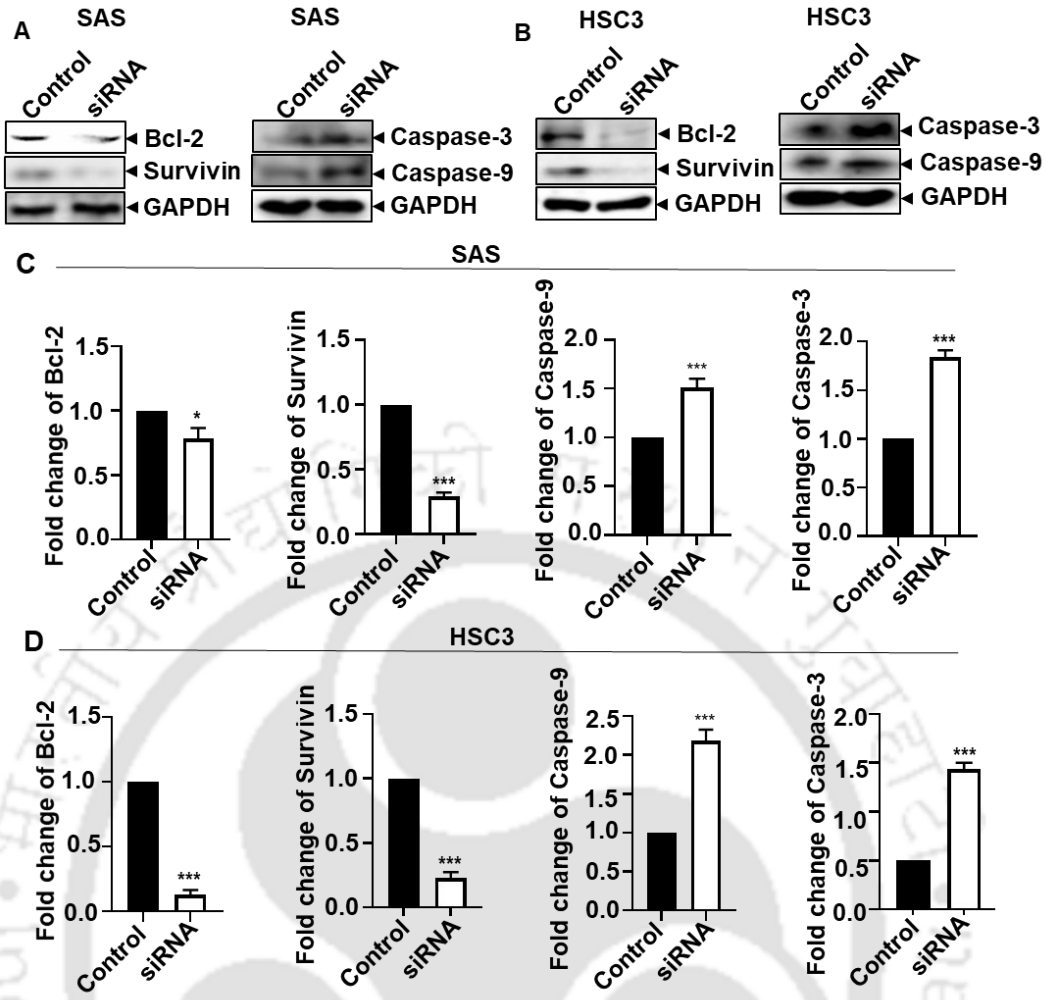

Fig. 3.5. Impact of Mortalin knockdown on proteins involved in cell death pathways. Knockdown of Mortalin decreased the expression of Bcl-2 and Survivin; and induced the expression of caspases in OC cells (Fig. 3.5A & 3.5B). C& D. shows the quantitative graphs for the given protein in fig. 3.5A & 3.5B, for SAS and HSC3 cells.

Moreover, our investigation extended on exploring the role of Mortalin in regulating autophagy (**Fig. 3.6**). Autophagy is a crucial degrative pathway that facilitates the removal of dysfunctional organelles and misfolded protein aggregates. It is also referred to as type II cell death, characterized by the presence of abundant autophagic structures in the dying cell that lacks phagocytic recruitment and in sometimes are caspase-independent, unlike the type I cell death, i.e., apoptosis [Das et al., 2012]. However, autophagy has been reported to suppress tumorigenesis by regulating mitochondrial damage and ROS levels and certain proteins of this pathway act as tumor suppressors by inducing the process of cell death [Yun et al., 2018]. In our study, we found that Mortalin knockdown induced the expression of LC3B and p62 in OC cells (**Fig. 3.6C & 3.6E**). LC3B is an autophagic marker that helps in autophagosome biogenesis, while p62 acts as a cargo receptor for ubiquitinated proteins for transporting them to autophagosomes for degradation [Hwang and Kim, 2023].

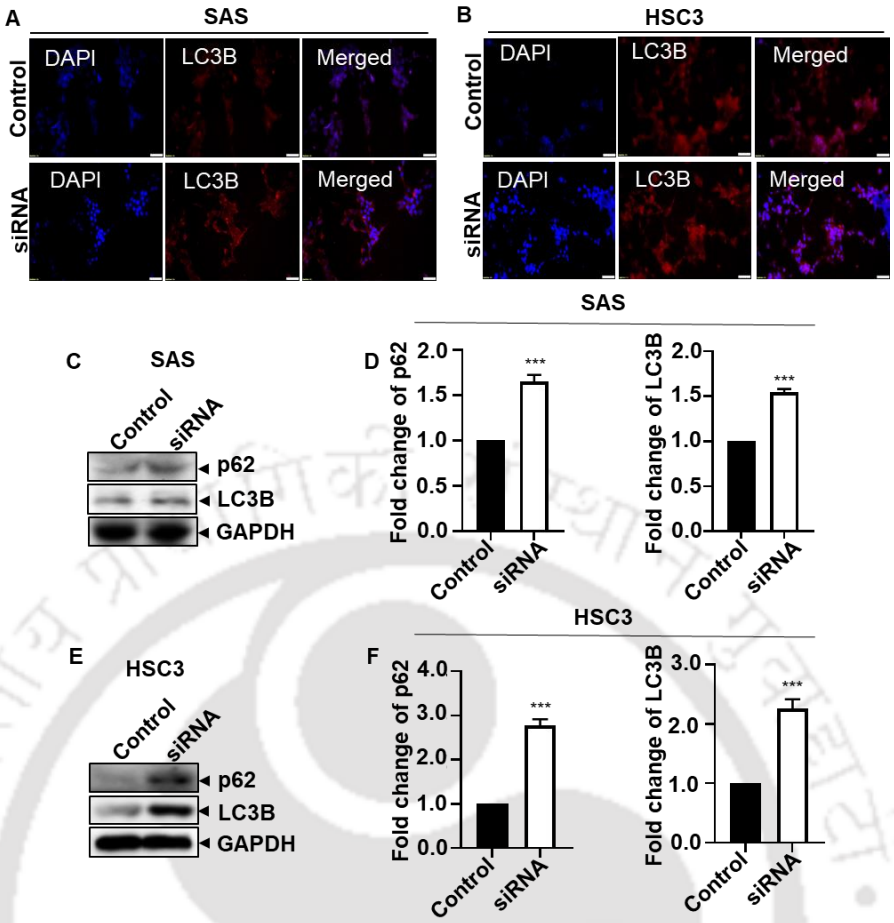

Fig. 3.6 Effect of Mortalin knockdown on autophagy in OC cells. A & B: Autophagic assay determined through fluorescence imaging, in control and siRNA treated samples for SAS and HSC3 cells; E & F: Knockdown of Mortalin induced the expression of LC3B and p62 in these cells; quantitative expression is given in fig. 3.6D & 3.6F for both OC cells.

In conclusion, our investigation demonstrated the significant role of Mortalin in regulating diverse cell death processes and associated molecules, which might potentially facilitate the evasion of cell death mechanisms in OC. In addition, these findings reveal the potential of Mortalin as a promising target in the management of OC.

3.4.4 Knockdown of Mortalin suppresses EMT, invasion, angiogenesis and migration of OC cells

Cancer progression is a complex process associated with a series of events, comprising of EMT, invasion, angiogenesis and migration [Fares et al., 2020; Ribatti et al., 2020]. These processes play a pivotal role in OC and are interconnected which collectively contribute to highly aggressive and metastatic nature of cancer cells [Jayanthi et al., 2020]. Therefore, this study examined the role of Mortalin in regulating these hallmark processes of oral carcinogenesis. For this, the Mortalin knockdown cells were subjected to different assays, pertaining to understand these processes. The Boyden chamber assay revealed a significant reduction in the invasive capacity of Mortalin knockdown in both SAS and HSC3

cells compared to their respective controls, indicating the potential role of Mortalin in regulating the invasive phenotype crucial for metastasis of OC (**Fig 3.7A-3.7D**). Additionally, the impact of Mortalin knockdown on directional migration was evaluated in OC cells by a cancer cell migration assay (**Fig. 3.7E-3.7H**). This assay demonstrated that Mortalin knockdown inhibited directional migration of OC cells, as evidenced by images captured at different time points (0 hr to 48 hr).

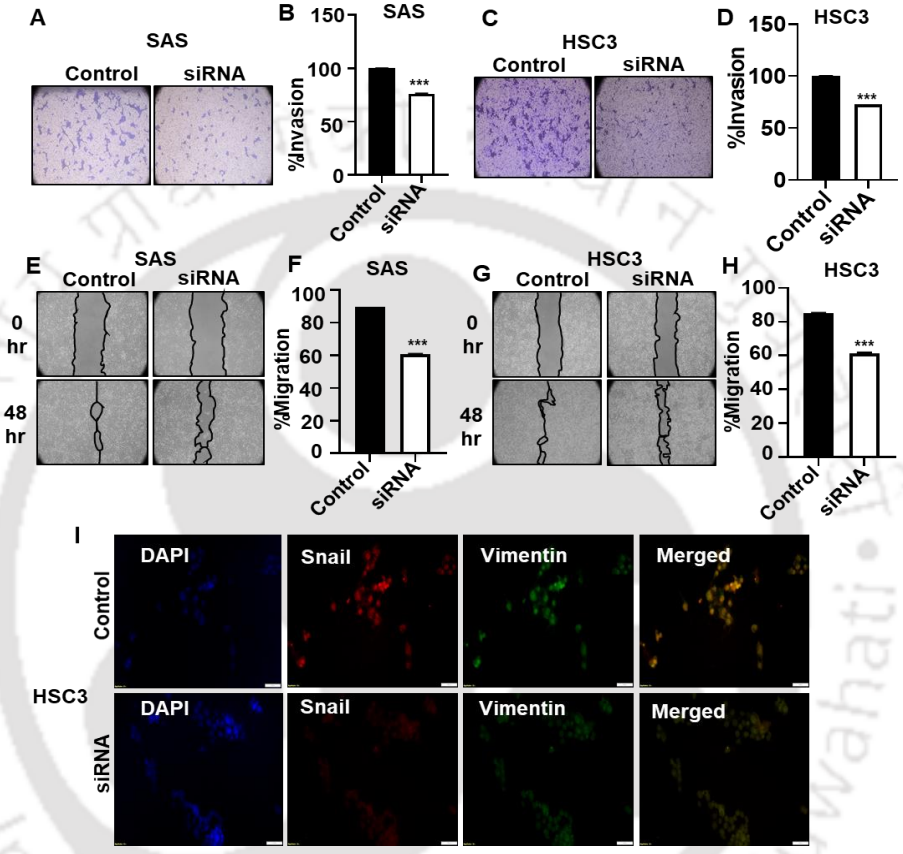

Fig. 3.7 Association of Mortalin in regulating EMT, invasion, and migration in OC. A & C: Shows invaded cells in SAS and HSC3 cells, respectively, in control as well as siRNA-mediated Mortalin knockdown samples; B & D: %Invasion in SAS and HSC3 cells after Mortalin knockdown. E & G: Migration images determined at 0 hr and 48 hr by wound healing/scratch assay in SAS and HSC3 cell lines; F & H: %Migration of OC cells (SAS and HSC3) after knockdown of Mortalin; I: EMT assay in HSC3 cells by fluorescence imaging.

Another important event influencing the metastatic ability of OC cells, EMT, was determined by immunofluorescence microscopy (**Fig. 3.7I**). This assay provided further insights into the role of Mortalin on the process of EMT. It was found that the expression levels of EMT markers, including Snail and vimentin were reduced in Mortalin knockdown cells relative to the control group. Snail is a master transcriptional regulator that causes aggressive and metastatic abilities of cancer cells, whereas vimentin regulates cell migration and adhesion [Paul et al., 2023].

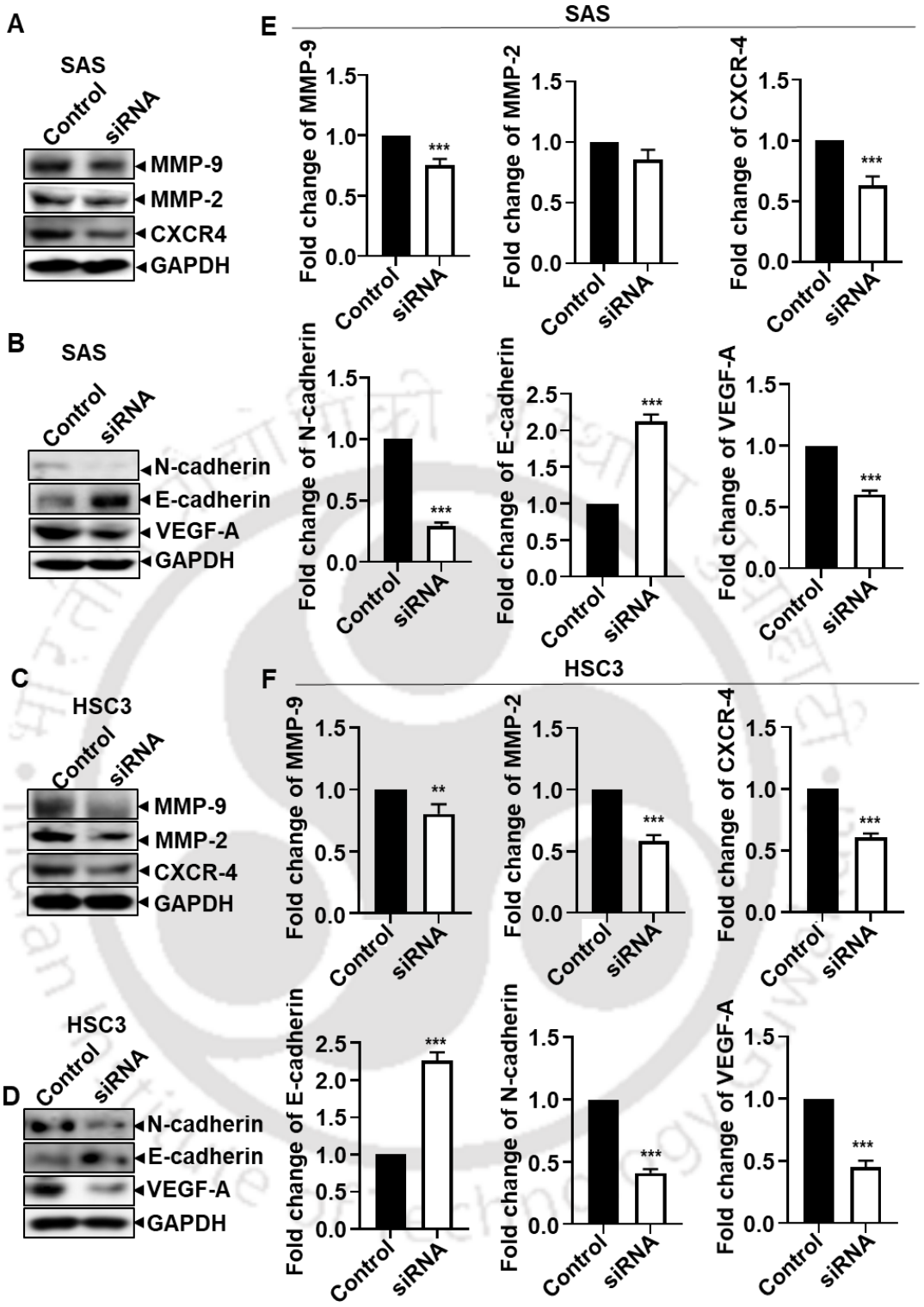

Fig. 3.8 Knockdown of Mortalin modulated proteins involved in EMT, invasion, angiogenesis and migration in OC cells (3.8A- 3.8D). Fig. 3.8E & 3.8F represents the densitometric graphs for the proteins depicted in 3.8A-3.8D. The knockdown of Mortalin was found to induce the expression of E-cadherin, while downregulating the expression of N-cadherin, VEGF-A and CXCR4. In addition, the knockdown of Mortalin also suppressed MMPs (-2 and -9) in OC cells.

In addition, Western blot analysis corroborated these findings, revealing modulation of key proteins associated with EMT, invasion, angiogenesis, and migration. Notably, Mortalin knockdown led to elevated expression of E-cadherin while suppressing N-cadherin expression in OC cells (**Fig. 3.8B & 3.8D**). E-cadherin is an epithelial marker which exerts tumor-suppressive effects by promoting cell adhesion and regulating cell maturity and movement, while N-cadherin is a mesenchymal marker that induces migration of cancer cells by promoting tumor cell motility [Zhu et al., 2021].

Moreover, our study showed that knockdown of Mortalin downregulated the expression angiogenesis marker, VEGF-A which stimulates endothelial cell invasion and vessel formation, along with the suppression of the invasion and metastasis-associated proteins C-X-C chemokine receptor type 4 (CXCR-4), matrix metalloproteinase (MMP)-2, and MMP-9 in both SAS and HSC3 cells (**Fig. 3.8**). In addition, CXCR-4 promotes intravasation and extravasation of cancer cells as well as tumor angiogenesis by recruiting the endothelial progenitor cells [Kim et al., 2017; Luker et al., 2021]. MMPs play important roles by promoting tumor growth and metastasis in OC [Thomas et al., 1999].

The findings of this study are consistent with previous investigations; for instance, studies demonstrated increased expression of N-cadherin, vimentin, MMP-2, and MMP-9 in Mortalin-overexpressing breast cancer, hepatocellular carcinoma, melanoma and osteosarcoma cells. However, the knockdown or inhibition of Mortalin in these cancer cells reversed the effects of its overexpression leading to the suppression of EMT, invasion and migration [Na et al., 2016; Teng et al., 2021]. In conclusion, our study delineated the significant association of Mortalin in regulating multiple hallmarks such as EMT, invasion, angiogenesis, and migration, by modulating the expression of proteins associated with these processes.

3.4.5 Knockdown of Mortalin inhibited Akt/mTOR signaling in OC cells

In our continued investigation, due to the well-established involvement of the Akt/mTOR pathway in oral carcinogenesis, we have evaluated the regulatory role of Mortalin on this pathway in OC cells [Harsha et al., 2020]. The current study unveiled a convincing link between Mortalin and Akt/mTOR signaling, as evidenced by the inhibition of p-Akt Ser473, p-mTOR Ser2448 and p-S6 Ser236 in Mortalin knockdown cells (**Fig. 3.9**). These findings strongly suggest that Mortalin exerts significant influence over the Akt/mTOR pathway, thereby impeding the survival, growth, proliferation, EMT, invasion, of OC cells.

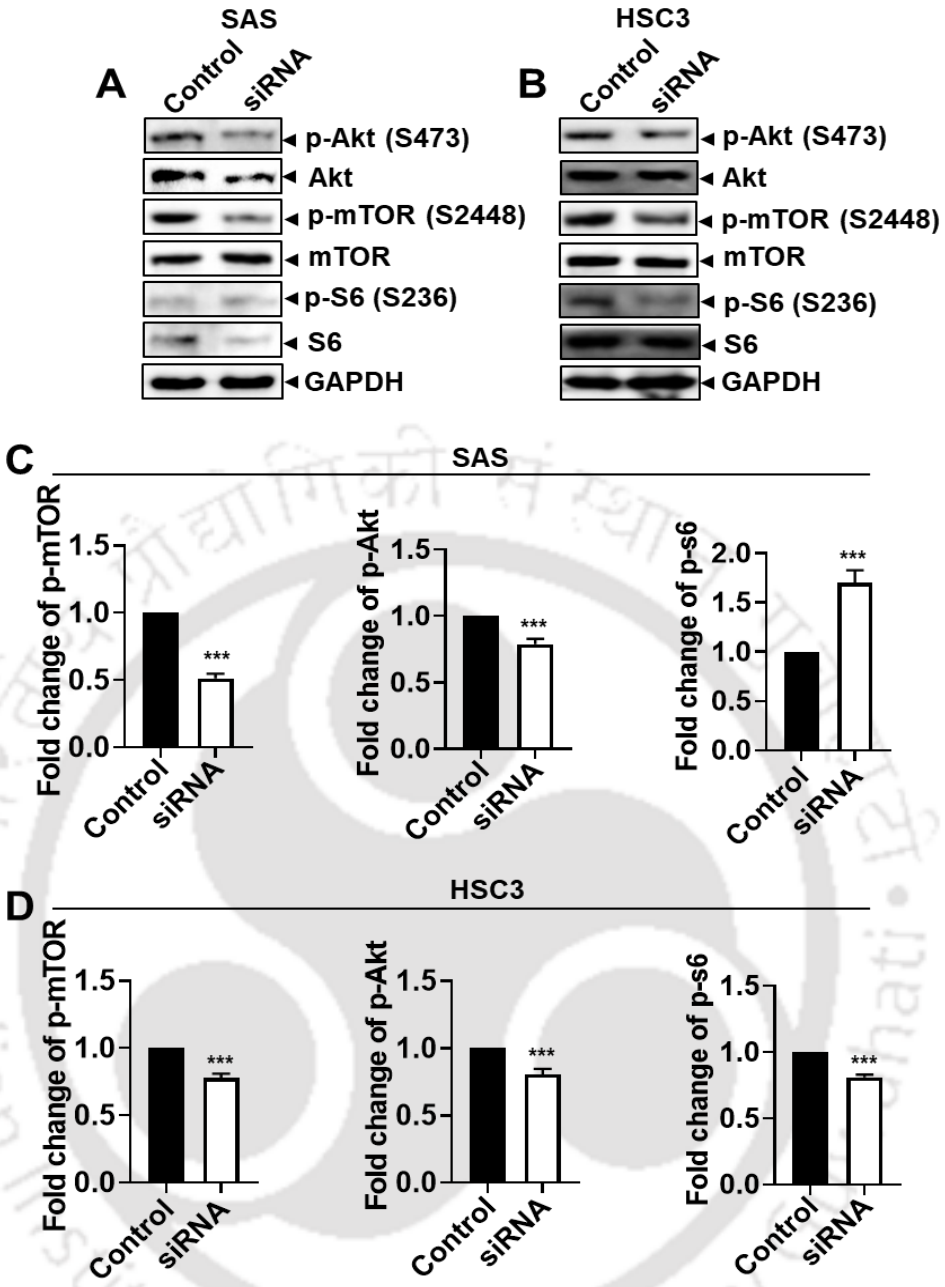

Fig. 3.9 Effect of Mortalin knockdown on Akt/mTOR signaling in OC. A & B: Expression of Akt signaling proteins in SAS and HSC3 cells, in control vs siRNA treated samples. C & D. Quantitative representation of the expression of Akt/mTOR signaling proteins upon Mortalin knockdown in OC cells.

3.5 Conclusion

In conclusion, our findings unravel the intricate role of Mortalin in OC pathogenesis, particularly its involvement in regulating key signaling pathways such as Akt/mTOR and regulating cellular processes including autophagy (**Fig. 3.10**). In conclusion, this study reveals new insights into the molecular mechanisms by which Mortalin influences the regulatory signaling pathways and molecules governing OC progression. In addition, the mechanistic association of Mortalin with Akt/mTOR signaling might possibly explain as one of the reasons for the modulation of different cellular processes of OC by Mortalin. Hence, these

observations suggest that Mortalin can be used as a potential molecular target for developing drugs for the treatment of OC.

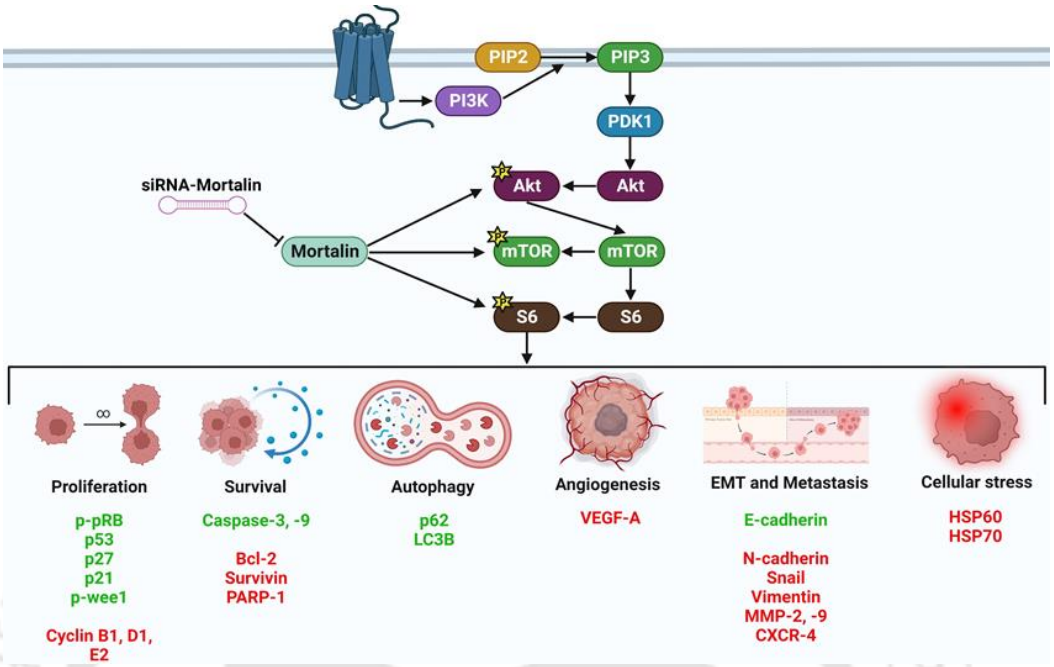

Fig. 3.10 Schematic representation of the putative mechanism of Mortalin in OC. (Image credit: BioRender.com)

Chapter 4

Effect of Withaferin A on Mortalin and OC Hallmarks

4.1 Introduction

The findings of the previous chapters, have established the potential role of Mortalin in regulating various hallmarks of OC and related pathways. Therefore, the suppression of Mortalin can be used as potential strategy in developing novel treatment modalities for the therapeutic management of this cancer. Various methods can be used for targeting Mortalin in OC cells such as small molecules inhibitors, monoclonal therapeutic antibodies, microRNAs (miRNAs), etc. Several small molecule inhibitors have been authorized by the FDA for the treatment of various malignancies including OC; however, most of these inhibitors show non-specific binding, undesirable toxicities, and unaffordability by majority of the patients in the world. Monoclonal antibodies are novel classes of anti-cancer drugs; however, their higher cost and toxicities makes them less suitable for the treatment of diverse pathological conditions. miRNAs emerged as novel therapeutics for the management of different diseases, including cancer. However, the non-specific binding of these molecules, and lesser acceptability are the main concern. Therefore, it is imperative to find out safe, affordable, and effective molecules for suppressing Mortalin for the treatment of OC.

It has been well established that natural products have high potential for the prevention and treatment of various malignancies, and many of these products such as vinca alkaloids, taxol, camptothecin and so on have been approved by FDA for the therapeutic application in this disease. Previous studies have shown that WiA isolated from Ashwagandha (*Withania somnifera*), has potential anti-cancer properties by modulating different signaling pathways such NF-κB and STAT3 [Mohan et al., 2004; Yco et al., 2014; Choi and Kim, 2015; Yang et al., 2015] Conversely, WiA treatment was found to induce expression of tumor suppressor p53 and its downstream target p21, along with upregulated expression of apoptotic proteins such as Bax, Bcl-2 interacting mediator of cell death, BH3 interacting-domain death agonist and caspases [Yang et al., 2015; Tang et al., 2019]. Moreover, studies have shown that WiA directly binds with Mortalin and suppresses different hallmarks of various malignancies such as breast and ovarian cancers and osteosarcoma. Therefore, in the current chapter we have investigated the therapeutic efficacy of WiA in suppressing Mortalin in OC cells and its downstream targets. Moreover, the ability of WiA in modulating different hallmarks of cancer has also been studied.

4.2 Materials methods

Several experimental assays were performed to assess the impact of WiA on Mortalin expression and different OC hallmarks. The detailed methodologies employed are outlined below:

4.2.1 Cell culture

The details regarding the maintenance of cell culture and its conditions were followed as per highlighted in Chapter 2, Section 2.2.1.

4.2.2 Western blot analysis

Western blot analysis was employed to analyze the expression of different proteins after the treatment of WiA. Briefly, the OC cells were treated with a range of concentrations of WiA and incubated for 24 hr. The cells were collected and whole cell lysates were prepared and western blotting was done as described in the previous Chapter 2.

4.2.3 Proliferation assay

The effect of different concentrations of WiA on the proliferation of OC cells and HaCaT cells was determined by MTT assay using the protocol mentioned in the previous Chapter 3, Section 3.2.4.

4.2.4 Cell cycle analysis

Similarly, the effect of WiA on cell cycle was investigated in OC cells. The cells were seeded with the density of 2×10^5 cells/ well and incubated for 24 hours and the cells were treated with 0.25µM and 5µM of WiA and kept for 24 hours. The cell cycle arrest was determined as mentioned in Chapter 3, Section 3.2.5.

4.2.5 Colony formation assay

The efficacy of WiA was assessed on the colony forming efficiency of SAS and HSC3 cells. To perform this experiment, cells were seeded at a density of 1000 cells/well. After 24 hours incubation, the cells were exposed to different concentrations of WiA. Then the colony forming ability of the cells were determined using the procedure outlined in Chapter 3, Section 3.2.6.

4.2.6 PI flow cytometric assay

The impact of WiA on the viability of SAS and HSC3 cells (seeded at a density of 5×10^4 cells/well) was evaluated by treating at varying dosage levels of the compound for 72 hours. The percentage cell death was calculated as mentioned in Chapter 3, Section 3.2.7.

4.2.7 Live/Dead assay

Live/Dead assay was performed to determine the impact of WiA treatment on cell viability of OC cells. This assay relies on calcein AM and ethidium homodimer to evaluate intracellular esterase activity and plasma membrane integrity. Calcein is permeable in living cells, interacts with intracellular esterases and emits green fluorescence, whereas, the later dye is only permeable in cells with compromised plasma membranes and intercalates with nucleic acids, thereby emitting bright red fluorescence [Ravindran et al., 2011]. For this experiment, 2×10^3 cells/well were seeded and subjected to a range of concentrations of WiA for 72 hours. Subsequently, the cells were stained with Live/Dead assay reagents following manufacturer's guidelines, and images were acquired using an inverted fluorescence microscope from Olympus, Japan.

4.2.8 Annexin V assay

The efficacy of WiA on apoptosis of OC cells was evaluated using annexin V assay. $1x10^5$ cells/well were plated and incubated with varying concentrations of WiA and incubated for 48 hours. The percentage of apoptosis was determined as specified in Chapter 3, Section 3.2.8.

4.2.9 Cancer cell migration assay

The impact of WiA on the directional migration of OC cells was investigated by seeding 5 x 10^5 cells/well in 6-well plates, allowing for confluent monolayer formation. Subsequently, cells underwent serum starvation and scratch was generated, followed by treatment with the 0, 0.1, 0.2, 0.3 μM of the drug. Images were captured by an inverted microscope, and migration potential was calculated using Image J software as detailed in Chapter 3, Section 3.2.10.

4.2.10 Invasion assay

The effect of WiA on the invasive potential of OC cells was evaluated by a Boyden chamber assay after the treatment of 0.3 μM of the compound for 24 hours. The percentage invasion was calculated as discussed in Chapter 3, Section 3.2.11.

4.2.11 Immunocytochemistry (ICC)

To determine the effect of WiA in inducing autophagy of OC cells was investigated by ICC after the treatment of 0.5 μM WiA for a duration of 24 hours. After incubation, cells were subjected to primary anti-LC3B antibody, accompanied by secondary antibody conjugated with fluorophore and the images were taken using the protocol outlined in Chapter 3, Section 3.2.11.

4.3 Statistical analysis

Statistical significance was determined as outlined in Chapter 2, Section 2.3.

4.4 Results and Discussion

In the current chapter, we have analyzed the potential of WiA in suppressing Mortalin, modulating different hallmarks of OC and the underlying mechanism.

4.4.1 WiA inhibited the expression of Mortalin in OC cells

The study examined the efficacy of WiA in modulating the expression of Mortalin in OC cells (**Fig. 4.1A- 4.1D**). Remarkably, WiA was found to downregulate the expression of Mortalin in both SAS and HSC3 OC cell lines dose-dependently as shown in fig. 4.1B- 4.1D. This dose-dependent inhibition of Mortalin could be attributed to its strong binding affinity with this protein, as reported previously [Vaishnavi K et al., 2012]. These findings demonstrated the promising potential of WiA in targeting Mortalin expression in OC.

4.4.2 WiA inhibited the survival and proliferation of OC cells

Subsequently, we determined the efficacy of WiA on the survival and proliferation of OC cells through various assays. In MTT assay, WiA significantly

repressed the proliferation of SAS and HSC3 cells, while the inhibition of proliferation in normal cells was lesser compared to OC cells (**Fig. 4.1E)**. The median inhibitory concentration (IC_{50}) for OC cells was determined to be approximately 0.35 µM, while normal cells exhibited no observable IC_{50} up to a dose of 1 µM WiA. In addition, colony formation assay was conducted to analyze the survival fraction of OC cells by WiA treatment (**Fig. 4.1H & Fig. 4.1I)**. It was found that WiA potentially suppressed the ability of SAS and HSC3 cells to form colonies in a concentration dependent manner.

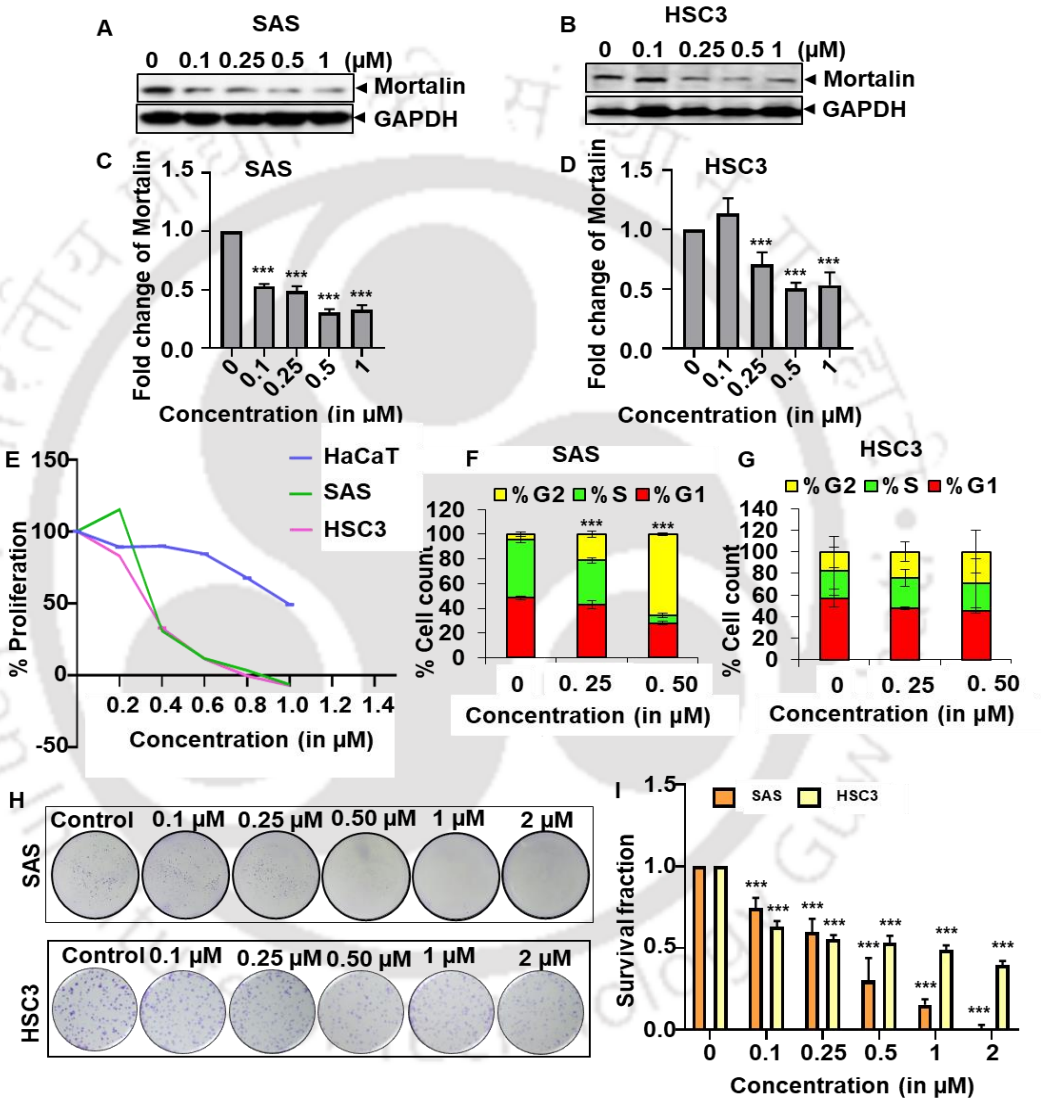

Fig. 4.1 Effect of Withaferin A (WiA) on Mortalin expression and on the survival and proliferation of OC cells. A & B: Effect of WiA on the expression of Mortalin in SAS and HSC3 cells; C & D: Densitometric analysis of Mortalin expression in WiA-treated OC cells; E: Inhibition in %Proliferation in SAS and HSC3 cells compared to normal HaCaT cells upon WiA treatment. F & G: Effect of WiA on the cell cycle in SAS and HSC3 cells. H: Represents the colony images in SAS and HSC3 cells upon treatment with different concentration of WiA; I: Survival fraction determined from colony formation assay in SAS and HSC3 cells.

Moreover, cell cycle analysis of the SAS and HSC3 cells treated with WiA was found to halt cell progression through G2/M phase (Fig. 4.1F & Fig. 4.1G). The result of the cell cycle analysis obtained from this study correlates with the previous reported potential of WiA in inducing G2/M phase cell cycle arrest in breast, gastric and osteosarcoma cancer cells by downregulating the expression of cyclin A, cyclin B1, Cdk2 and phospho-cell division cycle (p-Cdc)2 expression [Lv and Wang, 2015; Stan et al., 2008, Kim et al., 2017]. The G2/M cell cycle arrest by WiA might also be due to different other transcription factors and proteins modulated by this compound in OC cells.

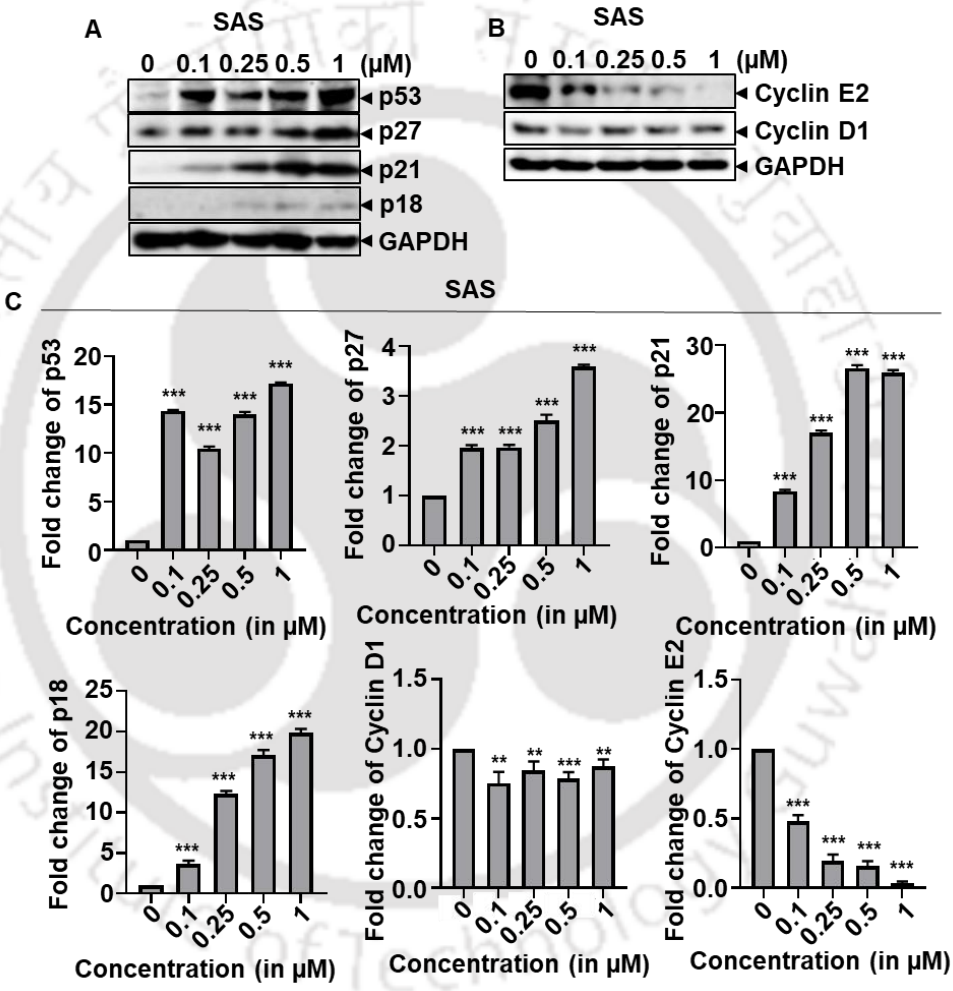

Fig. 4.2a Effect of WiA on the expression of different proteins associated with cell survival and proliferation in WiA treated SAS cells. Fig. 4.2a.C and 4.2a.D and depicts the densitometric graphs for proteins given in 4.2a.A & 4.2a.B. for SAS cells. It was found that the treatment of SAS cells with the given dose of WiA induced the expression of p53, and CDK inhibitors such as p27, p21 and p18. On the other hand, WiA treatment also led to the inhibition of cyclin D1 and cyclin E2.

Moreover, the treatment of WiA induced the expression of p53 in SAS and HSC3 cells (**Fig. 4.2a & Fig. 4.2b**). Further, WiA was also found to induce the levels of

CDK inhibitors such as p27, P21 and p18 in both the OC cell lines. On the other hand, OC cells subjected WiA treatment showed diminished expression of cyclins such as cyclin E2 and cyclin D1. As these proteins are involved in regulating the process of cell cycle and proliferation, the modulation of these given proteins by WiA, might explain its mechanistic effect in these processes. Thus, these results establish the potential of WiA in inhibiting the survival and proliferation of the OC cells.

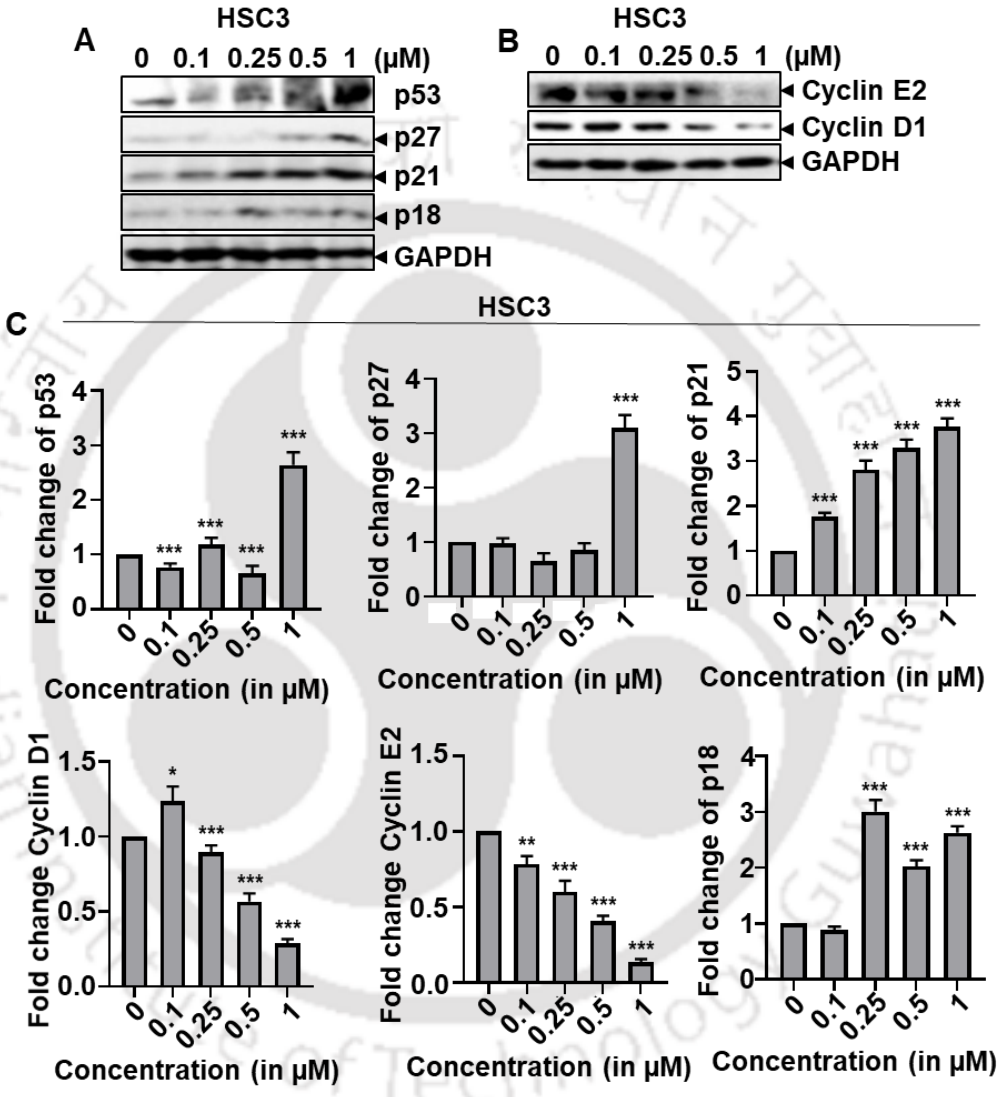

Fig. 4.2b Effect of WiA on the expression of different proteins associated with cell survival and proliferation in WiA treated HSC3 cells. Fig. 4.2b.C and 4.2b.D and depicts the densitometric graphs for proteins given in 4.2b.A & 4.2b.B. for SAS cells. It was found that the treatment of HSC3 cells with the given dose of WiA induced the expression of p53, and CDK inhibitors such as p27, p21 and p18. On the other hand, WiA treatment also led to the inhibition of cyclin D1 and cyclin E2.

4.4.3 WiA promoted cell death of OC cells

Extending our study, we evaluated the potential of WiA in modulating different types of cell death in OC cells by treating with different concentrations of WiA. In PI-FACs assay, the treatment of OC cells with 0, 0.5, 1 and 5µM concentrations of WiA triggered cellular death in a dose responsive manner (**Fig. 4.3A-4.3C**). This dose-response effect was further supported by Live/Dead assay, which demonstrated increased cytotoxicity in both SAS and HSC3 cell lines following WiA treatment (**Fig. 4.3G)**. Additionally, annexin V assay revealed a concentration-dependent induction of apoptosis by WiA at 0, 0.5, 1 and 5µM in these cells (**Fig. 4.3D-4.3F**).

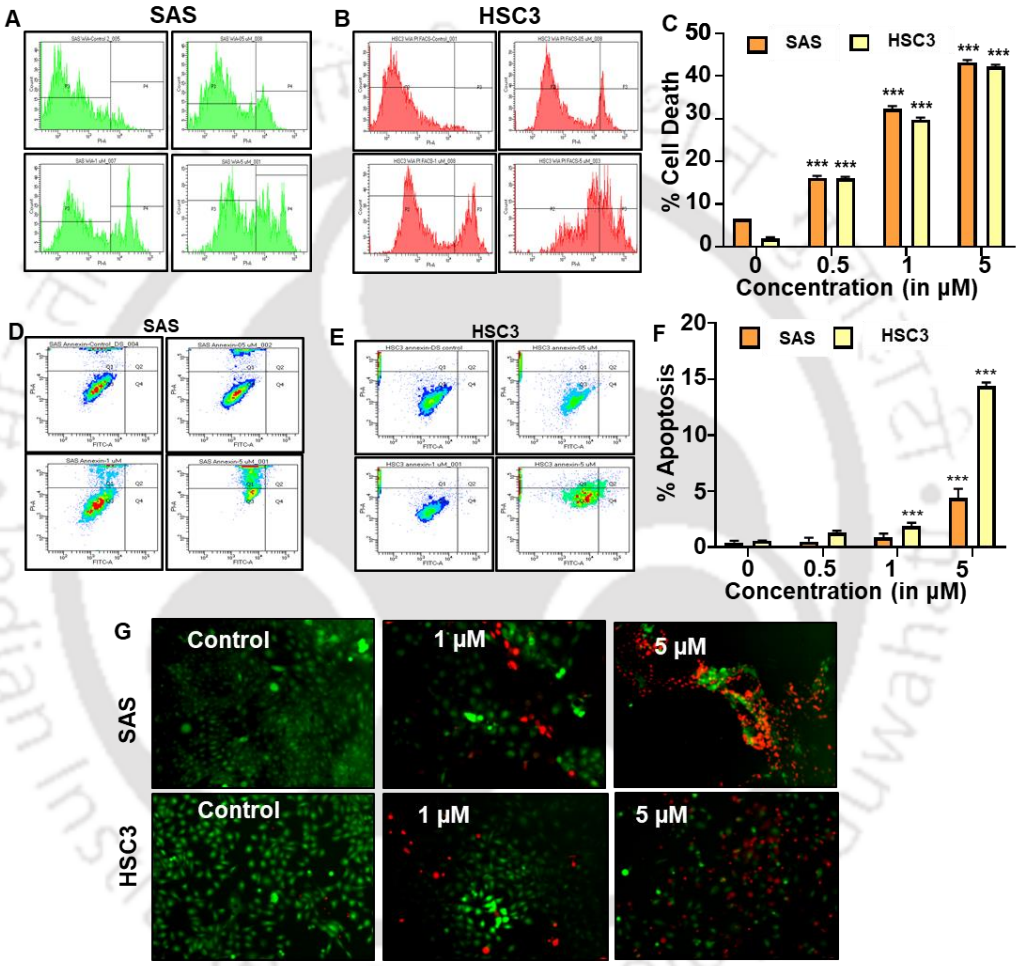

Fig. 4.3 Effect of WiA on cell death in OC. A & B: Histogram representation of PI-FACS assay in SAS and HSC3 cells treated with different concentration of WiA; C: %Cell death induced by WiA in SAS and HSC3 cells. D & E: Contour plots for Annexin V/ FITC assay in WiA treated SAS and HSC3 cells; F: %Apoptosis in SAS and HSC3 cell lines determined through Annexin V/ FITC assay; G: Cytotoxic effect of WiA treatment in SAS and HSC3 cells, determined by live/dead assay.

Notably, immunoblotting analyses unveiled the efficacy of WiA treatment in suppressing the levels of anti-apoptotic proteins cyclooxygenase 2 (COX-2), Bcl-2, and survivin (Fig. 4.4a), while also upregulating Caspase-3 and -9 expressions (Fig. 4.4b). These observations are consistent with prior investigations, for

instance, a study by Hsu et al., demonstrated the apoptotic effects of WiA in lung cancer cells by modulating various proteins involved in apoptosis pathways [Hsu et al., 2019]. Similar research conducted by Mayola et al. elucidated the potential of WiA to induce apoptosis in melanoma cells by modulating mitochondrial function, cytochrome c release, and DNA fragmentation, along with alterations in apoptotic protein expressions like Bcl-2, Bax, Caspase-3, and Caspase-9 [Mayola et al., 2011].

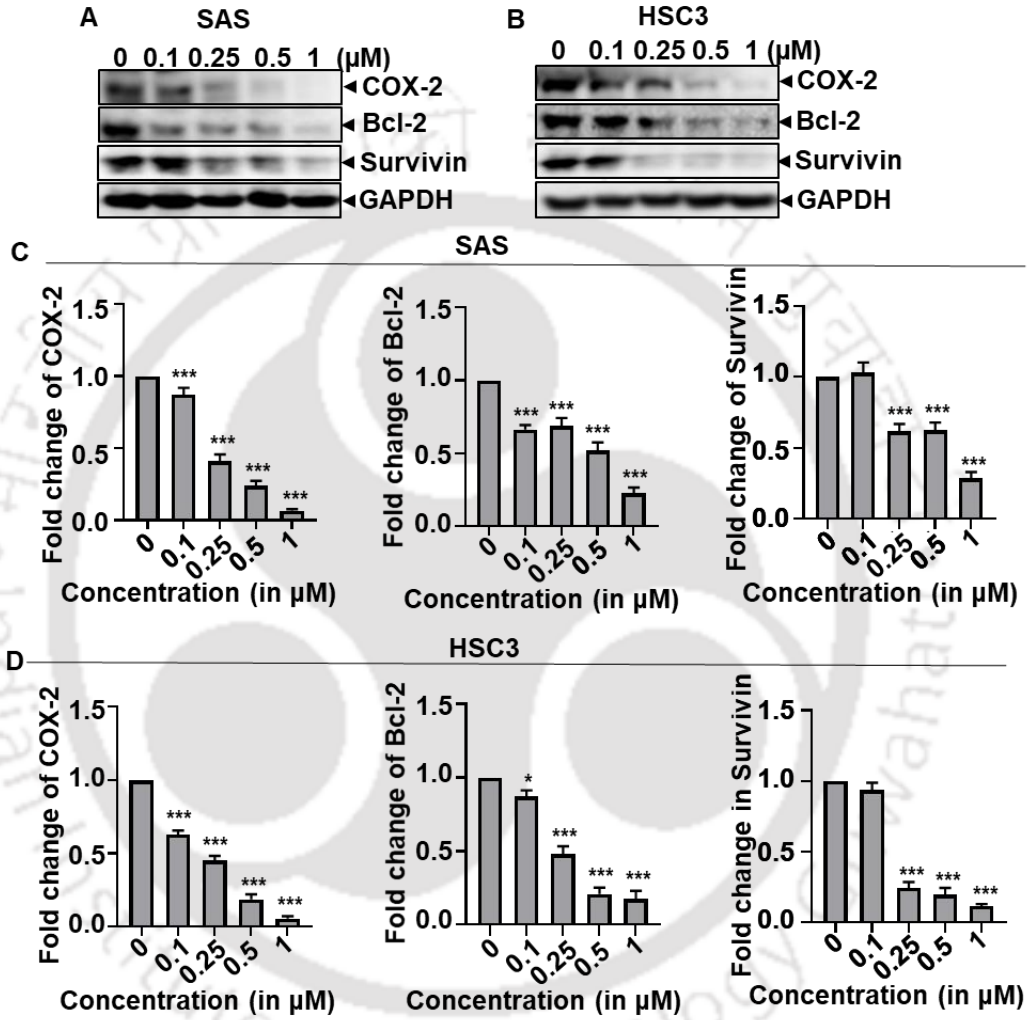

Fig. 4.4a Effect of WiA on the expression of anti-apoptotic proteins in OC cells. 4.4a.A & 4.4a.B represents the immunoblotting images of the proteins analyzed after WiA treatment in OC cells. 4.4C & 4.4D represents the densitometric graphs for the fig. 4.4a.C & 4.4a.B. WiA treatment was found to suppress the expression of COX-2, Bcl-2 and Survivin in a dose dependent manner in OC cells.

In addition, WiA treatment was found to induce the levels of proteins implicated in autophagy such as LC3B and p62 (**Fig. 4.5**). Thus, these results showed the efficacy of WiA in promoting cell death in OC cells through the modulation of various proteins associated with diverse cellular death processes.

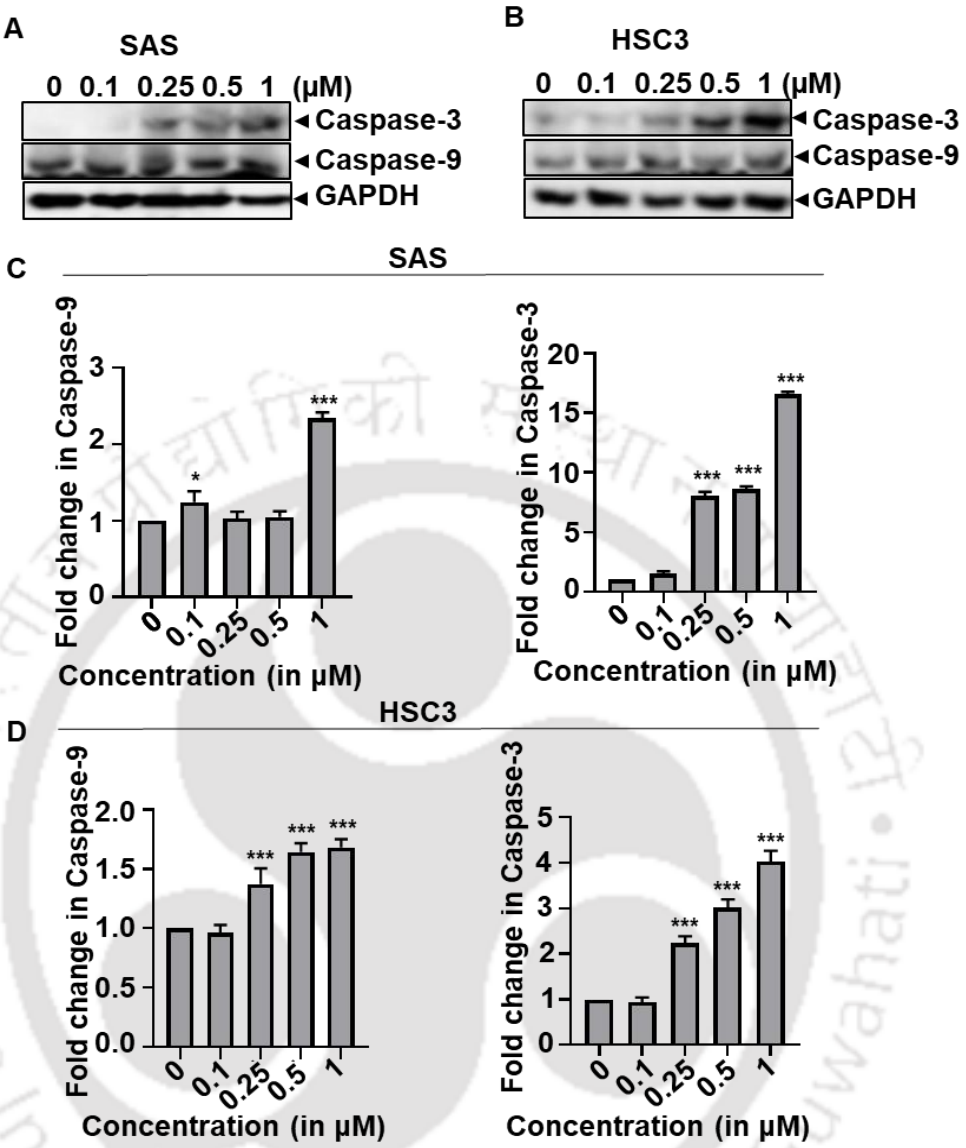

Fig. 4.4b Effect of WiA on the expression of apoptotic proteins in OC cells. 4.4b.A & 4.4b.B represents the immunoblotting images of the proteins analyzed after WiA treatment in OC cells. 4.4C & 4.4D represents the densitometric graphs for the fig. 4.4b.C & 4.4b.B. WiA treatment was found to induce the expression of Caspase-3 and Caspase-9 in a dose dependent manner in OC cells.

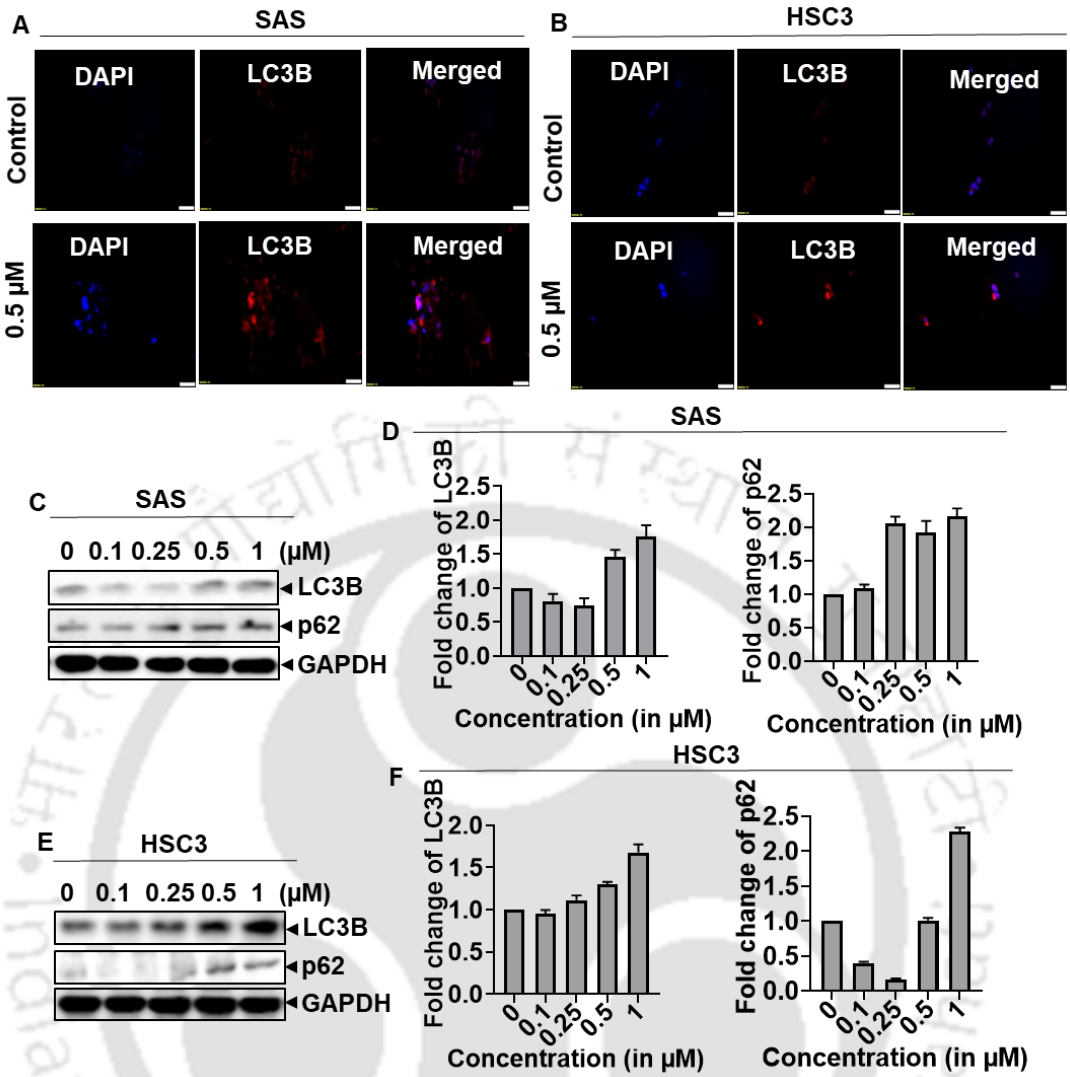

Fig. 4.5 Effect of WiA on autophagy in OC cells. A & B. Autophagic assay determined through fluorescence imaging, in control vs 0.5 µM WiA treated samples in SAS and HSC3 cells. C & E. Immunoblotting of autophagic proteins (LC3B and p62) in SAS and HSC3 cells; densitometric graph represented in fig 4.5D and 4.5F.

4.4.4 WiA suppressed EMT, invasion, angiogenesis and migration of OC cells

The efficacy of WiA was determined in regulating the hallmarks such as EMT, angiogenesis, invasion and migration in OC cells. In the Boyden chamber assay, it was found that WiA treatment remarkably inhibited the invasion of SAS and HSC3 cells (**Fig. 4.6A- 4.6D**). Additionally, cancer cell migration assay was performed which showed that increasing dose of WiA reduced the directional migration of SAS and HSC3 cells compared to control (**Fig. 4.6E & 4.6F**).

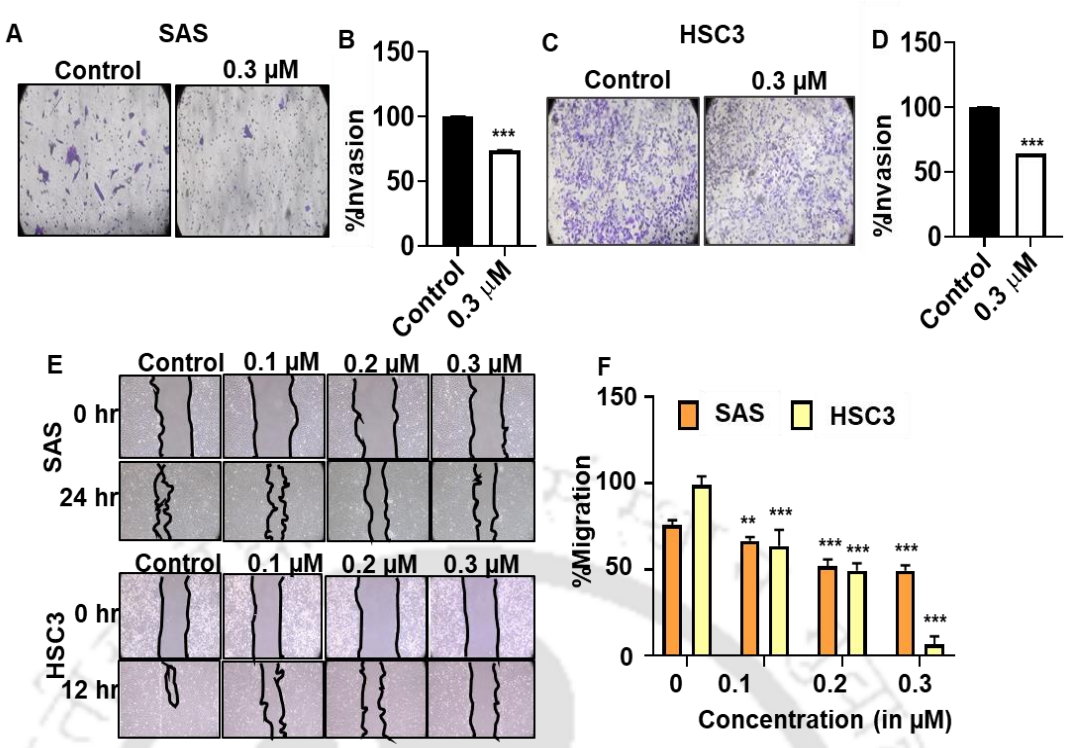

Fig. 4.6 Effect of WiA on invasion and migration in OC cells. A & C: Images representing invaded cells in control and WiA treated cells in SAS and HSC3 cells; B & D: %Invasion in WiA treated SAS and HSC3 cell lines; E: Images representing migration in SAS and HSC3 cells, determined through cancer cell migration assay; F: Representation of %Migration in SAS and HSC3 cells treated with WiA.

Mechanistically, it was found that WiA suppressed the expression of VEGF-A, MMP-2, MMP-9 and N-cadherin while inducing E-cadherin in these cells (**Fig 4.7a & 4.7b**). Previous studies reported the efficacy of WiA in attenuating EMT by inhibiting the phosphorylation and nuclear translocation of Smad2/3 and NF-κB, while also mechanistically overexpressing E-cadherin and repressing N-cadherin, fibronectin, vimentin, Snail, and claudin-1 in lung cancer cells [Kyakulaga et al., 2018]. Decisively, these experimental results accomplish the ability of WiA to reduce various processes linked to tumor invasion and metastasis of OC cells via modulating different proteins.

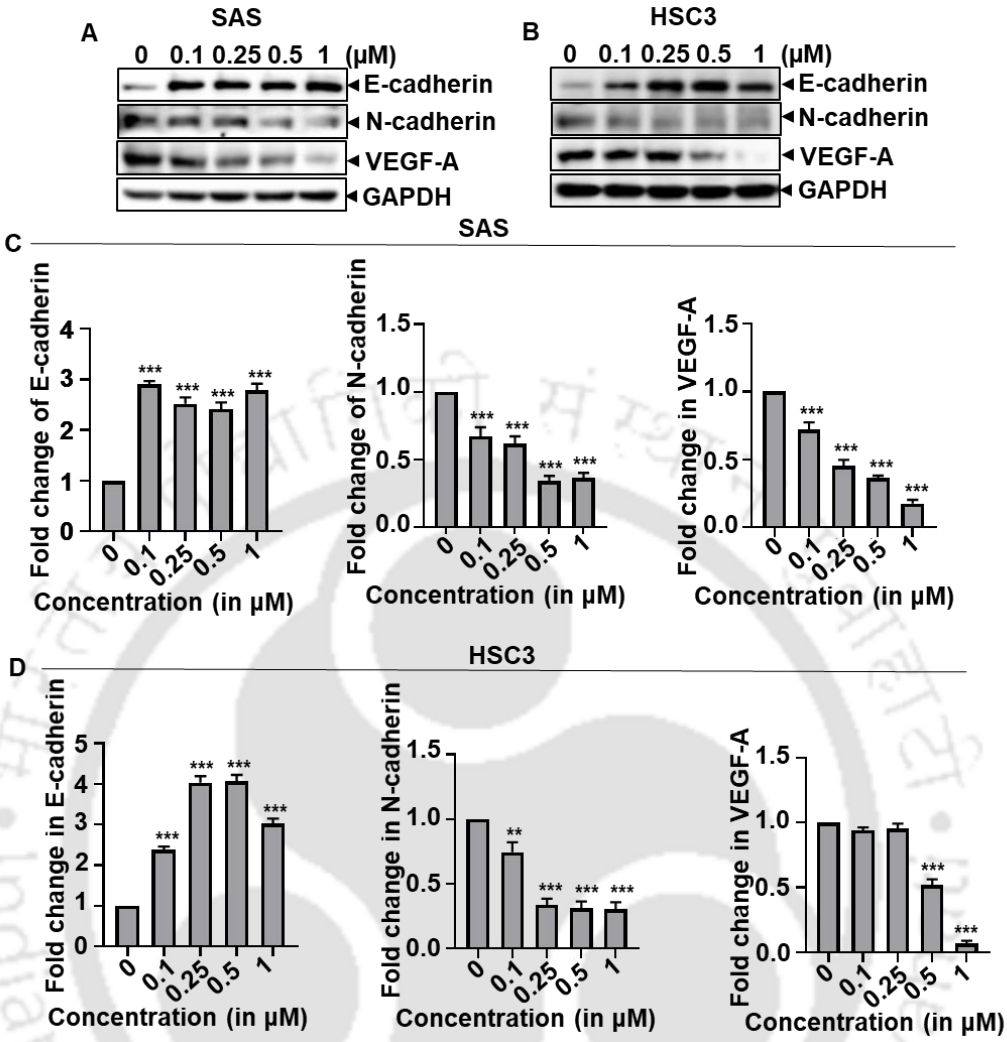

Fig. 4.7a Effect of WiA on the expression of proteins associated with EMT and angiogenesis of OC, determined by western blotting in WiA treated OC cells. The WiA treatment was found to suppress the expression of mesenchymal marker N-cadherin and angiogenic marker VEGF-A as shown in fig. 4.7a. A and 4.7.a.B. While on the other hand, the treatment of WiA induced the expression of epithelial marker E-cadherin. Fig. 4.7a. C and 4.7.a.D. represents the densitometric graphs for the protein blots shown in fig. 4.7a. A and 4.7.a.B, respectively.

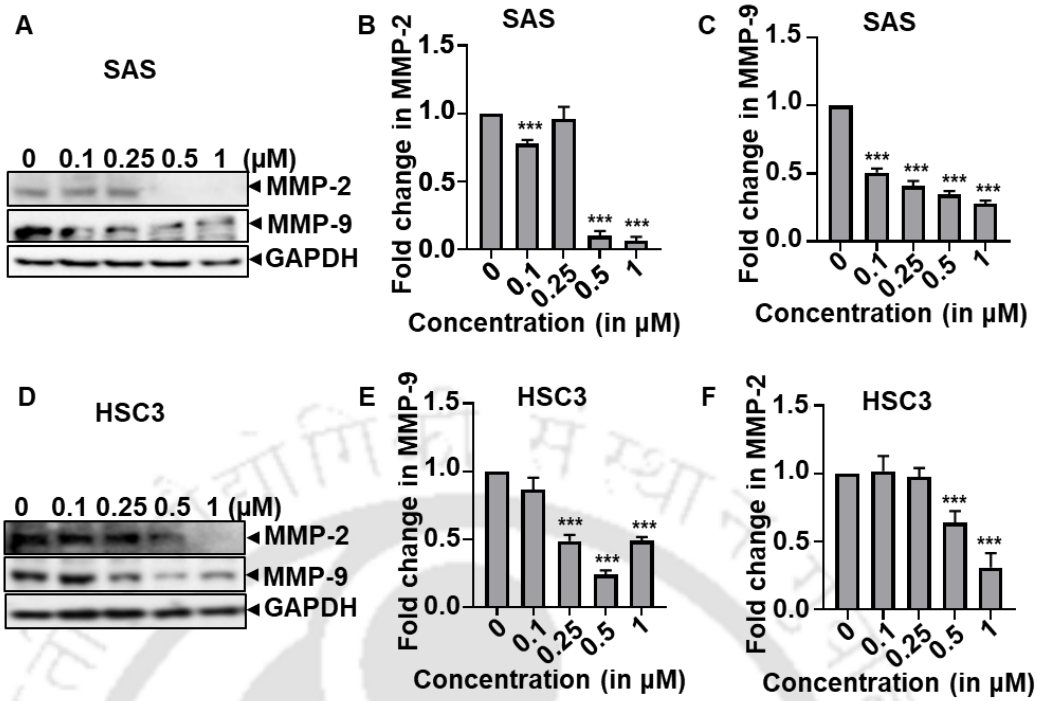

Fig. 4.7b Effect of WiA on the expression of proteins involved in invasion and migration in OC, determined by western blotting in WiA treated OC cells. It was shown that WiA treatment with the indicated concentrations suppressed the expression of MMP-2 and MMP-9 in OC cells. The subsequent plots in fig. 4.7b.C and 4.7b.D represents the quantification of the blots in fig. 4.7b.A; while for the immunoblots in fig. 4.7b.D, the densitometric are depicted in in fig. 4.7b.E and 4.7.b.F.

4.4.5 WiA modulated Akt/mTOR signaling cascades in OC cells

The Akt/mTOR signaling cascade plays a crucial function in OC development and has been associated with the promotion of several OC hallmarks. In addition, we have shown in the previous chapter that Mortalin inhibition led to the suppression of Akt/mTOR pathway. Therefore, in this chapter, we have determined the efficacy of WiA in suppressing this pathway. We observed that WiA inhibited p-Akt and its effector molecules, p-mTOR and p-s6 in OC cells (**Fig. 4.8**). Akt/mTOR axis regulates key processes such cellular survival, proliferation, invasion, angiogenesis, and migration, we have concluded that WiA inhibited this pathway via the suppression of Mortalin.

Thus, considering from this observation, it can be concluded that WiA has promising potential suppressing Mortalin and its regulated major signaling cascades and hallmarks in OC.

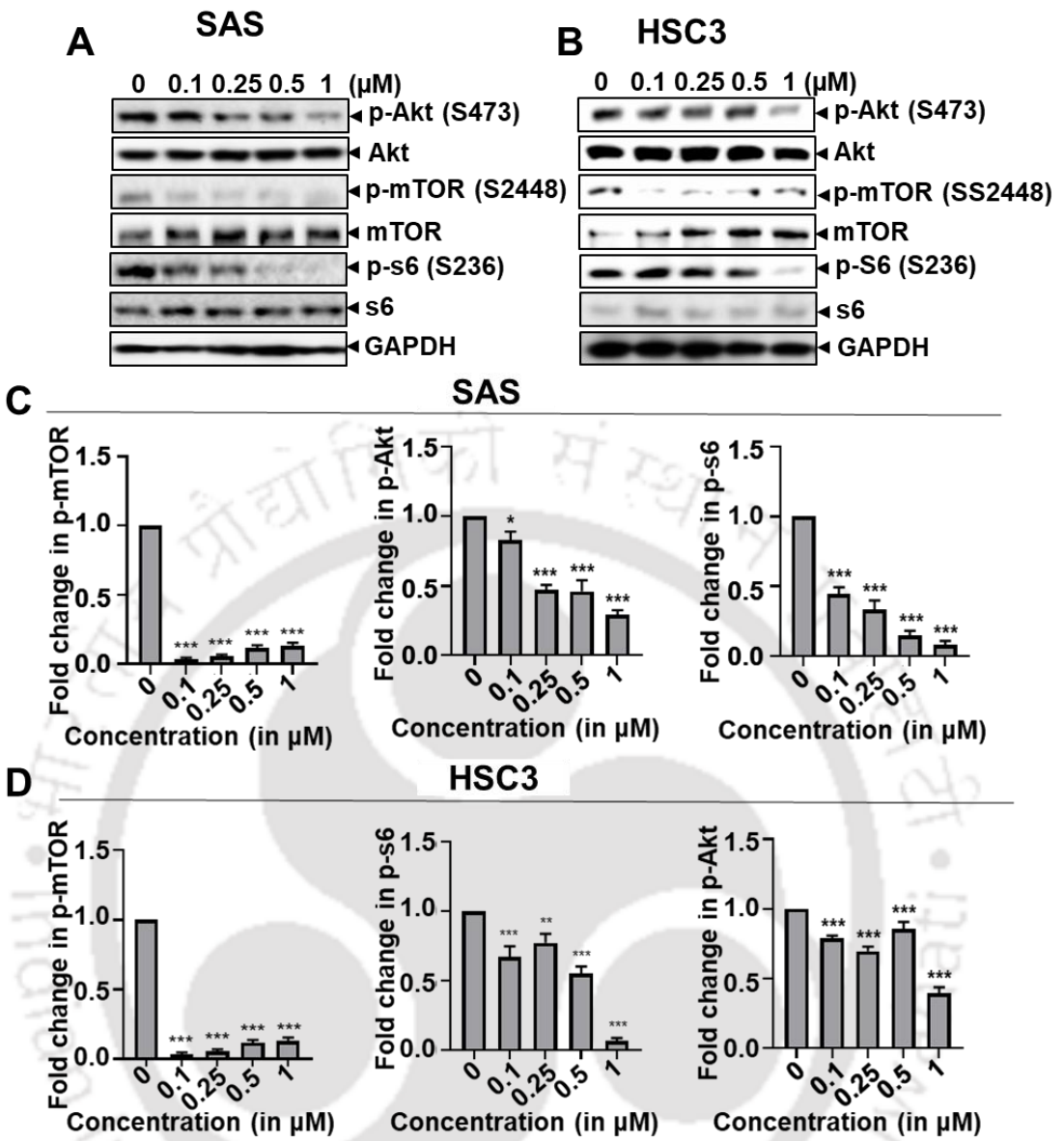

Fig. 4.8 Effect of WiA on Akt signaling in OC cells. A & B; Expression of Akt signaling proteins in SAS and HSC3 cells treated with different concentration of WiA. C & D. Represents the densitometric graphs for fig. 4.8A & 4.8B. The treatment of OC cells with different concentration of WiA was found to inhibit the expression of p-Akt, p-mTOR and pS6.

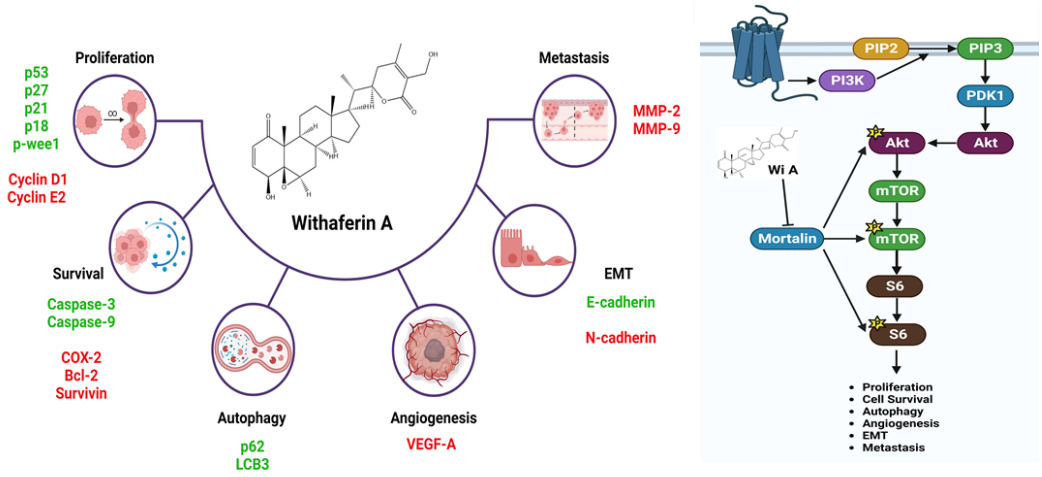

Fig. 4.9 Schematic representation of the mechanistic action of WiA in OC. (Image credit: BioRender.com).

4.5 Conclusion

The investigations of this chapter suggest that WiA inhibited Mortalin and its downstream effector Akt/mTOR pathway, and suppressed different hallmarks of OC (**Fig. 4.9**). Therefore, it can be concluded that WiA has a significant potential in developing safe, efficacious, and affordable drugs for the treatment of Mortalin-driven OC. However, in vivo and clinical studies are required to validate these findings.

Chapter 5

Discussion and Conclusion

5.1 Discussion and Conclusion

OC being one of the frequent causes of mortality is among the top three cancers in India, ranking as the most common malignancies in men [Harsha et al., 2020; Gangane et al., 2024]. Despite various treatment strategies, it is associated with unfavorable prognosis and diminished survival outcomes in patients. This necessitates the identification of novel therapeutic target for the management of OC. Hence, our study focused on establishing the role of Mortalin in OC, and investigating the potential of WiA as Mortalin inhibitor that would help in suppressing OC hallmarks and better management of OC.

In the current study, we have investigated the potential of Mortalin as a novel molecular target for OC. Firstly, we have determined the expression of Mortalin in different OC tissues and cell lines. Here, we observed that Mortalin was overexpressed in OC tissues and cell lines. The over expression of Mortalin was also observed in different subgroups of OC patients such as stage, grade, tumor size, and lymph nodes. In addition, this observation was also accompanied by the upregulated expression of Mortalin in different pathological conditions of oral carcinogenesis except in hyperplasic samples. Moreover, OC samples from different tissues of origin of the oral cavity was examined, and found that anatomic sites like UJ, tongue, gingiva and maxilla had higher expression of Mortalin compared to other sites. Similar to our investigations, studies reported the overexpression of Mortalin in breast cancer, pancreatic cancer, cholangiocarcinoma, colorectal, gastric cancer, hepatocellular carcinoma, lung cancer, etc. and was found to be linked with the histological grade, clinical stage, and nodal metastasis [Chen et al., 2014; Jin et al., 2016; Cui et al., 2017; Xu et al., 2020; Ando et al., 2014; Kang et al., 2017; Sun et al., 2017]. In addition, our study showed that overexpression of Mortalin affects the OS outcomes in OC patients. This overexpression of Mortalin also resulted in poor OS in patients having enriched mesenchymal stem cell markers, as well as patients with different grades and stages (except stage III). Parallel to our observations, previous studies have also shown that upregulated Mortalin leads to the compromised OS outcomes in patients affected with breast, colorectal, gastric, liver, lung and pancreatic cancers [Jin et al., 2016; Cui et al., 2017; Xu et al., 2020; Ando et al., 2014; Kang et al., 2017; Sun et al., 2017]. In addition, studies on breast cancer, osteosarcoma and melanoma cells showed that Mortalin overexpression promoted EMT and metastasis by upregulating various mesenchymal markers such as vimentin (VIM), β-catenin (CTNNB1), fibronectin (FN1), CK14 (KRT14), hnRNP-K and MMPs (-2.-3 and -9), while downregulating the expression of epithelial marker E-cadherin [Na et al., 2016]. This might explain the decrease OS in Mortalin overexpression group having enriched mesenchymal stem cell markers, compared to patients with decreased mesenchymal stem cell markers. In addition, overexpression of Mortalin in higher histological grades and stages might explain

the possibility of Mortalin in inducing the aggressive, invasive and metastatic ability in OC.

Secondly, in our study, we have explored the role of Mortalin in regulating multiple hallmarks of OC and its impact on the modulation of proteins and signaling pathways associated with these processes. We found that knockdown of Mortalin suppressed the survival and proliferation of OC cells, along with the suppression of other hallmarks such as EMT, invasion, angiogenesis and migration. Conversely, knockdown of Mortalin in normal HaCaT cells did not show much inhibition in its cell proliferation as compared to OC cells. In addition, knockdown of Mortalin resulted in S-phase cell cycle arrest in OC cells. Mechanistically, the regulation of Mortalin knockdown on these processes was associated with the induction of tumor suppressor p53, CDK inhibitors (p-wee1, p27, p21, and p18), and epithelial marker E-cadherin; while on the other hand suppressing the expression of cyclins (B1, D1, and E2), N-cadherin, Snail, vimentin, VEGF-A, CXCR-4 and MMPs (-2 and -9) in OC cells. Similarly, knockdown of Mortalin in breast cancer cell lines (SK-BR-3 and MCF-7), cholangiocarcinoma (ICC cells) and hepatoma cells (MHCC97H) demonstrated inhibitory effect on the proliferation, EMT, invasion, angiogenesis, metastasis and cancer progression by inducing the expression of zonula occludens (ZO-1) and E-cadherin while reducing the expression of MMP-2, VEGF, Vimentin, Twist, Snail, Slug, C-Myc, β-Catenin and Cyclin-D1 [Chen et al., 2014; Kang et al., 2017; Zhang et al., 2021]. Additionally, a study on ovarian cancer cells showed that knockdown of Mortalin inhibited cell proliferation and colony forming ability along with suppression in invasion and migration by significantly modulating the expression of Cyclin-D1, Cyclin-B1 and C-Myc [Hu et al., 2016]. Importantly correlating to our study, Lu et al., demonstrated a noteworthy observation wherein the knockdown of Mortalin in HCC cells resulted in a specific repression of cellular proliferation in cancer cells while exerting minimal inhibition on normal cell proliferation. This study showed that Mortalin knockdown disrupted the Mortalin-p53 interaction within the cancer cells, consequently activating p53 and its transcriptional function, thereby causing growth arrest and cell death. Conversely, this interaction was not observed in normal cells due to the absence of co-localization between these two proteins, hence, the suppression of Mortalin did not substantially affect the proliferation of normal cells [Lu et al., 2011]. Moreover, our study found that the knockdown of Mortalin induced cell death, apoptosis and autophagy in OC cells. This effect of Mortalin knockdown on different processes of cell death in OC cells was accompanied by the suppression of Bcl-2 and Survivin alongside elevating the levels of caspases-3 and -9. Further, the knockdown of Mortalin in these cells was associated with the upregulated expression of two critical proteins involved in autophagy such as LC3B and p62 (SQSTM1). Similarly, a study on HCC cells showed that shRNA-mediated inactivation of Mortalin triggered apoptosis through the activation of p53 and

increased expression of cleaved caspase-3 [Lu et al., 2011]. In another study, depletion of Mortalin was found to induce cell death in preclinical experimental settings, accompanied by the cleavage of PARP and increased expression of TP53, along with the modulation of rearranged during transfection (RET) and cell cycle and apoptosis regulators such as p21CIP1, p27KIP1, E2F-1, and Bcl-2 family proteins in thyroid cancer cells [Starenki et al., 2015]. In addition, a study by Abunimer et al., demonstrated that depletion of Mortalin led to the induction of autophagy in drug resistant breast cancer cells through the formation of mitochondrial autophagosomes and modulated expression of LC3B and p62 [Abunimer et al., 2018]. Extending our findings, we also observed that knockdown of Mortalin regulated the Akt/mTOR signaling via suppression of p-Akt, p-mTOR and p-S6 in OC cells. Akt/mTOR is one of the major pathways that is recognized as a key regulator of oncogenesis in OC, contributing to various hallmarks of this disease such as survival, proliferation, angiogenesis, migration and invasion [Roy et al., 2019; Harsha et al., 2020].

Thus, concluding from the second and third chapters, we found that Mortalin is associated with the development and progression of OC potentially by regulating Akt/mTOR signaling and various proteins involved in promoting the oncogenic hallmarks in OC. Therefore, Mortalin can be used as a novel molecular target for OC. These observations robustly provided a foundation for developing therapeutic strategies targeting Mortalin in OC. Furthermore, the identification of the compound WiA, which have high affinity with Mortalin as reported from previous studies, prompted an investigation into its potential as an inhibitor of Mortalin and its driven oncogenesis.

Therefore, in the fourth chapter, we have examined the role WiA in suppressing Mortalin and attenuating OC hallmarks, exploring its potential as a candidate for drug development for OC. We have found that WiA significantly suppressed the expression of Mortalin in a concentration-dependent manner. In addition, WiA effectively suppressed the proliferation and colony forming ability in OC cells, subsequently initiating G2/M phase cell cycle arrest. The suppressive effect of WiA on OC cell proliferation was noted with an IC_{50} of approximately 0.35 μM, while minimal inhibition of proliferation was observed in normal cells, with IC_{50} values remaining undetermined up to the highest tested dose of 1 μM used for this assay, suggesting the cancer specific inhibitory effect and the safety profile of this compound against the normal cells. Moreover, the inhibition of survival and proliferation by WiA was associated with augmented expression of tumor p53 and cell cycle checkpoint and CDK inhibitors such as p-wee1, p27, p21 and p18 in OC cells. Moreover, the treatment of WiA was found to trigger various forms of cell death, including necrosis, apoptosis, and autophagy in these cells, accompanied by the upregulation of caspases-3 and -9, LC3B and p62 (SQSTM1), and downregulation of COX-2, Bcl-2 and Survivin. Further, WiA was found to

suppress other hallmarks such as EMT, invasion, angiogenesis and migration of OC cells which was substantiated by an elevated expression of E-cadherin and repressed expression of N-cadherin, VEGF-A, MMP-2 and MM-9 in OC cells. Furthermore, WiA treatment suppressed the expression of Akt/mTOR signaling proteins such as p-Akt, p-mTOR and p-S6 in OC cells, suggesting the potential of this compound. Similarly, various cancer cell types including cervical carcinoma, glioblastoma, colorectal, and lung cancer has demonstrated that WiA induces apoptosis and suppresses cell viability, proliferation, motility, invasion, and migration through the upregulation of p53, Bim, Bax, Bad, and E-cadherin expression while downregulating the expression levels of p-STAT-3, Bcl-2, Bcl-xL, MMP-9, and vimentin [Lee et al., 2013; Choi et al., 2015; Tang et al., 2020; Sari et al., 2020; Lin et al., 2021]. In addition, WiA has been shown to halt cell cycle progression at the G2/M transition in different cancer cell types such as glioblastoma, osteosarcoma, breast, and gastric cancer mediated by the inhibition of cell cycle regulatory proteins, such as cyclin A, cyclin B1, Cdk1, Cdk2, p-Cdc2 (Tyr15), Cdc25C, and Cdc25B, coupled with the elevation of p21, p-Chk1 (Ser345), and p-Chk2 (Thr68) levels [Stan et al., 2008; Lv et al., 2015; Kyakulaga et al., 2018; Tang et al., 2020]. Moreover, WiA was found to suppress EMT in lung cancer cells by reducing vimentin expression and increasing E-cadherin levels [Wang et al., 2018]. Additionally, few studies have also shown the strong binding affinity of six hydrogen bonds of WiA with four specific amino acid residues on Mortalin, exhibiting binding energy of -9.8 kcal/mol. This binding ability might disrupt the interaction of Mortalin with its former binding partner p53, leading to p53 activation and induce cell growth arrest and cell death in cancer cells. Moreover, disruption of p53-Mortalin complex by WiA also resulted in inhibition of Bcl-2, Bcl-xL, pro PARP, and procaspase 3, while elevating the expression of Bax and cleaved PARP-1 [Vaishnavi et al., 2012; Huang et al., 2015; Behl et al., 2020]. Thus, this indicates the potential efficacy of WiA in targeting Mortalin in OC.

Considering the therapeutic efficacy of WiA, it is imperative to understand its safety and toxicity across diverse experimental models. In accordance with this, Gupta et al. performed a study to evaluate the acute and sub-acute toxicological effects of WiA in mice. The study revealed that WiA doses up to 2000 mg/kg were well tolerated, and repeated sub-acute doses of 10, 70, and 500 mg/kg showed no indications of toxicity at any dosage level. However, WiA was shown to be less bioavailable at a rate of 1.8% [Gupta et al., 2022]. Similarly, another study by Patel showed similar results where acute dose of 2000 mg/kg ashwagandha extract in mice model was considered to be safe and no adverse side effects were noted [Patel et al., 2016]. In addition, in another study, WiA at 10 mg/kg oral administration in rat was found to exhibit a half-life of 7.6 ± 3.3 h, bioavailability of 32.4 ± 4.8% with the C_{max} of 619 ± 125 ng/mL and T_{max} of 0.11 ± 0.07 h [Dai et al., 2019]. Further, in a phase I dose escalation study with

ashwagandha root extract (400 mg AshwaMAX capsule containing 18 mg of WiA) in advanced stage high-grade osteosarcoma daily divided dose of 72, 108, 144 and 216 mg of WiA was given to patients, which was found to be well tolerated. However, the oral bioavailability remains low [Pires et al., 2020]. Dai T et al., proposed that the first-pass metabolism of WA might contribute to its low bioavailability [Dai et al., 2019]. Therefore, monitoring the metabolism might be helpful in regulating the bioavailability of this compound. Thus, WiA could be considered as safe agent with no adverse toxicity being observed even at higher doses.

Based on our observations, WiA demonstrated as a promising candidate for OC management. The inhibitory concentrations identified in our study are in the nanomolar range, and considering the safety and the absence of toxicity of WiA even at high doses as reported by other studies previously, the concerns regarding its bioavailability are alleviated. Therefore, WiA emerges as a potential compound for the treatment and management of OC.

5.2 Limitations and future directions

Though the present study advanced our understanding on the role of Mortalin and its mechanistic pathways in OC, it also has certain limitations. Firstly, although expression analysis was conducted on patient samples collected from NECHRI, we could not investigate the OS outcomes of these patients. Additionally, examining Mortalin expression in OC tissues from various anatomical locations within the oral cavity and also correlating Mortalin expression with different mutational markers (such as TP53 mutations, PTEN mutations, HRAS mutations, etc.) could have provided further insights. Moreover, the samples collected from the Northeast Indian region may not adequately represent India's diverse ethnic groups and regional variations. Expanding the sample pool to include regions across India could offer a more comprehensive understanding on the role of Mortalin in OC across diverse populations, particularly considering the high prevalence of OC incidences. Even though we have shown the silencing of Mortalin inhibited OC cell survival, proliferation, EMT, invasion, migration, etc., in OC cell lines, these results must be validated through in vivo studies. In addition, investigating the regulatory role of Mortalin in inducing chemoresistance and radioresistance pathways would enhance our understanding of its involvement in OC progression. Moreover, this study involved the use of two OC cell lines, future research should consider employing a broader range of cell lines to strengthen the evidence supporting the role of Mortalin in OC. Furthermore, this study has focused on evaluating the efficacy of WiA as a potential agent using OC cell lines; however, in vivo validation of the results is imperative prior to clinical trials.

Agrawal A, Shindell E, Jordan F, Baeva L, Pfefer J, Godar DE. UV radiation increases carcinogenic risks for oral tissues compared to skin. Photochem Photobiol. 2013 Sep-Oct;89(5):1193-8. doi: 10.1111/php.12140. Epub 2013 Aug 17. PMID: 23855371.

Aguas SC, Quarracino MC, Lence AN, Lanfranchi-Tizeira HE. Primary melanoma of the oral cavity: ten cases and review of 177 cases from literature. Med Oral Patol Oral Cir Bucal. 2009 Jun 1;14(6):E265-71. PMID: 19300378.

Aksoy S, Orhan K, Kursun S, Kolsuz ME, Celikten B. Metastasis of prostate carcinoma in the mandible manifesting as numb chin syndrome. World J Surg Oncol. 2014 Dec 29;12:401. doi: 10.1186/1477-7819-12-401. PMID: 25547947; PMCID: PMC4326431.

Algazi AP, Grandis JR. Head and neck cancer in 2016: A watershed year for improvements in treatment? Nat Rev Clin Oncol. 2017 Feb;14(2):76-78. doi: 10.1038/nrclinonc.2016.196. Epub 2016 Dec 6. PMID: 27922045.

Ali S, El-Rayes BF, Heilbrun LK, Sarkar FH, Ensley JF, Kucuk O, Philip PA. Cytochrome p450 and glutathione transferase expression in squamous cell cancer. Clin Cancer Res. 2004 Jul 1;10(13):4412-6. doi: 10.1158/1078-0432.CCR-04-0145. PMID: 15240530.

Aljohani AI, Toss MS, Green AR, Rakha EA. The clinical significance of cyclin B1 (CCNB1) in invasive breast cancer with emphasis on its contribution to lymphovascular invasion development. Breast Cancer Res Treat. 2023 Apr;198(3):423-435. doi: 10.1007/s10549-022-06801-2. Epub 2022 Nov 22. PMID: 36418517; PMCID: PMC10036284.

Al-Zaher N, Obeid A, Al-Salam S, Al-Kayyali BS. Acinic cell carcinoma of the salivary glands: a literature review. Hematol Oncol Stem Cell Ther. 2009;2(1):259-64. doi: 10.1016/s1658-3876(09)50035-0. PMID: 20063555.

Amick J, Schlanger SE, Wachnowsky C, Moseng MA, Emerson CC, Dare M, Luo WI, Ithychanda SS, Nix JC, Cowan JA, Page RC, Misra S. Crystal structure of the nucleotide-binding domain of mortalin, the mitochondrial Hsp70 chaperone. Protein Sci. 2014 Jun;23(6):833-42. doi: 10.1002/pro.2466. Epub 2014 Apr 17. PMID: 24687350; PMCID: PMC4093958.

Ando K, Oki E, Zhao Y, Ikawa-Yoshida A, Kitao H, Saeki H, Kimura Y, Ida S, Morita M, Kusumoto T, Maehara Y. Mortalin is a prognostic factor of gastric cancer with normal p53 function. Gastric Cancer. 2014 Apr;17(2):255-62. doi: 10.1007/s10120-013-0279-1. Epub 2013 Jul 5. PMID: 23828548.

Arakeri G, Brennan PA. Oral submucous fibrosis: an overview of the aetiology, pathogenesis, classification, and principles of management. Br J Oral Maxillofac Surg. 2013 Oct;51(7):587-93. doi: 10.1016/j.bjoms.2012.08.014. Epub 2012 Oct 26. PMID: 23107623.

Argiris A, Karamouzis MV, Raben D, Ferris RL. Head and neck cancer. Lancet. 2008 May 17;371(9625):1695-709. doi: 10.1016/S0140-6736(08)60728-X. PMID: 18486742; PMCID: PMC7720415.

Armstrong LTD, Subramaniam SS, Borgna S. Adenoid cystic carcinoma of the palate with isolated cutaneous metastasis: a unique case and review of current and future treatment modalities of metastatic disease. Int J Oral Maxillofac Surg. 2020 Jun;49(6):700-708. doi: 10.1016/j.ijom.2019.11.012. Epub 2019 Dec 28. PMID: 31892444.

Arya S, Rane P, Deshmukh A. Oral cavity squamous cell carcinoma: role of pretreatment imaging and its influence on management. Clin Radiol. 2014 Sep;69(9):916-30. doi: 10.1016/j.crad.2014.04.013. Epub 2014 Jun 5. PMID: 24908285.

Baillie R, Tan ST, Itinteang T. Cancer Stem Cells in Oral Cavity Squamous Cell Carcinoma: A Review. Front Oncol. 2017 Jun 2;7:112. doi: 10.3389/fonc.2017.00112. PMID: 28626726; PMCID: PMC5454033.

Barve S, Chen SY, Kirpich I, Watson WH, Mcclain C. Development, Prevention, and Treatment of Alcohol-Induced Organ Injury: The Role of Nutrition. Alcohol Res. 2017;38(2):289-302. PMID: 28988580; PMCID: PMC5513692.

Baseler WA, Croston TL, Hollander JM. Functional characteristics of mortalin. Mortalin Biology: Life, Stress and Death. 2012:55-80.

Behl T, Sharma A, Sharma L, Sehgal A, Zengin G, Brata R, Fratila O, Bungau S. Exploring the Multifaceted Therapeutic Potential of Withaferin A and Its Derivatives. Biomedicines. 2020 Dec 6;8(12):571. doi: 10.3390/biomedicines8120571. PMID: 33291236; PMCID: PMC7762146.

Beyerle J, Holowatyj AN, Haffa M, Frei E, Gigic B, Schrotz-King P, Boehm J, Habermann N, Stiborova M, Scherer D, Kölsch T, Skender S, Becker N, Herpel E, Schneider M, Ulrich A, Schirmacher P, Chang-Claude J, Brenner H, Hoffmeister M, Haug U, Owen RW, Ulrich CM. Expression Patterns of Xenobiotic-Metabolizing Enzymes in Tumor and Adjacent Normal Mucosa Tissues among Patients with Colorectal Cancer: The ColoCare Study. Cancer Epidemiol Biomarkers Prev. 2020 Feb;29(2):460-469. doi: 10.1158/1055-9965.EPI-19-0449. Epub 2019 Nov 18. PMID: 31740522; PMCID: PMC7007332.

Bordoloi D, Banik K, Vikkurthi R, Thakur KK, Padmavathi G, Sailo BL, Girisa S, Chinnathambi A, Alahmadi TA, Alharbi SA, Buhrmann C, Shakibaei M, Kunnumakkara AB. Inflection of Akt/mTOR/STAT-3 cascade in TNF-α induced protein 8 mediated human lung carcinogenesis. Life Sci. 2020 Dec 1;262:118475. doi: 10.1016/j.lfs.2020.118475. Epub 2020 Sep 22. PMID: 32976884.

Bordoloi D, Monisha J, Roy NK, Padmavathi G, Banik K, Harsha C, Wang H, Kumar AP, Arfuso F, Kunnumakkara AB. An Investigation on the Therapeutic Potential of Butein, ATretrahydroxychalcone Against Human Oral Squamous Cell Carcinoma. Asian Pac J Cancer Prev. 2019 Nov 1;20(11):3437-3446. doi: 10.31557/APJCP.2019.20.11.3437. PubMed PMID: 31759370.

Buch SC, Nazar-Stewart V, Weissfeld JL, Romkes M. Case-control study of oral and oropharyngeal cancer in whites and genetic variation in eight metabolic enzymes. Head Neck. 2008 Sep;30(9):1139-47. doi: 10.1002/hed.20867. PMID: 18642288; PMCID: PMC3627181.

Burstein HJ, Elias AD, Rugo HS, Cobleigh MA, Wolff AC, Eisenberg PD, Lehman M, Adams BJ, Bello CL, DePrimo SE, Baum CM, Miller KD. Phase II study of sunitinib malate, an oral multitargeted tyrosine kinase inhibitor, in patients with metastatic breast cancer previously treated with an anthracycline and a taxane. J Clin Oncol. 2008 Apr 10;26(11):1810-6. doi: 10.1200/JCO.2007.14.5375. Epub 2008 Mar 17. PMID: 18347007.

Butler C, Dunmire M, Choi J, Szalai G, Johnson A, Lei W, Chen X, Liu L, Li W, Walter MJ, Liu T. HSPA9/mortalin inhibition disrupts erythroid maturation through a TP53-dependent mechanism in human CD34+ hematopoietic progenitor cells. Cell Stress Chaperones. 2024 Apr;29(2):300-311. doi: 10.1016/j.cstres.2024.03.006. Epub 2024 Mar 19. PMID: 38508444; PMCID: PMC10998001.

Cardoso IL, Leal F, Santos DR, Azevedo WS. Biochemical mechanisms of the effect of environmental factors (diet, fluorine, alcohol and tobacco) on oral health. European Journal of Biomedical and Pharmaceutical Sciences. 2020;7:52-63.

Carrington C. Oral targeted therapy for cancer. Aust Prescr. 2015 Oct;38(5):171-6. Epub 2015 Oct 1. Review. PubMed PMID: 26648656; PubMed Central PMCID: PMC4657306.

Cavaliere M, De Luca P, Scarpa A, Savignano L, Cassandro C, Cassandro E, Iemma M. Acinic cell carcinoma of the parotid gland: from pathogenesis to management: a literature review. Eur Arch Otorhinolaryngol. 2020 Oct;277(10):2673-2679. doi: 10.1007/s00405-020-05998-6. Epub 2020 May 4. PMID: 32367151.

Chandra S, Sah K, Bagewadi A, Keluskar V, Shetty A, Ammanagi R, Naik Z. Additive and synergistic effect of phytochemicals in prevention of oral cancer. European Journal of General Dentistry. 2012 Sep;1(03):142-7.

Chang LY, Wan HC, Lai YL, Kuo YF, Liu TY, Chen YT, Hung SL. Areca nut extracts increased expression of inflammatory cytokines, tumor necrosis factor-alpha, interleukin-1beta, interleukin-6 and interleukin-8, in peripheral blood mononuclear cells. J Periodontal Res. 2009 Apr;44(2):175-83. doi: 10.1111/j.1600-0765.2008.01104.x. Epub 2008 Oct 7. PMID: 18973534.

Chang YL, Lin CF, Ho NH. Treatment of Adenoid Cystic Carcinoma of the Palate and Reconstruction of Defect With Nasoseptal Flap: A Clinical Case and Review of Literature. Ear Nose Throat J. 2022 Nov 14:1455613221139407. doi: 10.1177/01455613221139407. Epub ahead of print. PMID: 36374156.

Chen J, Liu WB, Jia WD, Xu GL, Ma JL, Huang M, Deng YR, Li JS. Overexpression of Mortalin in hepatocellular carcinoma and its relationship with angiogenesis and epithelial to mesenchymal transition. Int J Oncol. 2014 Jan;44(1):247-55. doi: 10.3892/ijo.2013.2161. Epub 2013 Nov 1. PMID: 24190572.

Chen S, Lin Z, Chen J, Yang A, Zhang Q, Xie C, Zhang X, Yang Z, Chen W, Song M. Older age is a risk factor associated with poor prognosis of patients with squamous cell carcinoma of the oral cavity. Eur Arch Otorhinolaryngol. 2020 Sep;277(9):2573-2580. doi: 10.1007/s00405-020-05963-3. Epub 2020 Apr 22. PMID: 32322960; PMCID: PMC7410825.

Choi BY, Kim BW. Withaferin-A Inhibits Colon Cancer Cell Growth by Blocking STAT3 Transcriptional Activity. J Cancer Prev. 2015 Sep;20(3):185-92. doi: 10.15430/JCP.2015.20.3.185. PMID: 26473157; PMCID: PMC4597807.

Conway DI, Petticrew M, Marlborough H, Berthiller J, Hashibe M, Macpherson LM. Socioeconomic inequalities and oral cancer risk: a systematic review and meta-analysis of case-control studies. Int J Cancer. 2008 Jun 15;122(12):2811-9. doi: 10.1002/ijc.23430. PMID: 18351646.

Crabb DW, Liangpunsakul S. Acetaldehyde generating enzyme systems: roles of alcohol dehydrogenase, CYP2E1 and catalase, and speculations on the role of other enzymes and processes. Novartis Found Symp. 2007;285:4-16; discussion 16-22, 198-9. doi: 10.1002/9780470511848.ch2. PMID: 17590984.

Cretu B, Zamfir A, Bucurica S, Scheau AE, Savulescu Fiedler I, Caruntu C, Caruntu A, Scheau C. Role of Cannabinoids in Oral Cancer. Int J Mol Sci. 2024 Jan 12;25(2):969. doi: 10.3390/ijms25020969. PMID: 38256042; PMCID: PMC10815457.

Cui X, Li Z, Piao J, Li J, Li L, Lin Z, Jin A. Mortalin expression in pancreatic cancer and its clinical and prognostic significance. Hum Pathol. 2017 Jun;64:171-178. doi: 10.1016/j.humpath.2017.03.015. Epub 2017 Apr 12. PMID: 28412209.

Dai F, Dai L, Zheng X, Guo Y, Zhang Y, Niu M, Lu Y, Li H, Hou R, Zhang Y, Wen S, Hu W, An C, Wu Y, Gao W. Non-coding RNAs in drug resistance of head and neck cancers: A review. Biomed Pharmacother. 2020 Jul;127:110231. doi: 10.1016/j.biopha.2020.110231. Epub 2020 May 16. PMID: 32428836.

Dai T, Jiang W, Guo Z, Wang Z, Huang M, Zhong G, Liang C, Pei X, Dai R. Studies on oral bioavailability and first-pass metabolism of withaferin A in rats using LC-MS/MS and Q-TRAP. Biomed Chromatogr. 2019 Sep;33(9):e4573. doi: 10.1002/bmc.4573. Epub 2019 May 26. PMID: 31062367.

Dai T, Jiang W, Guo Z, Wang Z, Huang M, Zhong G, Liang C, Pei X, Dai R. Studies on oral bioavailability and first-pass metabolism of withaferin A in rats using LC-MS/MS and Q-TRAP. Biomed Chromatogr. 2019 Sep;33(9):e4573. doi: 10.1002/bmc.4573. Epub 2019 May 26. PMID: 31062367.

Dai Y, Li F, Jiao Y, Wang G, Zhan T, Xia Y, Liu H, Yang H, Zhang J, Tang L. Mortalin/glucose-regulated protein 75 promotes the cisplatin-resistance of gastric cancer via regulating anti-oxidation/apoptosis and metabolic reprogramming. Cell Death Discov. 2021 Jun 11;7(1):140. doi: 10.1038/s41420-021-00517-w. PMID: 34117210; PMCID: PMC8196146.

Das G, Shravage BV, Baehrecke EH. Regulation and function of autophagy during cell survival and cell death. Cold Spring Harb Perspect Biol. 2012 Jun 1;4(6):a008813. doi: 10.1101/cshperspect.a008813. PMID: 22661635; PMCID: PMC3367545.

Das H, Motghare S. India as "The Oral Cancer Capital of the World": The Rising Burden of Oral Malignancies across the Nation. International Journal of Science and Healthcare Research. May 2021; 6(2):99-107. April-June 2021 DOI: 10.52403/ijshr.20210419

Debela DT, Muzazu SG, Heraro KD, Ndalama MT, Mesele BW, Haile DC, Kitui SK, Manyazewal T. New approaches and procedures for cancer treatment: Current perspectives. SAGE Open Med. 2021 Aug 12;9:20503121211034366. doi: 10.1177/20503121211034366. PMID: 34408877; PMCID: PMC8366192.

Debela DT, Muzazu SG, Heraro KD, Ndalama MT, Mesele BW, Haile DC, Kitui SK, Manyazewal T. New approaches and procedures for cancer treatment: Current perspectives. SAGE Open Med. 2021 Aug 12;9:20503121211034366. doi: 10.1177/20503121211034366. PMID: 34408877; PMCID: PMC8366192.

Deocaris CC, Kaul SC, Wadhwa R. On the brotherhood of the mitochondrial chaperones mortalin and heat shock protein 60. Cell Stress Chaperones. 2006 Summer;11(2):116-28. doi: 10.1379/csc-144r.1. PMID: 16817317; PMCID: PMC1484513.

Deocaris CC, Lu WJ, Kaul SC, Wadhwa R. Druggability of mortalin for cancer and neuro-degenerative disorders. Curr Pharm Des. 2013;19(3):418-29. PMID: 22920904.

Devaraju R, Gantala R, Aitha H, Gotoor SG. Mucoepidermoid carcinoma. BMJ Case Rep. 2014 Aug 1;2014:bcr-2013-202776. doi: 10.1136/bcr-2013-202776. PMID: 25085946; PMCID: PMC4127757.

Dhanuthai K, Rojanawatsirivej S, Thosaporn W, Kintarak S, Subarnbhesaj A, Darling M, Kryshtalskyj E, Chiang CP, Shin HI, Choi SY, Lee SS, Aminishakib P. Oral cancer: A multicenter study. Med Oral Patol Oral Cir Bucal. 2018 Jan 1;23(1):e23-e29. doi: 10.4317/medoral.21999. PubMed PMID: 29274153; PubMed Central PMCID: PMC5822535.

Dhawan A. Adjunctive Therapy in Oral Cancer. Oral and Maxillofacial Surgery for the Clinician. 2021:1903-13.

D'Souza W, Saranath D. Clinical implications of epigenetic regulation in oral cancer. Oral Oncol. 2015 Dec;51(12):1061-8. doi: 10.1016/j.oraloncology.2015.09.006. Epub 2015 Oct 5. PMID: 26421863.

Dwivedi R, Pandey R, Chandra S, Mehrotra D. Apoptosis and genes involved in oral cancer - a comprehensive review. Oncol Rev. 2020 Jul 9;14(2):472. doi: 10.4081/oncol.2020.472. PMID: 32685111; PMCID: PMC7365992.

Edenberg HJ. The genetics of alcohol metabolism: role of alcohol dehydrogenase and aldehyde dehydrogenase variants. Alcohol Res Health. 2007;30(1):5-13. PMID: 17718394; PMCID: PMC3860432.

Elser C, Siu LL, Winquist E, Agulnik M, Pond GR, Chin SF, Francis P, Cheiken R, Elting J, McNabola A, Wilkie D. Phase II trial of sorafenib in patients with recurrent or metastatic squamous cell carcinoma of the head and neck or nasopharyngeal carcinoma. Journal of Clinical Oncology. 2007 Aug 20;25(24):3766-73.

Elwakeel A. Abrogating the Interaction Between p53 and Mortalin (Grp75/HSPA9/mtHsp70) for Cancer Therapy: The Story so far. Front Cell Dev Biol. 2022 Apr 14;10:879632. doi: 10.3389/fcell.2022.879632. PMID: 35493098; PMCID: PMC9047732.

Esfahanian N, Knoblich CD, Bowman GA, Rezvani K. Mortalin: Protein partners, biological impacts, pathological roles, and therapeutic opportunities. Front Cell Dev Biol. 2023 Feb 2;11:1028519. doi: 10.3389/fcell.2023.1028519. PMID: 36819105; PMCID: PMC9932541.

Fares J, Fares MY, Khachfe HH, Salhab HA, Fares Y. Molecular principles of metastasis: a hallmark of cancer revisited. Signal Transduct Target Ther. 2020 Mar 12;5(1):28. doi: 10.1038/s41392-020-0134-x. PMID: 32296047; PMCID: PMC7067809.

Feller L, Lemmer J. Oral Squamous Cell Carcinoma: Epidemiology, Clinical Presentation and Treatment,Journal of Cancer Therapy, 2012; 3: 263-268.

Fitzpatrick SG, Hirsch SA, Gordon SC. The malignant transformation of oral lichen planus and oral lichenoid lesions: a systematic review. J Am Dent Assoc. 2014 Jan;145(1):45-56. doi: 10.14219/jada.2013.10. PMID: 24379329.

Gangane NM, Ghongade PV, Patil BU, Atram M. Oral cavity cancer incidence and survival trends: A population-based study. J Cancer Res Ther. 2024 Jan 22. doi: 10.4103/jcrt.jcrt_2720_22. Epub ahead of print. PMID: 38261454.

Gangane NM, Ghongade PV, Patil BU, Atram M. Oral cavity cancer incidence and survival trends: A population-based study. J Cancer Res Ther. 2024 Jan 22. doi: 10.4103/jcrt.jcrt_2720_22. Epub ahead of print. PMID: 38261454.

Georgaki M, Theofilou VI, Pettas E, Stoufi E, Younis RH, Kolokotronis A, Sauk JJ, Nikitakis NG. Understanding the complex pathogenesis of oral cancer: A comprehensive review. Oral Surg Oral Med Oral Pathol Oral Radiol. 2021 Nov;132(5):566-579. doi: 10.1016/j.oooo.2021.04.004. Epub 2021 Apr 26. PMID: 34518141.

Ghantous Y, Abu Elnaaj I. Global incidence and risk factors of oral cancer. Harefuah. 2017 Oct;156(10):645-649. Hebrew. PMID: 29072384.

Giannini PJ, Shetty KV, Horan SL, Reid WD, Litchmore LL. Adenoid cystic carcinoma of the buccal vestibule: A case report and review of the literature. Oral Oncol. 2006 Nov;42(10):1029-32. doi: 10.1016/j.oraloncology.2006.06.005. Epub 2006 Sep 25. PMID: 16996786.

Goel A, Singla A, Prabhash K. Neoadjuvant chemotherapy in oral cancer: Current status and future possibilities. Cancer Research, Statistics, and Treatment. 2020 Jan 1;3(1):51-9.

Greene FL, Sobin LH. The staging of cancer: a retrospective and prospective appraisal. CA Cancer J Clin. 2008 May-Jun;58(3):180-90. doi: 10.3322/CA.2008.0001. PMID: 18460593.

Grover A, Priyandoko D, Gao R, Shandilya A, Widodo N, Bisaria VS, Kaul SC, Wadhwa R, Sundar D. Withanone binds to mortalin and abrogates mortalin-p53 complex: computational and experimental evidence. Int J Biochem Cell Biol. 2012 Mar;44(3):496-504. doi: 10.1016/j.biocel.2011.11.021. Epub 2011 Dec 2. PMID: 22155302.

Guha N, Warnakulasuriya S, Vlaanderen J, Straif K. Betel quid chewing and the risk of oral and oropharyngeal cancers: a meta-analysis with implications for cancer control. Int J Cancer. 2014 Sep 15;135(6):1433-43. doi: 10.1002/ijc.28643. Epub 2014 May 14. PMID: 24302487.

Guiglia R, Musciotto A, Compilato D, Procaccini M, Lo Russo L, Ciavarella D, Lo Muzio L, Cannone V, Pepe I, D'Angelo M, Campisi G. Aging and oral health: effects in hard and soft tissues. Curr Pharm Des. 2010;16(6):619-30. doi: 10.2174/138161210790883813. PMID: 20388072.

Gupta SK, Jadhav S, Gohil D, Panigrahi GC, Kaushal RK, Gandhi K, Patil A, Chavan P, Gota V. Safety, toxicity and pharmacokinetic assessment of oral Withaferin-A in mice. Toxicol Rep. 2022 May 18;9:1204-1212. doi: 10.1016/j.toxrep.2022.05.012. PMID: 36518386; PMCID: PMC9742883.

Gupta SK, Jadhav S, Gohil D, Panigrahi GC, Kaushal RK, Gandhi K, Patil A, Chavan P, Gota V. Safety, toxicity and pharmacokinetic assessment of oral Withaferin-A in mice. Toxicol Rep. 2022 May 18;9:1204-1212. doi: 10.1016/j.toxrep.2022.05.012. PMID: 36518386; PMCID: PMC9742883.

Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011 Mar 4;144(5):646-74. doi: 10.1016/j.cell.2011.02.013. PMID: 21376230.

Harsha C, Banik K, Ang HL, Girisa S, Vikkurthi R, Parama D, Rana V, Shabnam B, Khatoon E, Kumar AP, Kunnumakkara AB. Targeting AKT/mTOR in Oral Cancer: Mechanisms and Advances in Clinical Trials. Int J Mol Sci. 2020 May 6;21(9):3285. doi: 10.3390/ijms21093285. PMID: 32384682; PMCID: PMC7246494.

Hartner L. Chemotherapy for Oral Cancer. Dent Clin North Am. 2018 Jan;62(1):87-97. doi: 10.1016/j.cden.2017.08.006. Epub 2017 Oct 12. PMID: 29126496.

Hashibe M, Ritz B, Le AD, Li G, Sankaranarayanan R, Zhang ZF. Radiotherapy for oral cancer as a risk factor for second primary cancers. Cancer Lett. 2005 Apr 8;220(2):185-95. doi: 10.1016/j.canlet.2004.10.023. PMID: 15766594.

Helen-Ng LC, Razak IA, Ghani WM, Marhazlinda J, Norain AT, Raja Jallaludin RL, Rahman ZA, Abdullah N, Zain RB. Dietary pattern and oral cancer risk--a factor analysis study. Community Dent Oral Epidemiol. 2012 Dec;40(6):560-6. doi: 10.1111/j.1600-0528.2012.00704.x. Epub 2012 Jun 9. PMID: 22679921.

Hosseinpour S, Mashhadiabbas F, Ahsaie MG. Diagnostic Biomarkers in Oral Verrucous Carcinoma: A Systematic Review. Pathol Oncol Res. 2017 Jan;23(1):19-32. doi: 10.1007/s12253-016-0150-x. Epub 2016 Dec 6. PMID: 27924463.

Hsu JH, Chang PM, Cheng TS, Kuo YL, Wu AT, Tran TH, Yang YH, Chen JM, Tsai YC, Chu YS, Huang TH, Huang CF, Lai JM. Identification of Withaferin A as a Potential Candidate for Anti-Cancer Therapy in Non-Small Cell Lung Cancer. Cancers (Basel). 2019 Jul 17;11(7):1003. doi: 10.3390/cancers11071003. PMID: 31319622; PMCID: PMC6678286.

Hu Y, Yang L, Yang Y, Han Y, Wang Y, Liu W, Zuo J. Oncogenic role of mortalin contributes to ovarian tumorigenesis by activating the MAPK-ERK pathway. J Cell Mol Med. 2016 Nov;20(11):2111-2121. doi: 10.1111/jcmm.12905. Epub 2016 Jul 4. PMID: 27374312; PMCID: PMC5082394.

Huang C, Vaishnavi K, Kalra R, Zhang Z, Sekar K, Kaul SC, Wadhwa R. 3beta-methoxy derivation of Withaferin-a attenuates its anticancer potency: bioinformatics and molecular evidences. Med Aromat Plants. 2015;4(219):2167-0412.

Huang SH, O'Sullivan B. Oral cancer: Current role of radiotherapy and chemotherapy. Med Oral Patol Oral Cir Bucal. 2013 Mar 1;18(2):e233-40. Review. PubMed PMID: 23385513; PubMed Central PMCID: PMC3613874.

Huang SH, O'Sullivan B. Oral cancer: Current role of radiotherapy and chemotherapy. Med Oral Patol Oral Cir Bucal. 2013 Mar 1;18(2):e233-40. Review. PubMed PMID: 23385513; PubMed Central PMCID: PMC3613874.

Hudgins PA, Beitler JJ. Introduction to the imaging and staging of cancer. Neuroimaging Clin N Am. 2013 Feb;23(1):1-7. doi: 10.1016/j.nic.2012.08.003. PMID: 23199658.

Hwang HJ, Kim YK. The role of LC3B in autophagy as an RNA-binding protein. Autophagy. 2023 Mar;19(3):1028-1030. doi: 10.1080/15548627.2022.2111083. Epub 2022 Aug 13. PMID: 35968566; PMCID: PMC9980594.

Ilayaraja V, Prasad H, Anuthama K, Sruthi R. Acinic cell carcinoma of minor salivary gland showing features of high-grade transformation. J Oral Maxillofac Pathol. 2014 Jan;18(1):97-101. doi: 10.4103/0973-029X.131925. PMID: 24959046; PMCID: PMC4065458

Irani S. New Insights into Oral Cancer-Risk Factors and Prevention: A Review of Literature. Int J Prev Med. 2020 Dec 30;11:202. doi: 10.4103/ijpvm.IJPVM_403_18. PMID: 33815726; PMCID: PMC8000242.

Islam S, Abiko Y, Uehara O, Chiba I. Sirtuin 1 and oral cancer. Oncol Lett. 2019 Jan;17(1):729-738. doi: 10.3892/ol.2018.9722. Epub 2018 Nov 16. Review. PubMed PMID: 30655824; PubMed Central PMCID: PMC6313032.

Ismail SB, Kumar SK, Zain RB. Oral lichen planus and lichenoid reactions: etiopathogenesis, diagnosis, management and malignant transformation. J Oral Sci. 2007 Jun;49(2):89-106. doi: 10.2334/josnusd.49.89. PMID: 17634721.

Janbaz KH, Qadir MI, Basser HT, Bokhari TH, Ahmad B. Risk for oral cancer from smokeless tobacco. Contemp Oncol (Pozn). 2014;18(3):160-4. doi: 10.5114/wo.2014.40524. Epub 2014 Feb 10. PMID: 25520574; PMCID: PMC4268996.

Jayanthi P, Varun BR, Selvaraj J. Epithelial-mesenchymal transition in oral squamous cell carcinoma: An insight into molecular mechanisms and clinical implications. J Oral Maxillofac Pathol. 2020 Jan-Apr;24(1):189. doi: 10.4103/jomfp.JOMFP_334_19. Epub 2020 May 8. PMID: 32508481; PMCID: PMC7269282.

Jeng JH, Chang MC, Hahn LJ. Role of areca nut in betel quid-associated chemical carcinogenesis: current awareness and future perspectives. Oral Oncol. 2001 Sep;37(6):477-92. doi: 10.1016/s1368-8375(01)00003-3. PMID: 11435174.

Jiang Y, Zhang T, Kusumanchi P, Han S, Yang Z, Liangpunsakul S. Alcohol Metabolizing Enzymes, Microsomal Ethanol Oxidizing System, Cytochrome P450 2E1, Catalase, and Aldehyde Dehydrogenase in Alcohol-Associated Liver Disease. Biomedicines. 2020 Mar 4;8(3):50. doi: 10.3390/biomedicines8030050. PMID: 32143280; PMCID: PMC7148483.

Jin H, Ji M, Chen L, Liu Q, Che S, Xu M, Lin Z. The clinicopathological significance of Mortalin overexpression in invasive ductal carcinoma of breast. J Exp Clin Cancer Res. 2016 Mar 9;35:42. doi: 10.1186/s13046-016-0316-0. PMID: 26955804; PMCID: PMC4784366.

Johnson N. Tobacco use and oral cancer: a global perspective. J Dent Educ. 2001 Apr;65(4):328-39. PMID: 11336118.

Jones L, Ong EL, Okpokam A, Sloan P, Macleod I, Staines KS. Three cases of oral syphilis - an overview. Br Dent J. 2012 May 25;212(10):477-80. doi: 10.1038/sj.bdj.2012.420. PMID: 22627222.

Justesen MM, Stampe H, Jakobsen KK, Andersen AO, Jensen JM, Nielsen KJ, Gothelf AB, Wessel I, Christensen A, Grønhøj C, von Buchwald C. Impact of tumor subsite on survival outcomes in oral squamous cell carcinoma: A retrospective cohort study from 2000 to 2019. Oral Oncol. 2024 Feb;149:106684. doi: 10.1016/j.oraloncology.2024.106684. Epub 2024 Jan 10. PMID: 38211527.

Kademani D, Bagheri SC. 10 - Oral Cancer. Editor(s): Shahrokh C. Bagheri, Chris Jo, Clinical Review of Oral and Maxillofacial Surgery, Mosby, 2008, Pages 243-262, ISBN 9780323045742, doi: 10.1016/B978-0-323-04574-2.50015-6.

Kakabadze MZ, Paresishvili T, Karalashvili L, Chakhunashvili D, Kakabadze Z. Oral microbiota and oral cancer: Review. Oncol Rev. 2020 Jul 6;14(2):476. doi: 10.4081/oncol.2020.476. PMID: 32676172; PMCID: PMC7358985

Kang CJ, Chang JT, Chen TM, Chen IH, Liao CT. Surgical treatment of oral verrucous carcinoma. Chang Gung Med J. 2003 Nov;26(11):807-12. PMID: 14765750.

Kang Q, Cai JB, Dong RZ, Liu LX, Zhang C, Zhang PF, Zou H, Xie N, Zhang L, Zhang XY, Song ZJ, Dong ZR, Hu MY, Huang XY, Zhang XW, Ke AW, Shi GM. Mortalin promotes cell proliferation and epithelial mesenchymal transition of intrahepatic cholangiocarcinoma cells in vitro. J Clin Pathol. 2017 Aug;70(8):677-683. doi: 10.1136/jclinpath-2016-204251. Epub 2017 Jan 17. PMID: 28096273.

Kaul SC, Taira K, Pereira-Smith OM, Wadhwa R. Mortalin: present and prospective. Exp Gerontol. 2002 Oct-Nov;37(10-11):1157-64. doi: 10.1016/s0531-5565(02)00135-3. PMID: 12470827.

Khanna S, Udas AC, Kumar GK, Suvarna S, Karjodkar FR. Trace elements (copper, zinc, selenium and molybdenum) as markers in oral sub mucous fibrosis and oral squamous cell carcinoma. J Trace Elem Med Biol. 2013 Oct;27(4):307-11. doi: 10.1016/j.jtemb.2013.04.003. Epub 2013 Apr 12. PMID: 23664922.

Khatoon E, Banik K, Harsha C, Sailo BL, Thakur KK, Khwairakpam AD, Vikkurthi R, Devi TB, Gupta SC, Kunnumakkara AB. Phytochemicals in cancer cell chemosensitization: Current knowledge and future perspectives. Semin Cancer Biol.

2022 May;80:306-339. doi: 10.1016/j.semcancer.2020.06.014. Epub 2020 Jun 28. PMID: 32610149.

Kim ES, Yoon DY, Moon JY, Baek S, Han YM, Seo YL, Yun EJ. Detection of loco-regional recurrence in malignant head and neck tumors: a comparison of CT, MRI, and FDG PET-CT. Acta Radiol. 2019 Feb;60(2):186-195. doi: 10.1177/0284185118776504. Epub 2018 May 12. PMID: 29754496.

Kim G, Kim TH, Hwang EH, Chang KT, Hong JJ, Park JH. Withaferin A inhibits the proliferation of gastric cancer cells by inducing G2/M cell cycle arrest and apoptosis. Oncol Lett. 2017 Jul;14(1):416-422. doi: 10.3892/ol.2017.6169. Epub 2017 May 12. PMID: 28693185; PMCID: PMC5494816.

Kim M, Jang K, Miller P, Picon-Ruiz M, Yeasky TM, El-Ashry D, Slingerland JM. VEGFA links self-renewal and metastasis by inducing Sox2 to repress miR-452, driving Slug. Oncogene. 2017 Sep 7;36(36):5199-5211. doi: 10.1038/onc.2017.4. Epub 2017 May 15. PMID: 28504716; PMCID: PMC5596211.

Kirby AM, A'Hern RP, D'Ambrosio C, Tanay M, Syrigos KN, Rogers SJ, Box C, Eccles SA, Nutting CM, Harrington KJ. Gefitinib (ZD1839, Iressa) as palliative treatment in recurrent or metastatic head and neck cancer. Br J Cancer. 2006 Mar 13;94(5):631-6. doi: 10.1038/sj.bjc.6602999. PMID: 16495923; PMCID: PMC2361202.

Koopman G, Reutelingsperger CP, Kuijten GA, Keehnen RM, Pals ST, van Oers MH. Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. Blood. 1994 Sep 1;84(5):1415-20. PMID: 8068938.

Krishna A, Singh S, Kumar V, Pal US. Molecular concept in human oral cancer. Natl J Maxillofac Surg. 2015 Jan-Jun;6(1):9-15. doi: 10.4103/0975-5950.168235. PMID: 26668446; PMCID: PMC4668742.

Krishnan PA. Fungal infections of the oral mucosa. Indian J Dent Res. 2012 Sep-Oct;23(5):650-9. doi: 10.4103/0970-9290.107384. PMID: 23422613.

Kudpaje A, Gupta S, Naik S, Halkud R, Prashanth V, Nanjundappa A, Biswas S. Acinic cell carcinoma of the minor salivary glands of the tongue: A case study with review of literature. International Journal of Head and Neck Surgery. 2014 Aug 1;4(1):24-8.

Kumar G, Manjunatha B. Metastatic tumors to the jaws and oral cavity. J Oral Maxillofac Pathol. 2013 Jan;17(1):71-5. doi: 10.4103/0973-029X.110737. PMID: 23798834; PMCID: PMC3687193.

Kumar M, Nanavati R, Modi TG, Dobariya C. Oral cancer: Etiology and risk factors: A review. J Cancer Res Ther. 2016 Apr-Jun;12(2):458-63. doi: 10.4103/0973-1482.186696. PMID: 27461593.

Kumar SS, Zain RB. Aetiology and risk factors for oral cancer-A brief overview. Annals of Dentistry University of Malaya. 2004 Dec 31;11(1):41-50.

Kyakulaga AH, Aqil F, Munagala R, Gupta RC. Withaferin A inhibits Epithelial to Mesenchymal Transition in Non-Small Cell Lung Cancer Cells. Sci Rep. 2018 Oct

24;8(1):15737. doi: 10.1038/s41598-018-34018-1. PMID: 30356176; PMCID: PMC6200817.

Lambertini M, Patrizi A, Fanti PA, Melotti B, Caliceti U, Magnoni C, Misciali C, Baraldi C, Ravaioli GM, Dika E. Oral melanoma and other pigmentations: when to biopsy? J Eur Acad Dermatol Venereol. 2018 Feb;32(2):209-214. doi: 10.1111/jdv.14574. Epub 2017 Sep 14. PMID: 28862771.

Lang K, Baur M, Held T, Shafie RE, Moratin J, Freudlsperger C, Zaoui K, Bougatf N, Hoffmann J, Plinkert PK, Debus J, Adeberg S. Definitive radiotherapy for squamous cell carcinoma of the oral cavity: a single-institution experience. Radiol Oncol. 2021 Nov 19;55(4):467-473. doi: 10.2478/raon-2021-0041. PMID: 34821134; PMCID: PMC8647789.

Lee IC, Choi BY. Withaferin-A-A Natural Anticancer Agent with Pleitropic Mechanisms of Action. Int J Mol Sci. 2016 Mar 4;17(3):290. doi: 10.3390/ijms17030290. Review. PubMed PMID: 26959007; PubMed Central PMCID: PMC4813154.

Leemans CR, Braakhuis BJ, Brakenhoff RH. The molecular biology of head and neck cancer. Nat Rev Cancer. 2011 Jan;11(1):9-22. doi: 10.1038/nrc2982. Epub 2010 Dec 16. PMID: 21160525.

Li J, Sulman E, Aldape K. Molecular biology of brain tumors. Handb Clin Neurol. 2012;104:23-34. doi: 10.1016/B978-0-444-52138-5.00002-5. PMID: 22230432.

Li Q, Tie Y, Alu A, Ma X, Shi H. Targeted therapy for head and neck cancer: signaling pathways and clinical studies. Signal Transduct Target Ther. 2023 Jan 16;8(1):31. doi: 10.1038/s41392-022-01297-0. PMID: 36646686; PMCID: PMC9842704.

Li Z, Song W, Rubinstein M, Liu D. Recent updates in cancer immunotherapy: a comprehensive review and perspective of the 2018 China Cancer Immunotherapy Workshop in Beijing. J HematolOncol. 2018 Dec 21;11(1):142. doi: 10.1186/s13045-018-0684-3. Review. PubMed PMID: 30577797; PubMed Central PMCID: PMC6303854.

Liang CC, Park AY, Guan JL. In vitro scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro. Nat Protoc. 2007;2(2):329-33. doi: 10.1038/nprot.2007.30. PMID: 17406593.

Lin CC, Yang TY, Lu HJ, Wan CK, Hsu SL, Wu CC. Attenuating role of withaferin A in the proliferation and migration of lung cancer cells via a p53-miR-27a/miR-10b pathway. Oncol Lett. 2021 Mar;21(3):232. doi: 10.3892/ol.2021.12493. Epub 2021 Jan 26. PMID: 33613721; PMCID: PMC7856700.

Liu C, Wang M, Zhang H, Li C, Zhang T, Liu H, Zhu S, Chen J. Tumor microenvironment and immunotherapy of oral cancer. Eur J Med Res. 2022 Oct 8;27(1):198. doi: 10.1186/s40001-022-00835-4. PMID: 36209263; PMCID: PMC9547678.

Liu H, Yu Z, Xu Z, Liu T, Liu W. A scientometric study of tobacco and alcohol use as risk factors for oral cavity health. J Dent Sci. 2023 Oct;18(4):1883-1888. doi:

10.1016/j.jds.2023.05.016. Epub 2023 May 23. PMID: 37799876; PMCID: PMC10547978.

Liu LX, Lu JC, Zeng HY, Cai JB, Zhang PF, Guo XJ, Huang XY, Dong RZ, Zhang C, Kang Q, Zou H, Zhang XY, Zhang L, Zhang XW, Ke AW, Shi GM. Mortalin stabilizes CD151-depedent tetraspanin-enriched microdomains and implicates in the progression of hepatocellular carcinoma. J Cancer. 2019 Oct 15;10(25):6199-6206. doi: 10.7150/jca.36301. PMID: 31772652; PMCID: PMC6856732.

Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am J Clin Nutr. 2003 Sep;78(3 Suppl):517S-520S. doi: 10.1093/ajcn/78.3.517S. PMID: 12936943.

López F, Rodrigo JP, Cardesa A, Triantafyllou A, Devaney KO, Mendenhall WM, Haigentz M Jr, Strojan P, Pellitteri PK, Bradford CR, Shaha AR, Hunt JL, de Bree R, Takes RP, Rinaldo A, Ferlito A. Update on primary head and neck mucosal melanoma. Head Neck. 2016 Jan;38(1):147-55. doi: 10.1002/hed.23872. Epub 2015 May 22. PMID: 25242350; PMCID: PMC4986507.

Lu WJ, Lee NP, Kaul SC, Lan F, Poon RT, Wadhwa R, Luk JM. Mortalin-p53 interaction in cancer cells is stress dependent and constitutes a selective target for cancer therapy. Cell Death Differ. 2011 Jun;18(6):1046-56. doi: 10.1038/cdd.2010.177. Epub 2011 Jan 14. PMID: 21233847; PMCID: PMC3131943.

Luker GD, Yang J, Richmond A, Scala S, Festuccia C, Schottelius M, Wester HJ, Zimmermann J. At the Bench: Pre-clinical evidence for multiple functions of CXCR4 in cancer. J Leukoc Biol. 2021 May;109(5):969-989. doi: 10.1002/JLB.2BT1018-715RR. Epub 2020 Oct 26. PMID: 33104270; PMCID: PMC8254203.

Luo WI, Dizin E, Yoon T, Cowan JA. Kinetic and structural characterization of human mortalin. Protein Expr Purif. 2010 Jul;72(1):75-81. doi: 10.1016/j.pep.2010.02.003. Epub 2010 Feb 10. PMID: 20152901; PMCID: PMC2887293.

Lv TZ, Wang GS. Antiproliferation potential of withaferin A on human osteosarcoma cells via the inhibition of G2/M checkpoint proteins. Exp Ther Med. 2015 Jul;10(1):323-329. doi: 10.3892/etm.2015.2480. Epub 2015 May 7. PMID: 26170956; PMCID: PMC4487066.

Mallya S, Mallya S. Candida and oral candidosis—a review. Journal of Health and Allied Sciences NU. 2019 Apr;9(02):39-44.

Mangalath U, Aslam SA, Abdul Khadar AH, Francis PG, Mikacha MS, Kalathingal JH. Recent trends in prevention of oral cancer. J Int Soc Prev Community Dent. 2014 Dec;4(Suppl 3):S131-8. doi: 10.4103/2231-0762.149018. PMID: 25625069; PMCID: PMC4304049.

Manoharan S, Karthikeyan S, Essa MM, Manimaran A, Selvasundram R. An overview of oral carcinogenesis. International Journal of Nutrition, Pharmacology, Neurological Diseases. 2016 Apr 1;6(2):51-62.

Markopoulos AK. Current aspects on oral squamous cell carcinoma. Open Dent J. 2012;6:126-30. doi: 10.2174/1874210601206010126. Epub 2012 Aug 10. PMID: 22930665; PMCID: PMC3428647.

Mayola E, Gallerne C, Esposti DD, Martel C, Pervaiz S, Larue L, Debuire B, Lemoine A, Brenner C, Lemaire C. Withaferin A induces apoptosis in human melanoma cells through generation of reactive oxygen species and down-regulation of Bcl-2. Apoptosis. 2011 Oct;16(10):1014-27. doi: 10.1007/s10495-011-0625-x. PMID: 21710254.

Meurman JH. Infectious and dietary risk factors of oral cancer. Oral oncology. 2010 Jun 1;46(6):411-3.

Mihajlovic M, Vlajkovic S, Jovanovic P, Stefanovic V. Primary mucosal melanomas: a comprehensive review. Int J Clin Exp Pathol. 2012;5(8):739-53. Epub 2012 Oct 1. PMID: 23071856; PMCID: PMC3466987.

Miller CS, Johnstone BM. Human papillomavirus as a risk factor for oral squamous cell carcinoma: a meta-analysis, 1982-1997. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontology. 2001 Jun 1;91(6):622-35.

Miller JA, An D, Shafique K, Song S, Rao RA, Viswanathan K, Eykman E, Wiles A, Ali SZ, Field A, Fadda G, Barkan GA, Layfield LJ, Rossi ED, Powers CN, Siddiqui MT, Kholova I, Baloch Z, Maleki Z. Mucoepidermoid carcinoma, acinic cell carcinoma, and adenoid cystic carcinoma on fine-needle aspiration biopsy and The Milan System: an international multi-institutional study. J Am Soc Cytopathol. 2019 Sep-Oct;8(5):270-277. doi: 10.1016/j.jasc.2019.04.001. Epub 2019 Apr 9. PMID: 31104944.

Mira ES, Sapri AM, Aljehanı RF, Jambı BS, Bashir T, El-Kenawy ES, Saber M. Early Diagnosis of Oral Cancer Using Image Processing and Artificial Intelligence. Fusion: Practice and Applications. 2024;14(1):293-308.

Mohamad I, Glaun MDE, Prabhash K, Busheri A, Lai SY, Noronha V, Hosni A. Current Treatment Strategies and Risk Stratification for Oral Carcinoma. Am Soc Clin Oncol Educ Book. 2023 May;43:e389810. doi: 10.1200/EDBK_389810. PMID: 37200591.

Mohan R, Hammers HJ, Bargagna-Mohan P, Zhan XH, Herbstritt CJ, Ruiz A, Zhang L, Hanson AD, Conner BP, Rougas J, Pribluda VS. Withaferin A is a potent inhibitor of angiogenesis. Angiogenesis. 2004;7(2):115-22. PubMed PMID: 15516832.

Moin A, Shetty AD. Management of Non-Hodgkin's Lymphoma in Maxillofacial Region with Chemotherapy. Ann Maxillofac Surg. 2017 Jul-Dec;7(2):312-315. doi: 10.4103/ams.ams_85_17. PMID: 29264306; PMCID: PMC5717915.

Montalto FI, De Amicis F. Cyclin D1 in Cancer: A Molecular Connection for Cell Cycle Control, Adhesion and Invasion in Tumor and Stroma. Cells. 2020 Dec 9;9(12):2648. doi: 10.3390/cells9122648. PMID: 33317149; PMCID: PMC7763888.

Mummudi N, Agarwal JP, Chatterjee S, Mallick I, Ghosh-Laskar S. Oral Cavity Cancer in the Indian Subcontinent - Challenges and Opportunities. Clin Oncol (R Coll Radiol). 2019 Aug;31(8):520-528. doi: 10.1016/j.clon.2019.05.013. Epub 2019 Jun 4. PMID: 31174947.

Na Y, Kaul SC, Ryu J, Lee JS, Ahn HM, Kaul Z, Kalra RS, Li L, Widodo N, Yun CO, Wadhwa R. Stress chaperone mortalin contributes to epithelial-mesenchymal

transition and cancer metastasis. Cancer Res. 2016 May;76(9):2754-2765. doi: 10.1158/0008-5472.CAN-15-2704. Epub 2016 Mar 9. PMID: 26960973.

Nandini DB, Rao RS, Hosmani J, Khan S, Patil S, Awan KH. Novel therapies in the management of oral cancer: An update. Dis Mon. 2020 Dec;66(12):101036. doi: 10.1016/j.disamonth.2020.101036. Epub 2020 Jun 25. PMID: 32594997.

Damm DD, Bouquot JE, Neville BW, Allen C. Oral and maxillofacial pathology. Chap. 2002;1:66.

Nigam N, Grover A, Goyal S, Katiyar SP, Bhargava P, Wang PC, Sundar D, Kaul SC, Wadhwa R. Targeting Mortalin by Embelin Causes Activation of Tumor Suppressor p53 and Deactivation of Metastatic Signaling in Human Breast Cancer Cells. PLoS One. 2015 Sep 16;10(9):e0138192. doi: 10.1371/journal.pone.0138192. PMID: 26376435; PMCID: PMC4574062.

Oliveira DT, de Moraes RV, Fiamengui Filho JF, Fanton Neto J, Landman G, Kowalski LP. Oral verrucous carcinoma: a retrospective study in São Paulo Region, Brazil. Clin Oral Investig. 2006 Sep;10(3):205-9. doi: 10.1007/s00784-006-0050-7. PMID: 16738900.

Olson MA, Rogers RS 3rd, Bruce AJ. Oral lichen planus. Clin Dermatol. 2016 Jul-Aug;34(4):495-504. doi: 10.1016/j.clindermatol.2016.02.023. Epub 2016 Mar 2. PMID: 27343965.

Omura K. Current status of oral cancer treatment strategies: surgical treatments for oral squamous cell carcinoma. Int J Clin Oncol. 2014;19(3):423-30. doi: 10.1007/s10147-014-0689-z. Epub 2014 Apr 1. PMID: 24682763.

Ozawa H, Tomita T, Sakamoto K, Tagawa T, Fujii R, Kanzaki S, Ogawa K, Kameyama K, Fujii M. Mucoepidermoid carcinoma of the head and neck: clinical analysis of 43 patients. Jpn J Clin Oncol. 2008 Jun;38(6):414-8. doi: 10.1093/jjco/hyn045. PMID: 18573852.

Pachuau L, Zami Z, Nunga T, Zodingliana R, Zoramthari R, Lalnuntluanga R, Sangi Z, Rinmawii L, Kumar NS, Lalhruaitluanga H. First-degree family history of cancer can be a potential risk factor among head and neck cancer patients in an isolated Mizo tribal population, northeast India. Clinical Epidemiology and Global Health. 2022 Jan 1;13:100954.

Pandey M, Thomas G, Mathew A, Abraham EK, Somanathan T, Ramadas K, Iype EM, Ahamed IM, Sebastian P, Nair MK. Sarcoma of the oral and maxillofacial soft tissue in adults. Eur J Surg Oncol. 2000 Mar;26(2):145-8. doi: 10.1053/ejso.1999.0758. PMID: 10744932.

Pandya S, Chaudhary AK, Singh M, Singh M, Mehrotra R. Correlation of histopathological diagnosis with habits and clinical findings in oral submucous fibrosis. Head Neck Oncol. 2009 May 2;1:10. doi: 10.1186/1758-3284-1-10. PMID: 19409103; PMCID: PMC2691735.

Papalouka C, Adamaki M, Batsaki P, Zoumpourlis P, Tsintarakis A, Goulielmaki M, Fortis SP, Baxevanis CN, Zoumpourlis V. DNA Damage Response Mechanisms in Head and Neck Cancer: Significant Implications for Therapy and Survival. Int J Mol

Sci. 2023 Feb 1;24(3):2760. doi: 10.3390/ijms24032760. PMID: 36769087; PMCID: PMC9917521.

Papke RL, Horenstein NA, Stokes C. Nicotinic Activity of Arecoline, the Psychoactive Element of "Betel Nuts", Suggests a Basis for Habitual Use and Anti-Inflammatory Activity. PLoS One. 2015;10(10):e0140907 PubMed Central PMCID: PMCPMC4619380. 10.1371/journal.pone.0140907

Parlatescu I, Gheorghe C, Coculescu E, Tovaru S. Oral leukoplakia - an update. Maedica (Bucur). 2014 Mar;9(1):88-93. PMID: 25553134; PMCID: PMC4268300.

Patel SB, Rao NJ, Hingorani LL. Safety assessment of Withania somnifera extract standardized for Withaferin A: Acute and sub-acute toxicity study. J Ayurveda Integr Med. 2016 Mar;7(1):30-7. doi: 10.1016/j.jaim.2015.08.001. Epub 2016 May 24. PMID: 27297507; PMCID: PMC4910650.

Patel SG, Lydiatt WM. Staging of head and neck cancers: is it time to change the balance between the ideal and the practical? J Surg Oncol. 2008 Jun 15;97(8):653-7. doi: 10.1002/jso.21021. PMID: 18493945.

Pathak A, Soni Verma J. ASSOCIATION OF ORAL CANCER WITH CONSUMPTION OF TOBACCO, SMOKING AND ALCOHOL: A PROSPECTIVE COHORT STUDY OF HOSPITAL PATIENTS. Int J Acad Med Pharm. 2023;5(2):1420-8

Patil DJ, Nagaraju R. Personalised Precision Medicine - A Novel Approach for Oral Cancer Management. Submitted: 20 June 2021 Reviewed: 19 July 2021 Published: 17 August 2021. intechopen.99558. Oral Cancer - Current Concepts and Future Perspectives

Paul MC, Schneeweis C, Falcomatà C, Shan C, Rossmeisl D, Koutsouli S, Klement C, Zukowska M, Widholz SA, Jesinghaus M, Heuermann KK, Engleitner T, Seidler B, Sleiman K, Steiger K, Tschurtschenthaler M, Walter B, Weidemann SA, Pietsch R, Schnieke A, Schmid RM, Robles MS, Andrieux G, Boerries M, Rad R, Schneider G, Saur D. Non-canonical functions of SNAIL drive context-specific cancer progression. Nat Commun. 2023 Mar 7;14(1):1201. doi: 10.1038/s41467-023-36505-0. PMID: 36882420; PMCID: PMC9992512.

Peraza A, Gómez R, Beltran J, Amarista FJ. Mucoepidermoid carcinoma. An update and review of the literature. J Stomatol Oral Maxillofac Surg. 2020 Dec;121(6):713-720. doi: 10.1016/j.jormas.2020.06.003. Epub 2020 Jun 18. PMID: 32565266.

Pérez-Sayáns M, Somoza-Martín JM, Barros-Angueira F, Reboiras-López MD, Gándara Rey JM, García-García A. Genetic and molecular alterations associated with oral squamous cell cancer (Review). Oncol Rep. 2009 Dec;22(6):1277-82. doi: 10.3892/or_00000565. PMID: 19885577.

Petersen PE. Oral cancer prevention and control--the approach of the World Health Organization. Oral Oncol. 2009 Apr-May;45(4-5):454-60. doi: 10.1016/j.oraloncology.2008.05.023. Epub 2008 Sep 18. PMID: 18804412.

Pires N, Gota V, Gulia A, Hingorani L, Agarwal M, Puri A. Safety and pharmacokinetics of Withaferin-A in advanced stage high grade osteosarcoma: A phase I trial. J Ayurveda Integr Med. 2020 Jan-Mar;11(1):68-72. doi:

10.1016/j.jaim.2018.12.008. Epub 2019 Mar 21. PMID: 30904387; PMCID: PMC7125369.

Pöschl G, Seitz HK. Alcohol and cancer. Alcohol Alcohol. 2004 May-Jun;39(3):155-65. doi: 10.1093/alcalc/agh057. PMID: 15082451.

Prelec J, Laronde DM. Treatment modalities of oral cancer. Can J Dent Hyg. 2014;48(1):13-9.

Putri JF, Bhargava P, Dhanjal JK, Yaguchi T, Sundar D, Kaul SC, Wadhwa R. Mortaparib, a novel dual inhibitor of mortalin and PARP1, is a potential drug candidate for ovarian and cervical cancers. J Exp Clin Cancer Res. 2019 Dec 19;38(1):499. doi: 10.1186/s13046-019-1500-9. PMID: 31856867; PMCID: PMC6923857.

Rajendran V, Jain MV. In Vitro Tumorigenic Assay: Colony Forming Assay for Cancer Stem Cells. Methods Mol Biol. 2018;1692:89-95. doi: 10.1007/978-1-4939-7401-6_8. PMID: 28986889.

Ram H, Sarkar J, Kumar H, Konwar R, Bhatt ML, Mohammad S. Oral cancer: risk factors and molecular pathogenesis. J Maxillofac Oral Surg. 2011 Jun;10(2):132-7. doi: 10.1007/s12663-011-0195-z. Epub 2011 Apr 22. PMID: 22654364; PMCID: PMC3177522.

Ranganathan K, Quail G. 77 - Oral Disease in the Tropics, Editor(s): Jeremy Farrar, Patricia Garcia, Peter Hotez, Thomas Junghanss, Gagandeep Kang, David Lalloo, Nicholas White, Manson's Tropical Diseases (Twenty-Fourth Edition), Elsevier, 2024, Pages 1077-1086, ISBN 9780702079597

Rapidis AD, Gullane P, Langdon JD, Lefebvre JL, Scully C, Shah JP. Major advances in the knowledge and understanding of the epidemiology, aetiopathogenesis, diagnosis, management and prognosis of oral cancer. Oral Oncol. 2009 Apr-May;45(4-5):299-300. doi: 10.1016/j.oraloncology.2009.04.001. PMID: 19411038.

Reibel J. Prognosis of oral pre-malignant lesions: significance of clinical, histopathological, and molecular biological characteristics. Crit Rev Oral Biol Med. 2003;14(1):47-62. doi: 10.1177/154411130301400105. PMID: 12764019.

Reidy J, McHugh E, Stassen LF. A review of the relationship between alcohol and oral cancer. Surgeon. 2011 Oct;9(5):278-83. doi: 10.1016/j.surge.2011.01.010. Epub 2011 Feb 22. PMID: 21843823.

Renu T, Iyengar AR, Nagesh KS. GSTM1 null polymorphism–a possible key for oral carcinogenesis. RSBO. 2013;10(1):76-81.

Rezazadeh F, Andisheh-Tadbir A, Malek Mansouri Z, Khademi B, Bayat P, Sedarat H, Tabesh A, Tayebi Khorami E. Evaluation of recurrence, mortality and treatment complications of oral squamous cell carcinoma in public health centers in Shiraz during 2010 to 2020. BMC Oral Health. 2023 May 30;23(1):341. doi: 10.1186/s12903-023-03071-2. PMID: 37254138; PMCID: PMC10227788.

Ribatti D, Tamma R, Annese T. Epithelial-Mesenchymal Transition in Cancer: A Historical Overview. Transl Oncol. 2020 Jun;13(6):100773. doi:

10.1016/j.tranon.2020.100773. Epub 2020 Apr 22. PMID: 32334405; PMCID: PMC7182759.

Rivera C, Venegas B. Histological and molecular aspects of oral squamous cell carcinoma (Review). Oncol Lett. 2014 Jul;8(1):7-11. doi: 10.3892/ol.2014.2103. Epub 2014 Apr 29. PMID: 24959211; PMCID: PMC4063640.

Rivera C. Essentials of oral cancer. Int J Clin Exp Pathol. 2015 Sep 1;8(9):11884-94. PMID: 26617944; PMCID: PMC4637760.

Rogers S, Gloss BS, Lee CS, Sergio CM, Dinger ME, Musgrove EA, Burgess A, Caldon CE. Cyclin E2 is the predominant E-cyclin associated with NPAT in breast cancer cells. Cell Div. 2015 Feb 19;10:1. doi: 10.1186/s13008-015-0007-9. PMID: 25741376; PMCID: PMC4349318.

Roy NK, Monisha J, Padmavathi G, Lalhruaitluanga H, Kumar NS, Singh AK, Bordoloi D, Baruah MN, Ahmed GN, Longkumar I, Arfuso F, Kumar AP, Kunnumakkara AB. Isoform-Specific Role of Akt in Oral Squamous Cell Carcinoma. Biomolecules. 2019 Jun 27;9(7):253. doi: 10.3390/biom9070253. PMID: 31252679; PMCID: PMC6681224.

Ryu J, Kaul Z, Yoon AR, Liu Y, Yaguchi T, Na Y, Ahn HM, Gao R, Choi IK, Yun CO, Kaul SC, Wadhwa R. Identification and functional characterization of nuclear mortalin in human carcinogenesis. J Biol Chem. 2014 Sep 5;289(36):24832-44. doi: 10.1074/jbc.M114.565929. Epub 2014 Jul 10. PMID: 25012652; PMCID: PMC4155653.

Sama S, Komiya T, Guddati AK. Advances in the Treatment of Mucoepidermoid Carcinoma. World J Oncol. 2022 Feb;13(1):1-7. doi: 10.14740/wjon1412. Epub 2021 Dec 8. PMID: 35317327; PMCID: PMC8913015.

Sand L, Jalouli J. Viruses and oral cancer. Is there a link? Microbes Infect. 2014 May;16(5):371-8. doi: 10.1016/j.micinf.2014.02.009. Epub 2014 Mar 5. PMID: 24613199.

Sankaranarayanan R, Ramadas K, Amarasinghe H, Subramanian S, Johnson N. Oral Cancer: Prevention, Early Detection, and Treatment. In: Gelband H, Jha P, Sankaranarayanan R, Horton S, editors. Cancer: Disease Control Priorities, Third Edition (Volume 3). Washington (DC): The International Bank for Reconstruction and Development / The World Bank; 2015 Nov 1. Chapter 5. PMID: 26913350.

Sari AN, Bhargava P, Dhanjal JK, Putri JF, Radhakrishnan N, Shefrin S, Ishida Y, Terao K, Sundar D, Kaul SC, Wadhwa R. Combination of Withaferin-A and CAPE Provides Superior Anticancer Potency: Bioinformatics and Experimental Evidence to Their Molecular Targets and Mechanism of Action. Cancers (Basel). 2020 May 5;12(5):1160. doi: 10.3390/cancers12051160. PMID: 32380701; PMCID: PMC7281427.

Sarrión Pérez MG, Bagán JV, Jiménez Y, Margaix M, Marzal C. Utility of imaging techniques in the diagnosis of oral cancer. J Craniomaxillofac Surg. 2015 Nov;43(9):1880-94. doi: 10.1016/j.jcms.2015.07.037. Epub 2015 Aug 7. PMID: 26325616.

Scully C, Porter S. Oral cancer. West J Med. 2001 May;174(5):348-51. doi: 10.1136/ewjm.174.5.348. PMID: 11342519; PMCID: PMC1071397.

Shah JP, Gil Z. Current concepts in management of oral cancer--surgery. Oral Oncol. 2009 Apr-May;45(4-5):394-401. doi: 10.1016/j.oraloncology.2008.05.017. Epub 2008 Jul 31. PMID: 18674952; PMCID: PMC4130348.

Shah NG, Trivedi TI, Tankshali RA, Goswami JA, Shah JS, Jetly DH, Kobawala TP, Patel KC, Shukla SN, Shah PM, Verma RJ. Molecular alterations in oral carcinogenesis: significant risk predictors in malignant transformation and tumor progression. Int J Biol Markers. 2007 Apr-Jun;22(2):132-43. doi: 10.1177/172460080702200207. PMID: 17549669.

Shah P, Feng Q, Carey B, Diniz-Freitas M, Limeres J, Monteiro L, Silva L, Fricain JC, Catros S, Fenelon M, Lombardi N. Actinic cheilitis: guidance on monitoring and management in primary care. Journal of Oral Medicine and Oral Surgery. 2023;29(3):30.

Shapira A, Livney YD, Broxterman HJ, Assaraf YG. Nanomedicine for targeted cancer therapy: towards the overcoming of drug resistance. Drug Resist Updat. 2011 Jun;14(3):150-63. doi: 10.1016/j.drup.2011.01.003. Epub 2011 Feb 16. PMID: 21330184.

Sharma A, Boise LH, Shanmugam M. Cancer Metabolism and the Evasion of Apoptotic Cell Death. Cancers (Basel). 2019 Aug 9;11(8):1144. doi: 10.3390/cancers11081144. PMID: 31405035; PMCID: PMC6721599.

Sharma P, Wadhwan V, Aggarwal P, Sharma A. Oral verrucous hyperplasia versus oral verrucous carcinoma: A clinicopathologic dilemma revisited using p53 as immunohistochemical marker. J Oral Maxillofac Pathol. 2016 Sep-Dec;20(3):362-368. doi: 10.4103/0973-029X.190902. PMID: 27721598; PMCID: PMC5051281.

Shergill AK, Solomon MC, Carnelio S, Kamath AT, Aramanadka C, Shergill GS. Verrucous carcinoma of the oral cavity: Current concepts. International Journal of Scientific Study. 2015;3(3):115-9.

Shi W, Law T, Brumund KT, Chang J, Patel C, Lin G, Hu J. Low-grade mucoepidermoid carcinoma mimicking benign cystic lesions in the salivary gland: A diagnostic dilemma. Rare Tumors. 2024 Mar 23;16:20363613241242397. doi: 10.1177/20363613241242397. PMID: 38525087; PMCID: PMC10960343.

Shin BK, Wang H, Yim AM, Le Naour F, Brichory F, Jang JH, Zhao R, Puravs E, Tra J, Michael CW, Misek DE, Hanash SM. Global profiling of the cell surface proteome of cancer cells uncovers an abundance of proteins with chaperone function. J Biol Chem. 2003 Feb 28;278(9):7607-16. doi: 10.1074/jbc.M210455200. Epub 2002 Dec 18. PMID: 12493773.

Silva JPN, Pinto B, Monteiro L, Silva PMA, Bousbaa H. Combination Therapy as a Promising Way to Fight Oral Cancer. Pharmaceutics. 2023 Jun 4;15(6):1653. doi: 10.3390/pharmaceutics15061653. PMID: 37376101; PMCID: PMC10301495.

Silva TD, Ferreira CB, Leite GB, de Menezes Pontes JR, Antunes HS. Oral manifestations of lymphoma: a systematic review. Ecancermedicalscience. 2016 Aug 17;10:665. doi: 10.3332/ecancer.2016.665. PMID: 27594910; PMCID: PMC4990057.

Singhania N, Mishra A. Alcohol Consumption, Tobacco Use, and Viral Infections: A Multifactorial Approach to Understanding Head and Neck Cancer Risk. International Journal of Applied Health Care Analytics. 2024 Feb 14;9(2):44-57.

Sinha DN, Gupta PC, Pednekar M. Tobacco water: a special form of tobacco use in the Mizoram and Manipur states of India. Natl Med J India. 2004 Sep-Oct;17(5):245-7. PMID: 15638304.

Sivandzade F, Bhalerao A, Cucullo L. Analysis of the Mitochondrial Membrane Potential Using the Cationic JC-1 Dye as a Sensitive Fluorescent Probe. Bio Protoc. 2019 Jan 5;9(1):e3128. doi: 10.21769/BioProtoc.3128. PMID: 30687773; PMCID: PMC6343665.

Soulieres D, Senzer NN, Vokes EE, Hidalgo M, Agarwala SS, Siu LL. Multicenter phase II study of erlotinib, an oral epidermal growth factor receptor tyrosine kinase inhibitor, in patients with recurrent or metastatic squamous cell cancer of the head and neck. J Clin Oncol. 2004 Jan 1;22(1):77-85. doi: 10.1200/JCO.2004.06.075. PMID: 14701768.

Stan SD, Zeng Y, Singh SV. Ayurvedic medicine constituent withaferin a causes G2 and M phase cell cycle arrest in human breast cancer cells. Nutr Cancer. 2008;60 Suppl 1(Suppl 1):51-60. doi: 10.1080/01635580802381477. PMID: 19003581; PMCID: PMC2597540.

Starenki D, Hong SK, Lloyd RV, Park JI. Mortalin (GRP75/HSPA9) upregulation promotes survival and proliferation of medullary thyroid carcinoma cells. Oncogene. 2015 Aug 27;34(35):4624-34. doi: 10.1038/onc.2014.392. Epub 2014 Dec 1. PMID: 25435367; PMCID: PMC4451452.

Starenki D, Sosonkina N, Hong SK, Lloyd RV, Park JI. Mortalin (GRP75/HSPA9) Promotes Survival and Proliferation of Thyroid Carcinoma Cells. Int J Mol Sci. 2019 Apr 26;20(9):2069. doi: 10.3390/ijms20092069. PMID: 31027376; PMCID: PMC6540051.

Strączek A, Szałkowska J, Sutkowska P, Srebrna A, Puzio N, Piasecka A, Piskorz N, Błaszczyk J, Thum-Tyzo K. Impact of nutrition on the condition of the oral mucosa and periodontium: A narrative review. Dent Med Probl. 2023 Oct-Dec;60(4):697-707. doi: 10.17219/dmp/156466. PMID: 38133993.

Stransky N, Egloff AM, Tward AD, Kostic AD, Cibulskis K, Sivachenko A, Kryukov GV, Lawrence MS, Sougnez C, McKenna A, Shefler E, Ramos AH, Stojanov P, Carter SL, Voet D, Cortés ML, Auclair D, Berger MF, Saksena G, Guiducci C, Onofrio RC, Parkin M, Romkes M, Weissfeld JL, Seethala RR, Wang L, Rangel-Escareño C, Fernandez-Lopez JC, Hidalgo-Miranda A, Melendez-Zajgla J, Winckler W, Ardlie K, Gabriel SB, Meyerson M, Lander ES, Getz G, Golub TR, Garraway LA, Grandis JR. The mutational landscape of head and neck squamous cell carcinoma. Science. 2011 Aug 26;333(6046):1157-60. doi: 10.1126/science.1208130. Epub 2011 Jul 28. PMID: 21798893; PMCID: PMC3415217.

Stransky N, Egloff AM, Tward AD, Kostic AD, Cibulskis K, Sivachenko A, Kryukov GV, Lawrence MS, Sougnez C, McKenna A, Shefler E, Ramos AH, Stojanov P, Carter SL, Voet D, Cortés ML, Auclair D, Berger MF, Saksena G, Guiducci C, Onofrio RC, Parkin M, Romkes M, Weissfeld JL, Seethala RR, Wang L, Rangel-

Sudeep HV, Gouthamchandra K, Shyamprasad K. Molecular docking analysis of Withaferin A from Withania somnifera with the Glucose regulated protein 78 (GRP78) receptor and the SARS-CoV-2 main protease. Bioinformation. 2020 Apr 30;16(5):411-417. doi: 10.6026/97320630016411. PMID: 32831523; PMCID: PMC7434955.

Sultana T, Okla MK, Ahmed M, Akhtar N, Al-Hashimi A, Abdelgawad H, Haq IU. Withaferin A: From Ancient Remedy to Potential Drug Candidate. Molecules. 2021 Dec 20;26(24):7696. doi: 10.3390/molecules26247696. PMID: 34946778; PMCID: PMC8705790.

Suman S, Das TP, Sirimulla S, Alatassi H, Ankem MK, Damodaran C. Withaferin-A suppress AKT induced tumor growth in colorectal cancer cells. Oncotarget. 2016 Mar 22;7(12):13854-64. doi: 10.18632/oncotarget.7351. PMID: 26883103; PMCID: PMC4924683.

Sumida T, Otawa N, Kamata YU, Yamada T, Uchida K, Nakano H, Hamakawa H, Yamamoto T, Ueyama Y, Mori Y. A Clinical Investigation of Oral Sarcomas at Multi-institutions Over the Past 30 Years. Anticancer Res. 2015 Aug;35(8):4551-5. PMID: 26168500.

Sun J, Che SL, Piao JJ, Xu M, Chen LY, Lin ZH. Mortalin overexpression predicts poor prognosis in early stage of non-small cell lung cancer. Tumour Biol. 2017 Mar;39(3):1010428317695918. doi: 10.1177/1010428317695918. PMID: 28349826.

Sun R, Dou W, Liu W, Li J, Han X, Li S, Wu X, Wang F, Xu X, Li J. Global, regional, and national burden of oral cancer and its attributable risk factors from 1990 to 2019. Cancer Med. 2023 Jun;12(12):13811-13820. doi: 10.1002/cam4.6025. Epub 2023 May 2. PMID: 37132165; PMCID: PMC10315711.

Sundberg J, Korytowska M, Holmberg E, Bratel J, Wallström M, Kjellström E, Blomgren J, Kovács A, Öhman J, Sand L, Hirsch JM, Giglio D, Kjeller G, Hasséus B. Recurrence rates after surgical removal of oral leukoplakia-A prospective longitudinal multi-centre study. PLoS One. 2019 Dec 6;14(12):e0225682. doi: 10.1371/journal.pone.0225682. eCollection 2019. PubMed PMID: 31810078.

Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021 May;71(3):209-249. doi: 10.3322/caac.21660. Epub 2021 Feb 4. PMID: 33538338.

Sutton DN, Brown JS, Rogers SN, Vaughan ED, Woolgar JA. The prognostic implications of the surgical margin in oral squamous cell carcinoma. Int J Oral Maxillofac Surg. 2003 Feb;32(1):30-4. doi: 10.1054/ijom.2002.0313. PMID: 12653229.

Swain SK. Premalignant lesions of the oral cavity: Current perspectives. Int J Res Med Sci. 2021 Jun;9:1816-22.

Taghavi N, Yazdi I. Type of food and risk of oral cancer. Arch Iran Med. 2007 Apr;10(2):227-32. PMID: 17367228.

Tang Q, Ren L, Liu J, Li W, Zheng X, Wang J, Du G. Withaferin A triggers G2/M arrest and intrinsic apoptosis in glioblastoma cells via ATF4-ATF3-CHOP axis. Cell

Prolif. 2020 Jan;53(1):e12706. doi: 10.1111/cpr.12706. Epub 2019 Oct 23. PMID: 31642559; PMCID: PMC6985693.

Teng M, Hu C, Yang B, Xiao W, Zhou Q, Li Y, Li Z. Salvianolic acid B targets mortalin and inhibits the migration and invasion of hepatocellular carcinoma via the RECK/STAT3 pathway. Cancer Cell Int. 2021 Dec 7;21(1):654. doi: 10.1186/s12935-021-02367-z. PMID: 34876128; PMCID: PMC8650508.

Thaiparambil JT, Bender L, Ganesh T, Kline E, Patel P, Liu Y, Tighiouart M, Vertino PM, Harvey RD, Garcia A, Marcus AI. Withaferin A inhibits breast cancer invasion and metastasis at sub-cytotoxic doses by inducing vimentin disassembly and serine 56 phosphorylation. Int J Cancer. 2011 Dec 1;129(11):2744-55. doi: 10.1002/ijc.25938. Epub 2011 May 2. PMID: 21538350.

Thomas GT, Lewis MP, Speight PM. Matrix metalloproteinases and oral cancer. Oral Oncol. 1999 May;35(3):227-33. doi: 10.1016/s1368-8375(99)00004-4. PMID: 10621841.

Tsai YS, Lee KW, Huang JL, Liu YS, Juo SH, Kuo WR, Chang JG, Lin CS, Jong YJ. Arecoline, a major alkaloid of areca nut, inhibits p53, represses DNA repair, and triggers DNA damage response in human epithelial cells. Toxicology. 2008 Jul 30;249(2-3):230-7. doi: 10.1016/j.tox.2008.05.007. Epub 2008 May 21. PMID: 18585839.

Ullah A, Ahmed A, Lee KT, Yasinzai AQK, Lewis JS Jr. Salivary gland adenoid cystic carcinoma in the U.S population: Importance of grade, site of metastases, and adjuvant radiation for survival. Am J Otolaryngol. 2024 May-Jun;45(3):104266. doi: 10.1016/j.amjoto.2024.104266. Epub 2024 Mar 20. PMID: 38522261.

Vaishnavi K, Saxena N, Shah N, Singh R, Manjunath K, Uthayakumar M, Kanaujia SP, Kaul SC, Sekar K, Wadhwa R. Differential activities of the two closely related withanolides, Withaferin A and Withanone: bioinformatics and experimental evidences. PLoS One. 2012;7(9):e44419. doi: 10.1371/journal.pone.0044419. Epub 2012 Sep 4. PMID: 22973447; PMCID: PMC3433425.

Valentin A, Goetz M, Hetzel J, Reinert S, Hoefert S. Routine panendoscopy in oral squamous cell cancer patients: mandatory or facultative? Clin Oral Investig. 2021 Mar;25(3):1245-1254. doi: 10.1007/s00784-020-03429-8. Epub 2020 Jul 1. PMID: 32607829; PMCID: PMC7878265.

van der Schroeff MP, Baatenburg de Jong RJ. Staging and prognosis in head and neck cancer. Oral Oncol. 2009 Apr-May;45(4-5):356-60. doi: 10.1016/j.oraloncology.2008.05.022. Epub 2008 Aug 19. PMID: 18715810.

van der Waal I. Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management. Oral Oncol. 2009 Apr-May;45(4-5):317-23. doi: 10.1016/j.oraloncology.2008.05.016. Epub 2008 Jul 31. PMID: 18674954.

Vermes I, Haanen C, Steffens-Nakken H, Reutelingsperger C. A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. J Immunol Methods. 1995 Jul 17;184(1):39-51. doi: 10.1016/0022-1759(95)00072-i. PMID: 7622868.

Vila T, Sultan AS, Montelongo-Jauregui D, Jabra-Rizk MA. Oral Candidiasis: A Disease of Opportunity. J Fungi (Basel). 2020 Jan 16;6(1):15. doi: 10.3390/jof6010015. PMID: 31963180; PMCID: PMC7151112.

Vishak S, Rangarajan B, Kekatpure VD. Neoadjuvant chemotherapy in oral cancers: Selecting the right patients. Indian J Med Paediatr Oncol. 2015 Jul-Sep;36(3):148-53. doi: 10.4103/0971-5851.166716. PMID: 26855522; PMCID: PMC4743181.

Vladimir Beljanski. Pazopanib. Editor(s): S.J. Enna, David B. Bylund,xPharm: The Comprehensive Pharmacology Reference, Elsevier, 2010, Pages 1-5, ISBN 9780080552323, doi: 10.1016/B978-008055232-3.64523-4.

Wadhwa R, Kaul SC, Ikawa Y, Sugimoto Y. Identification of a novel member of mouse hsp70 family. Its association with cellular mortal phenotype. J Biol Chem. 1993a Mar 25;268(9):6615-21. PubMed PMID: 8454632.

Wadhwa R, Kaul SC, Mitsui Y, Sugimoto Y. Differential subcellular distribution of mortalin in mortal and immortal mouse and human fibroblasts. Exp Cell Res. 1993b Aug;207(2):442-8. PubMed PMID: 8344392.

Walvekar RR, Chaukar DA, Deshpande MS, Pai PS, Chaturvedi P, Kakade A, Kane SV, D'Cruz AK. Verrucous carcinoma of the oral cavity: A clinical and pathological study of 101 cases. Oral Oncol. 2009 Jan;45(1):47-51. doi: 10.1016/j.oraloncology.2008.03.014. Epub 2008 Jul 11. PMID: 18620896.

Wang L, Zeng Y, Liu Y, Hu X, Li S, Wang Y, Li L, Lei Z, Zhang Z. Fucoxanthin induces growth arrest and apoptosis in human bladder cancer T24 cells by up-regulation of p21 and down-regulation of mortalin. Acta Biochim Biophys Sin (Shanghai). 2014 Oct;46(10):877-84. doi: 10.1093/abbs/gmu080. Epub 2014 Sep 3. PMID: 25187415.

Wang P, Liu F, Wu X, Sun R. Effect of Withaferin A on epithelial-mesenchymal transition and apoptosis in cells of lung squamous cell carcinoma. Cancer Research and Clinic. 2018:825-9.

Wang YX, Ding WB, Dong CW. Withaferin A suppresses liver tumor growth in a nude mouse model by downregulation of cell signaling pathway leading to invasion and angiogenesis. Tropical Journal of Pharmaceutical Research. 2015;14(6):1005-11.

Warnakulasuriya S. Causes of oral cancer--an appraisal of controversies. Br Dent J. 2009 Nov 28;207(10):471-5. doi: 10.1038/sj.bdj.2009.1009. PubMed PMID: 19946320.

Warnakulasuriya S. Living with oral cancer: epidemiology with particular reference to prevalence and life-style changes that influence survival. Oral Oncol. 2010 Jun;46(6):407-10. doi: 10.1016/j.oraloncology.2010.02.015. Epub 2010 Apr 18. PMID: 20403722.

Warszawik-Hendzel O, Słowińska M, Olszewska M, Rudnicka L. Melanoma of the oral cavity: pathogenesis, dermoscopy, clinical features, staging and management. J Dermatol Case Rep. 2014 Sep 30;8(3):60-6. doi: 10.3315/jdcr.2014.1175. PMID: 25324906; PMCID: PMC4195501.

Weaver CH. Overview of Lip & Oral Cavity Cancer. news.cancerconnect.com. Dr. C.H. Weaver M.D. Updated: Oct 2, 2023; Original: Aug 8, 2018

Wei B, Cao J, Tian JH, Yu CY, Huang Q, Yu JJ, Ma R, Wang J, Xu F, Wang LB. Mortalin maintains breast cancer stem cells stemness via activation of Wnt/GSK3β/β-catenin signaling pathway. Am J Cancer Res. 2021 Jun 15;11(6):2696-2716. PMID: 34249423; PMCID: PMC8263651.

Wijeratne EMK, Oliveira MCF, Mafezoli J, Xu YM, Minguzzi S, Batista PHJ, Pessoa ODL, Whitesell L, Gunatilaka AAL. Withaferin A and Withanolide D Analogues with Dual Heat-Shock-Inducing and Cytotoxic Activities: Semisynthesis and Biological Evaluation. J Nat Prod. 2018 Apr 27;81(4):825-837. doi: 10.1021/acs.jnatprod.7b00918. Epub 2018 Mar 14. PMID: 29537263.

Wong T, Wiesenfeld D. Oral Cancer. Aust Dent J. 2018 Mar;63 Suppl 1:S91-S99. doi: 10.1111/adj.12594. PMID: 29574808.

Wood NH, Khammissa R, Meyerov R, Lemmer J, Feller L. Actinic cheilitis: a case report and a review of the literature. Eur J Dent. 2011 Jan;5(1):101-6. PMID: 21228959; PMCID: PMC3019754.

Xu M, Zhang Y, Cui M, Wang X, Lin Z. Mortalin contributes to colorectal cancer by promoting proliferation and epithelial-mesenchymal transition. IUBMB Life. 2020 Apr;72(4):771-781. doi: 10.1002/iub.2176. Epub 2019 Oct 24. PMID: 31647608.

Xu Z, Peng C, Zhang Y, Chen L, Zhai J. Adenoid cystic carcinoma of the retromolar pad region: A case report. Int J Surg Case Rep. 2024 Apr;117:109567. doi: 10.1016/j.ijscr.2024.109567. Epub 2024 Mar 20. PMID: 38522303; PMCID: PMC10973809.

Yamaguchi S, Nagasawa H, Suzuki T, Fujii E, Iwaki H, Takagi M, Amagasa T. Sarcomas of the oral and maxillofacial region: a review of 32 cases in 25 years. Clin Oral Investig. 2004 Jun;8(2):52-5. doi: 10.1007/s00784-003-0233-4. PMID: 15281217.

Yaming P, Urs AB, Saxena A, Zuberi M. Roles of CYP1A1 and CYP2E1 Gene Polymorphisms in Oral Submucous Fibrosis. Asian Pac J Cancer Prev. 2016;17(7):3335-40. PMID: 27509973.

Yang H, Shi G, Dou QP. The tumor proteasome is a primary target for the natural anticancer compound Withaferin A isolated from "Indian winter cherry". Mol Pharmacol. 2007 Feb;71(2):426-37. doi: 10.1124/mol.106.030015. Epub 2006 Nov 8. Erratum in: Mol Pharmacol. 2015 May;87(5):865. PMID: 17093135.

Yang J, Wang ZY, Huang L, Yu TL, Wan SQ, Song J, Zhang BL, Hu M. Do betel quid and areca nut chewing deteriorate prognosis of oral cancer? A systematic review, meta-analysis, and research agenda. Oral Dis. 2021 Sep;27(6):1366-1375. doi: 10.1111/odi.13456. Epub 2020 Jun 26. PMID: 32492239.

Yang N, Zhu L, Tan T, Hou C. [Study of the relationship among expression of Survivin and MRP and the drug resistance in human nasopharyngeal carcinoma]. Lin Chuang Er Bi Yan Hou Tou Jing Wai Ke Za Zhi. 2015 Feb;29(3):235-9. Chinese. PMID: 26012295.

Yardimci G, Kutlubay Z, Engin B, Tuzun Y. Precancerous lesions of oral mucosa. World J Clin Cases. 2014 Dec 16;2(12):866-72. doi: 10.12998/wjcc.v2.i12.866. PMID: 25516862; PMCID: PMC4266835.

Yco LP, Mocz G, Opoku-Ansah J, Bachmann AS. Withaferin A Inhibits STAT3 and Induces Tumor Cell Death in Neuroblastoma and Multiple Myeloma. Biochem Insights. 2014 Nov 9;7:1-13. doi: 10.4137/BCI.S18863. PMID: 25452693; PMCID: PMC4226392.

Yoon AR, Wadhwa R, Kaul SC, Yun CO. Why is Mortalin a Potential Therapeutic Target for Cancer? Front Cell Dev Biol. 2022 Jun 29;10:914540. doi: 10.3389/fcell.2022.914540. PMID: 35859897; PMCID: PMC9290191.

Yun CO, Bhargava P, Na Y, Lee JS, Ryu J, Kaul SC, Wadhwa R. Relevance of mortalin to cancer cell stemness and cancer therapy. Sci Rep. 2017 Feb 6;7:42016. doi: 10.1038/srep42016. PMID: 28165047; PMCID: PMC5292728.

Yun CW, Lee SH. The Roles of Autophagy in Cancer. Int J Mol Sci. 2018 Nov 5;19(11):3466. doi: 10.3390/ijms19113466. PMID: 30400561; PMCID: PMC6274804.

Zhang R, Meng Z, Wu X, Zhang M, Zhang S, Jin T. Mortalin promotes breast cancer malignancy. Exp Mol Pathol. 2021 Feb;118:104593. doi: 10.1016/j.yexmp.2020.104593. Epub 2020 Dec 9. PMID: 33309613.

Zhang Y, He J, He B, Huang R, Li M. Effect of tobacco on periodontal disease and oral cancer. Tob Induc Dis. 2019 May 9;17:40. doi: 10.18332/tid/106187. PMID: 31516483; PMCID: PMC6662776.

Zhao M, Ding L, Yang Y, Chen S, Zhu N, Fu Y, Ni Y, Wang Z. Aberrant Expression Of PDCD4/eIF4A1 Signal Predicts Postoperative Recurrence For Early-Stage Oral Squamous Cell Carcinoma. Cancer Manag Res. 2019 Nov 11;11:9553-9562. doi: 10.2147/CMAR.S223273. eCollection 2019. PubMed PMID: 31807078; PubMed Central PMCID: PMC6857661.

Zhou X, Cai X, Jing F, Li X, Zhang J, Zhang H, Li T. Genomic alterations in oral multiple primary cancers. Int J Oral Sci. 2024 Feb 18;16(1):13. doi: 10.1038/s41368-023-00265-w. PMID: 38368361; PMCID: PMC10874441.

Zhu X, Wang X, Gong Y, Deng J. E-cadherin on epithelial-mesenchymal transition in thyroid cancer. Cancer Cell Int. 2021 Dec 20;21(1):695. doi: 10.1186/s12935-021-02344-6. PMID: 34930256; PMCID: PMC8690896.

Zito PM, Brizuela M, Mazzoni T. Oral Melanoma. 2023 Apr 10. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2024 Jan–. PMID: 30020648.

ΔΨm:	Mitochondrial membrane potential
5-FU:	5-Fluorouracil
ABCG2:	ATP-binding cassette super-family G member 2
ACCa:	Acinic cell carcinoma
ACC:	Adenoid cystic carcinoma
ADHs:	Alcohol dehydrogenases
ADP:	Adenosine diphosphate
AFM:	Anterior floor of mouth
ALDH:	Aldehyde dehydrogenases
AIDS:	Acquired immunodeficiency syndrome
AIST:	National Institute of Advanced Industrial Science and Technology
AJCC:	American Joint Committee on Cancer
ALDH1:	Aldehyde dehydrogenase
ATP:	Adenosine triphosphate
BaP:	Benzo[a]pyrene
Bax:	Bcl-2-associated protein x
Bcl-2:	B-cell lymphoma-2
BSA:	Bovine serum albumin
BT:	Base of tongue
CAR:	Chimeric antigen receptor
CCCP:	Carbonyl cyanide m-chlorophenyl hydrazone
CD9:	Cluster of differentiation 9
CDK:	Cyclin-dependent kinase
CDKN2A:	Cyclin-dependent kinase inhibitor 2A
CIS:	Carcinoma-in situ
CM:	Cheek mucosa
COX-2:	Cyclooxygenase 2
CRT:	Chemoradiation therapy
CT:	Computed tomography
cTNM:	Clinical staging TNM
CXCR-4:	C-X-C chemokine receptor type 4
DAB:	3,3'-diaminobenzidine
DAPI:	4',6-diamidino-2-phenylindole
DDX3X:	Human Dead-box protein 3
DMEM:	Dulbecco's Modified Eagle Medium
DPX:	Dibutylphthalate polystyrene xylene
DSS:	Disease-specific survival
EBRT:	External beam radiotherapy
EGFR:	Epidermal growth factor receptor
EMT:	Epithelial-to-mesenchymal transition
EZH2:	Enhancer of zeste homolog 2
FBS:	Fetal bovine serum
FDA:	Food and Drug Administration
FM:	Floor of mouth
GAPDH:	Glyceraldehyde 3-phosphate dehydrogenase
GEO:	Gene Expression Omnibus

GST:	Glutathione-S-transferase
HCC:	Hepatocellular carcinoma
HNSCC:	Head and neck squamous cell carcinoma
HP:	Hard palate
HPV:	Human papillomavirus
HR:	Hazard ratio
HRP-:	Horseradish peroxidase
HS3ST6:	Heparan sulfate-glucosamine 3-sulfotransferase 6
HSCTs:	Hematopoietic stem cell transplants
HSP70:	Heat shock 70 kDa protein
IC_{50}:	Median inhibitory concentration
ICC:	Immunocytochemistry
IL:	Interleukin
JC dye:	5,5,6,6'-tetrachloro-1,1',3,3'tetraethylbenzimidazoylcarbocyanine iodide
LJ:	Lower jaw
MEC:	Mucoepidermoid carcinoma
MEF:	Mouse embryonic fibroblasts
miRNAs:	microRNAs
MRI:	Magnetic resonance imaging
MRP1:	Multidrug resistance protein 1
MSC:	Mesenchymal stem cells
MTT:	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
mTOR:	Mammalian target of rapamycin
MU:	Mouth
NBD:	Nucleotide-Binding Domain
NCCS:	National Centre for Cell Science
NECHRI:	North-East Cancer Hospital and Research Institute
NF-κB:	Nuclear factor kappa B
NHL:	Non-Hodgkin's lymphoma
NK:	Natural killer
NNK:	4-methylnitrosamino-1-(3-pyridyl)-1-butanone
NNN:	N'-nitrosonornicotine
OC:	Oral cancer
Oct-4:	Octamer-binding transcription factor 4
OLP:	Oral lichen planus
OS:	Overall survival
OSCC:	Oral squamous cell carcinoma
OSF:	Oral submucous fibrosis
PARP:	Poly (ADP-ribose) polymerase
PDGF:	Platelet-derived growth factor receptor
PET:	Positron emission tomography
PI3K:	Phosphoinositide 3-kinase
PMDs:	Potentially malignant disorders
pRB:	Retinoblastoma protein
pTNM:	Pathologic staging TNM

RET:	Rearranged during transfection
RFPL4A:	Ret finger protein like 4A
RGCB:	Rajiv Gandhi Centre for Biotechnology
ROS:	Reactive oxygen species
SBD:	Substrate-Binding Domain
SCC:	Squamous cell carcinoma
siRNAs:	Small interfering RNAs
Smad:	Mothers against decapentaplegic homolog
STAT-3:	Signal transducer and activator of transcription-3
TCGA:	The Cancer Genome Atlas
TMA:	Tissue microarray
TNM:	Tumor-node-metastasis
TSNs:	Tobacco-specific nitrosamines
UJ:	Upper jaw
UV:	Ultra-violet
VC:	Verrucous Carcinoma
VEGF:	Vascular endothelial growth factor
WHO:	World Health Organization
XMEs:	Xenobiotic metabolizing enzymes
ZO-1:	Zonula occludens

Supplementary Table 1.1: American Joint Committee on Cancer (AJCC) TNM system for OC [www.cancer.org/cancer/types/oral-cavity-and-oropharyngeal-cancer]

AJCC stage	Stage grouping	Description
0	Tis N0 M0	Cancer is still within the epithelium (the top layer of cells lining the oral cavity and oropharynx) and has not yet grown into deeper layers. It has not spread to nearby lymph nodes (N0) or distant sites (M0). This stage is also known as carcinoma in situ (Tis).
I	T1 N0 M0	The cancer is 2 cm or smaller. It is not grown into nearby tissues (T1). It has not spread to nearby lymph nodes (N0) or to distant sites (M0).
II	T2 N0 M0	The cancer is larger than 2 cm but no larger than 4 cm. It's not growing into nearby tissues (T2). It has not spread to nearby lymph nodes (N0) or to distant sites (M0).
III	T3 N0 M0	The cancer is larger than 4 cm (T3). For cancers of the oropharynx, T3 also includes tumors that are growing into the epiglottis (the base of the tongue). It has not spread to nearby lymph nodes (N0) or to distant sites (M0).
	T1, T2, T3 N1 M0	Cancer is any size and may have grown into nearby structures if oropharynx cancer (T1-T3) And has spread to 1 lymph node on the same side as the primary tumor. The cancer has not grown outside of the lymph node and the lymph node is no larger than 3 cm (N1). It has not spread to distant sites (M0).
IVA	T4a N0 or N1 M0	The cancer is any size and is growing into nearby structures such as: For lip cancers: nearby bone, the inferior alveolar nerve (the nerve to the jawbone), the floor of the mouth, or the skin of the chin or nose (T4a) For oral cavity cancers: the bones of the jaw or face, deep muscle of the tongue, skin of the face, or the maxillary sinus (T4a)

		For oropharyngeal cancers: the larynx (voice box), the tongue muscle, or bones such as the medial pterygoid, the hard palate, or the jaw (T4a). This is known as moderately advanced local disease (T4a). AND either of the following: It has not spread to nearby lymph nodes (N0) It has spread to 1 lymph node on the same side as the primary tumor, but has not grown outside of the lymph node and the lymph node is no larger than 3 cm (N1). It has not spread to distant sites (M0).
	T1, T2, T3 or T4a N2 M0	The cancer is any size and may have grown into nearby structures (T0-T4a). It has not spread to distant organs (M0). It has spread to one of the following: One lymph node on the same side as the primary tumor, but it has not grown outside of the lymph node and the lymph node is larger than 3 cm but not larger than 6 cm (about 2½ inches) (N2a) OR It has spread to more than 1 lymph node on the same side as the primary tumor, but it has not grown outside any of the lymph nodes and none are larger than 6 cm (N2b) OR It has spread to 1 or more lymph nodes either on the opposite side of the primary tumor or on both sides of the neck, but has not grown outside any of the lymph nodes and none are larger than 6 cm (N2c).
IVB	Any T N3 M0	The cancer is any size and may have grown into nearby soft tissues or structures (Any T) AND any of the

		following: It has spread to 1 lymph node that's larger than 6 cm but has not grown outside of the lymph node (N3a) OR It has spread to 1 lymph node that's larger than 3 cm and has clearly grown outside the lymph node (N3b) OR It has spread to more than 1 lymph node on the same side, the opposite side, or both sides of the primary cancer with growth outside of the lymph node(s) (N3b) OR It has spread to 1 lymph node on the opposite side of the primary cancer that's 3 cm or smaller and has grown outside of the lymph node (N3b). It has not spread to distant organs (M0).
	T4b Any N M0	The cancer is any size and is growing into nearby structures such as the base of the skull or other bones nearby, or it surrounds the carotid artery. This is known as very advanced local disease (T4b). It might or might not have spread to nearby lymph nodes (Any N). It has not spread to distant organs (M0).
IVC	Any T Any N M1	The cancer is any size and may have grown into nearby soft tissues or structures (Any T) AND it might or might not have spread to nearby lymph nodes (Any N). It has spread to distant sites such as the lungs (M1).

Supplementary Table 2.1: Patient specification for IHC analysis

Cores- 208; Cases- 99; Row number-13;
Column number-16; Core Diameter (mm)-1;
Thickness (µm)-5

Pos	Age	Sex	Organ/ Anatomic site	Pathology diagnosis	TNM	Grade	Stage	Type
A1	40	M	Upper jaw	Squamous cell carcinoma	T4N0 M0	1	IV	Malignant
A2	40	M	Lower jaw	Squamous cell carcinoma	T4N0 M0	1	IV	Malignant
A3	79	M	Cheek	Squamous cell carcinoma	T2N0 M0	1	II	Malignant
A4	79	M	Cheek	Squamous cell carcinoma	T2N0 M0	1	II	Malignant
A5	81	M	Lip	Squamous cell carcinoma	T2N0 M0	1	II	Malignant
A6	81	M	Lip	Squamous cell carcinoma	T2N0 M0	1	II	Malignant
A7	57	M	Tongue	Squamous cell carcinoma	T1N0 M0	1	I	Malignant
A8	57	M	Tongue	Squamous cell carcinoma	T1N0 M0	1	I	Malignant
A9	66	M	Tongue	Squamous cell carcinoma	T1N0 M0	1	I	Malignant
A10	66	M	Tongue	Squamous cell carcinoma	T1N0 M0	1	I	Malignant
A11	51	F	Cheek	Squamous cell carcinoma of left cheek	T4N0 M0	1	IV	Malignant
A12	51	F	Cheek	Squamous cell carcinoma of left cheek	T4N0 M0	1	IV	Malignant

A13	65	F	Cheek	Squamous cell carcinoma of right cheek	T2N0 M0	1	II	Malignant
A14	65	F	Cheek	Squamous cell carcinoma of right cheek	T2N0 M0	1	II	Malignant
A15	62	F	Cheek	Squamous cell carcinoma of left cheek	T1N0 M0	1	I	Malignant
A16	62	F	Cheek	Squamous cell carcinoma of left cheek (sparse)	T1N0 M0	1	I	Malignant
B1	46	F	Upper jaw	Squamous cell carcinoma of left upper jaw	T1N0 M0	1	I	Malignant
B2	46	F	Upper jaw	Squamous cell carcinoma of left upper jaw	T1N0 M0	1	I	Malignant
B3	68	M	Palate	Squamous cell carcinoma of right palate	T2N0 M0	1	II	Malignant
B4	68	M	Palate	Squamous cell carcinoma of right palate	T2N0 M0	1	II	Malignant
B5	56	F	Cheek	Squamous cell carcinoma of left cheek	T2N0 M0	1	II	Malignant
B6	56	F	Cheek	Squamous cell carcinoma	T2N0 M0	1	II	Malignant

				of left cheek				
B7	60	M	Gingiva	Squamous cell carcinoma	T1N0 M0	1	I	Malignant
B8	60	M	Gingiva	Squamous cell carcinoma	T1N0 M0	1	I	Malignant
B9	55	M	Cheek	Squamous cell carcinoma of right cheek	T1N0 M0	1	I	Malignant
B10	55	M	Cheek	Squamous cell carcinoma of right cheek	T1N0 M0	1	I	Malignant
B11	78	M	Lip	Squamous cell carcinoma of lower lip	T1N0 M0	1	I	Malignant
B12	78	M	Lip	Squamous cell carcinoma of lower lip	T1N0 M0	1	I	Malignant
B13	41	F	Gingiva	Squamous cell carcinoma	T1N0 M0	1	I	Malignant
B14	41	F	Gingiva	Squamous cell carcinoma	T1N0 M0	1	I	Malignant
B15	46	F	Tongue	Squamous cell carcinoma	T1N0 M0	1	I	Malignant
B16	46	F	Tongue	Squamous cell carcinoma	T1N0 M0	1	I	Malignant
C1	78	F	Lip	Squamous cell carcinoma of lower lip	T1N0 M0	1	I	Malignant
C2	78	F	Lip	Squamous cell carcinoma of lower	T1N0 M0	1	I	Malignant

				lip				
C3	70	F	Lip	Squamous cell carcinoma of lower lip	T1N0 M0	1	I	Malignant
C4	70	F	Lip	Squamous cell carcinoma of lower lip	T1N0 M0	1	I	Malignant
C5	35	F	Tongue	Squamous cell carcinoma	T2N0 M0	1	II	Malignant
C6	35	F	Tongue	Squamous cell carcinoma	T2N0 M0	1	II	Malignant
C7	39	F	Tongue	Squamous cell carcinoma	T1N0 M0	1	I	Malignant
C8	39	F	Tongue	Squamous cell carcinoma (sparse)	T1N0 M0	1	I	Malignant
C9	44	M	Gingiva	Squamous cell carcinoma	T2N0 M0	1	II	Malignant
C10	44	M	Gingiva	Squamous cell carcinoma	T2N0 M0	1	II	Malignant
C11	78	M	Tongue	Squamous cell carcinoma	T2N0 M0	1	II	Malignant
C12	78	M	Tongue	Squamous cell carcinoma	T2N0 M0	1	II	Malignant
C13	60	M	Gingiva	Squamous cell carcinoma of right lower gingiva (sparse)	T1N0 M0	-	I	Malignant
C14	60	M	Gingiva	Squamous cell carcinoma of right lower gingiva	T1N0 M0	1	I	Malignant

C15	54	F	Lip	Squamous cell carcinoma of lower lip	T1N0 M0	1	I	Malignant
C16	54	F	Lip	Squamous cell carcinoma of lower lip	T1N0 M0	1	I	Malignant
D1	75	F	Lip	Squamous cell carcinoma of lower lip	T1N0 M0	1	I	Malignant
D2	75	F	Lip	Squamous cell carcinoma of lower lip	T1N0 M0	1	I	Malignant
D3	70	M	Cheek	Squamous cell carcinoma	T2N0 M0	1	II	Malignant
D4	70	M	Cheek	Squamous cell carcinoma	T2N0 M0	1	II	Malignant
D5	73	M	Lip	Squamous cell carcinoma of lower lip	T1N0 M0	1	I	Malignant
D6	73	M	Lip	Squamous cell carcinoma of lower lip	T1N0 M0	1	I	Malignant
D7	48	F	Tongue	Squamous cell carcinoma	T2N0 M0	1	II	Malignant
D8	48	F	Tongue	Squamous cell carcinoma	T2N0 M0	1	II	Malignant
D9	60	M	Tongue	Squamous cell carcinoma	T1N0 M0	1	I	Malignant
D10	60	M	Tongue	Squamous cell carcinoma	T1N0 M0	1	I	Malignant
D11	86	F	Lip	Squamous	T4N0	1	IV	Malignant

				cell carcinoma of lower lip	M0			
D12	86	F	Lip	Squamous cell carcinoma of lower lip	T4N0 M0	1	IV	Malignant
D13	55	M	Gingiva	Squamous cell carcinoma	T2N0 M0	2	II	Malignant
D14	55	M	Gingiva	Squamous cell carcinoma	T2N0 M0	2	II	Malignant
D15	57	M	Gingiva	Squamous cell carcinoma	T1N0 M0	2	I	Malignant
D16	57	M	Gingiva	Squamous cell carcinoma	T1N0 M0	2	I	Malignant
E1	41	M	Gingiva	Squamous cell carcinoma of left upper gingiva	T1N0 M0	3	I	Malignant
E2	41	M	Gingiva	Squamous cell carcinoma of left upper gingiva	T1N0 M0	3	I	Malignant
E3	47	M	Lower jaw	Squamous cell carcinoma of left lower jaw	T2N0 M0	3	II	Malignant
E4	47	M	Lower jaw	Squamous cell carcinoma of left lower jaw	T2N0 M0	3	II	Malignant
E5	45	F	Tongue	Squamous cell carcinoma	T3N0 M0	3	III	Malignant
E6	45	F	Tongue	Squamous cell carcinoma	T3N0 M0	3	III	Malignant

E7	48	F	Oral cavity	Mucinous adenocarcinoma	T2N0 M0	2	II	Malignant
E8	48	F	Oral cavity	Mucinous adenocarcinoma	T2N0 M0	2	II	Malignant
E9	60	M	Upper jaw	Adenocarcinoma	T1N0 M0	1--2	I	Malignant
E10	60	M	Upper jaw	Adenocarcinoma	T1N0 M0	1--2	I	Malignant
E11	8	F	Salivary gland	Adenocarcinoma	T2N0 M0	*	II	Malignant
E12	8	F	Salivary gland	Adenocarcinoma	T2N0 M0	*	II	Malignant
E13	28	M	Maxillary sinus	Adenocarcinoma of right maxillary sinus	T1N0 M0	3	I	Malignant
E14	28	M	Maxillary sinus	Adenocarcinoma of right maxillary sinus	T1N0 M0	3	I	Malignant
E15	71	M	Mouth	Mucoepidermoid carcinoma of left mouth floor	T1N0 M0	1	I	Malignant
E16	71	M	Mouth	Mucoepidermoid carcinoma of left mouth floor	T1N0 M0	1	I	Malignant
F1	50	F	Cheek	Mucoepidermoid carcinoma (sparse)	T2N0 M0	1	II	Malignant
F2	50	F	Cheek	Mucoepidermoid carcinoma (sparse)	T2N0 M0	1	II	Malignant
F3	57	M	Lip	Mucoepidermoid carcinoma of upper	T1N0 M0	1--2	I	Malignant

				lip				
F4	57	M	Lip	Mucoepidermoid carcinoma of upper lip	T1N0M0	1--2	I	Malignant
F5	48	F	Lower jaw	Mucoepidermoid carcinoma of right lower jaw	T1N0M0	2	I	Malignant
F6	48	F	Lower jaw	Mucoepidermoid carcinoma of right lower jaw	T1N0M0	2	I	Malignant
F7	57	M	Palate	Mucoepidermoid carcinoma of upper palate	T2N0M0	2	II	Malignant
F8	57	M	Palate	Mucoepidermoid carcinoma of upper palate	T2N0M0	2	II	Malignant
F9	32	F	Palate	Mucoepidermoid carcinoma (sparse)	T2N0M0	3	II	Malignant
F10	32	F	Palate	Mucoepidermoid carcinoma (sparse)	T2N0M0	3	II	Malignant
F11	55	M	Gingiva	Mucoepidermoid carcinoma of left upper gingiva	T1N0M0	3	I	Malignant
F12	55	M	Gingiva	Mucoepidermoid carcinoma of left upper gingiva	T1N0M0	3	I	Malignant
F13	50	M	Tongue	Mucoepidermoid carcinoma	T1N0M0	2--3	I	Malignant

F14	50	M	Tongue	Mucoepidermoid carcinoma	T1N0M0	2--3	I	Malignant
F15	53	M	Maxilla	Mucoepidermoid carcinoma of left maxilla	T2N0M0	3	II	Malignant
F16	53	M	Maxilla	Mucoepidermoid carcinoma of left maxilla	T2N0M0	3	II	Malignant
G1	32	F	Tongue	Mucoepidermoid carcinoma	T1N0M0	2--3	I	Malignant
G2	32	F	Tongue	Mucoepidermoid carcinoma	T1N0M0	2--3	I	Malignant
G3	79	F	Lip	Basal cell carcinoma	T2N0M0	-	II	Malignant
G4	79	F	Lip	Basal cell carcinoma	T2N0M0	-	II	Malignant
G5	60	F	Lip	Basal cell carcinoma of right lower lip	T1N0M0	-	I	Malignant
G6	60	F	Lip	Basal cell carcinoma of right lower lip	T1N0M0	-	I	Malignant
G7	58	M	Lymph node	Metastatic squamous cell carcinoma of neck	-	1	-	Metastasis
G8	58	M	Lymph node	Metastatic squamous cell carcinoma of neck	-	1	-	Metastasis
G9	55	M	Lymph node	Metastatic squamous cell carcinoma of neck	-	1--2	-	Metastasis
G10	55	M	Lymph node	Metastatic squamous cell	-	1--2	-	Metastasis

				carcinoma of neck				
G11	40	F	Lymph node	Metastatic squamous cell carcinoma of left lower mandible	-	*	-	Metastasis
G12	40	F	Lymph node	Metastatic squamous cell carcinoma of left lower mandible	-	3	-	Metastasis
G13	52	F	Lymph node	Metastatic acinic cell carcinoma of neck	-	-	-	Metastasis
G14	52	F	Lymph node	Metastatic acinic cell carcinoma of neck	-	-	-	Metastasis
G15	11	M	Mandible	Adamantinoma	-	-	-	Benign
G16	11	M	Mandible	Adamantinoma	-	-	-	Benign
H1	62	M	Mandible	Adamantinoma	-	-	-	Benign
H2	62	M	Mandible	Adamantinoma	-	-	-	Benign
H3	28	M	Lower jaw	Adamantinoma of left lower jaw	-	-	-	Benign
H4	28	M	Lower jaw	Adamantinoma of left lower jaw	-	-	-	Benign
H5	38	M	Maxilla	Adamantinoma	-	-	-	Benign
H6	38	M	Maxilla	Adamantinoma	-	-	-	Benign
H7	37	F	Mandible	Adamantinoma	-	-	-	Benign
H8	37	F	Mandible	Adamantinoma	-	-	-	Benign

H9	34	M	Mandible	Adamantinoma with necrosis of left mandible (sparse)	-	-	-	Benign
H10	34	M	Mandible	Adamantinoma of left mandible	-	-	-	Benign
H11	40	M	Lower jaw	Adamantinoma	-	-	-	Benign
H12	40	M	Lower jaw	Adamantinoma	-	-	-	Benign
H13	47	F	Mandible	Adamantinoma	-	-	-	Benign
H14	47	F	Mandible	Adamantinoma	-	-	-	Benign
H15	70	F	Mandible	Adamantinoma of right mandible	-	-	-	Benign
H16	70	F	Mandible	Adamantinoma of right mandible	-	-	-	Benign
I1	51	M	Mandible	Adamantinoma of right mandible	-	-	-	Benign
I2	51	M	Mandible	Adamantinoma of right mandible	-	*	-	Benign
I3	60	M	Gingiva	Ulcer of right lower gingiva mucosa of No.45	-	-	-	Ulcer
I4	60	M	Gingiva	Ulcer of right lower gingiva mucosa of No.46	-	-	-	Ulcer
I5	76	M	Palate	Cyst of right	-	*	-	Cyst

				upper palate				
I6	76	M	Palate	Cyst of right upper palate	-	-	-	Cyst
I7	22	F	Mandible	Cyst of left mandible	-	*	-	Cyst
I8	22	F	Mandible	Cyst of left mandible	-	*	-	Cyst
I9	86	M	Lip	Mild hyperplasia of squamous epithelium of right upper lip	-	-	-	Hyperplasia
I10	86	M	Lip	Mild hyperplasia of squamous epithelium of right upper lip	-	-	-	Hyperplasia
I11	48	F	Tongue	Mild hyperplasia of squamous epithelium of No.55	-	-	-	Hyperplasia
I12	48	F	Tongue	Mild hyperplasia of squamous epithelium of No.56	-	-	-	Hyperplasia
I13	46	M	Tongue	Mild hyperplasia of squamous epithelium (sparse)	-	-	-	Hyperplasia
I14	46	M	Tongue	Mild hyperplasia of squamous epithelium	-	-	-	Hyperplasia

				(sparse)				
I15	40	M	Lip	Mild hyperplasia of squamous epithelium of right lower lip	-	*	-	Hyperplasia
I16	40	M	Lip	Mild hyperplasia of squamous epithelium of right lower lip	-	-	-	Hyperplasia
J1	53	M	Cheek	Mild-moderate atypical hyperplasia of left cheek	-	-	-	Hyperplasia
J2	53	M	Cheek	Mild-moderate atypical hyperplasia of left cheek	-	-	-	Hyperplasia
J3	62	F	Cheek	Chronic Inflammation of mucosa squamous epithelium	-	-	-	Inflammation
J4	62	F	Cheek	Chronic Inflammation of mucosa squamous epithelium	-	-	-	Inflammation
J5	58	F	Salivary gland	Chronic Inflammation of right palate	-	-	-	Inflammation
J6	58	F	Salivary gland	Chronic Inflammation of right	-	-	-	Inflammation

				palate				
J7	32	M	Salivary gland	Chronic Inflammation of right submandibular gland (sparse)	-	-	-	Inflammation
J8	32	M	Salivary gland	Chronic Inflammation of right submandibular gland	-	-	-	Inflammation
J9	63	M	Cheek	Chronic Inflammation of mucosa with focal epithelium hyperplasia of left cheek	-	-	-	Inflammation
J10	63	M	Cheek	Chronic Inflammation of mucosa with focal epithelium hyperplasia of left cheek	-	-	-	Inflammation
J11	63	M	Lower jaw	Chronic Inflammation of salivary gland of right lower jaw	-	-	-	Inflammation
J12	63	M	Lower jaw	Chronic Inflammation of salivary gland of right lower jaw	-	-	-	Inflammation

J13	45	F	Tongue	Chronic Inflammation with mild hyperplasia of squamous epithelium of No.69	-	-	-	Inflammation
J14	45	F	Tongue	Chronic Inflammation with mild hyperplasia of squamous epithelium of No.70	-	-	-	Inflammation
J15	63	M	Salivary gland	Chronic Inflammation of right submandibular gland	-	-	-	Inflammation
J16	63	M	Salivary gland	Chronic Inflammation of right submandibular gland	-	-	-	Inflammation
K1	32	F	Upper jaw	Chronic mucositis of right upper jaw	-	-	-	Inflammation
K2	32	F	Upper jaw	Chronic mucositis of right upper jaw	-	-	-	Inflammation
K3	75	F	Lip	Chronic Inflammation of skin of lower lip of No.49	-	-	-	Inflammation
K4	75	F	Lip	Chronic Inflammation of skin	-	-	-	Inflammation

				of lower lip of No.50				
K5	52	F	Tongue	Chronic Inflammation	-	*	-	Inflammation
K6	52	F	Tongue	Chronic Inflammation	-	*	-	Inflammation
K7	15	M	Salivary gland	Chronic Inflammation	-	-	-	Inflammation
K8	15	M	Salivary gland	Chronic Inflammation	-	-	-	Inflammation
K9	48	F	Salivary gland	Adjacent normal salivary gland tissue of No.55	-	-	-	NAT
K10	48	F	Salivary gland	Adjacent normal salivary gland tissue of No.56	-	-	-	NAT
K11	37	M	Salivary gland	Adjacent normal salivary gland tissue	-	-	-	NAT
K12	37	M	Salivary gland	Adjacent normal salivary gland tissue	-	-	-	NAT
K13	63	M	Salivary gland	Adjacent normal salivary gland tissue	-	-	-	NAT
K14	63	M	Salivary gland	Adjacent normal salivary gland tissue	-	-	-	NAT
K15	45	M	Salivary gland	Adjacent normal	-	-	-	NAT

				salivary gland tissue				
K16	45	M	Salivary gland	Adjacent normal salivary gland tissue	-	-	-	NAT
L1	45	M	Salivary gland	Cancer adjacent salivary gland tissue	-	-	-	AT
L2	45	M	Salivary gland	Cancer adjacent salivary gland tissue	-	-	-	AT
L3	47	M	Salivary gland	Adjacent normal salivary gland tissue	-	-	-	NAT
L4	47	M	Salivary gland	Adjacent normal salivary gland tissue	-	-	-	NAT
L5	13	F	Salivary gland	Adjacent normal salivary gland tissue	-	-	-	NAT
L6	13	F	Salivary gland	Adjacent normal salivary gland tissue	-	-	-	NAT
L7	33	F	Salivary gland	Adjacent normal salivary gland tissue (sparse)	-	-	-	NAT
L8	33	F	Salivary gland	Adjacent normal salivary gland tissue	-	-	-	NAT

L9	39	F	Tongue	Adjacent normal tongue tissue of No.39	-	-	-	NAT
L10	39	F	Tongue	Adjacent normal tongue tissue of No.40	-	-	-	NAT
L11	51	F	Tongue	Adjacent normal tongue tissue	-	-	-	NAT
L12	51	F	Tongue	Adjacent normal tongue tissue	-	-	-	NAT
L13	43	M	Tongue	Tongue tissue	-	-	-	Normal
L14	43	M	Tongue	Tongue tissue	-	-	-	Normal
L15	42	F	Tongue	Tongue tissue	-	-	-	Normal
L16	42	F	Tongue	Tongue tissue	-	-	-	Normal
M1	21	F	Tongue	Tongue tissue	-	-	-	Normal
M2	21	F	Tongue	Tongue tissue	-	-	-	Normal
M3	16	M	Tongue	Tongue tissue	-	-	-	Normal
M4	16	M	Tongue	Tongue tissue	-	-	-	Normal
M5	48	M	Tongue	Tongue tissue	-	-	-	Normal
M6	48	M	Tongue	Tongue tissue	-	-	-	Normal
M7	22	M	Salivary gland	Salivary gland tissue	-	-	-	Normal
M8	22	M	Salivary gland	Salivary gland tissue	-	-	-	Normal
M9	50	M	Salivary gland	Salivary gland tissue	-	-	-	Normal
M10	50	M	Salivary gland	Salivary gland	-	-	-	Normal

				tissue				
M11	15	F	Tongue	Tongue tissue	-	-	-	Normal
M12	15	F	Tongue	Tongue tissue	-	-	-	Normal
M13	45	M	Salivary gland	Salivary gland tissue	-	-	-	Normal
M14	45	M	Salivary gland	Salivary gland tissue	-	-	-	Normal
M15	38	F	Salivary gland	Salivary gland tissue	-	-	-	Normal
M16	38	F	Salivary gland	Salivary gland tissue	-	-	-	Normal
-	58	M	Skin	Malignant melanoma (tissue marker)		-		Malignant

Supplementary Table 2.2: Scoring method for IHC

Score (P)	**0**	**1+**	**2+**	**3+**	**4+**
Positive cells	**<10%**	**10-25%**	**25-50%**	**50-75%**	**>75%**
Score (I)	**1**	**2**	**3**	**Total expression score** **Q= P*I**	
Intensity of Stain	**Weak stain**	**Moderate stain**	**Strong stain**		

Supplementary Table 2.3: Details of tissue samples derived from OC patients from NECHRI

Patients Sl. No.	Age	Sex	Anatomic Site
1	40	M	Tongue
2	25	M	Nasopharynx
3	52	M	Tongue
4	48	M	Buccal mucosa
5	68	F	Palate
6	65	M	BOT (L) tonsil, soft palate
7	43	M	Oral cavity
8	43	M	Oral cavity
9	66	M	Lateral Pharyngel Wall
10	52	F	Tongue
11	56	M	Tongue
12	49	M	Tongue
13	49	M	Tongue
14	57	M	Tongue
15	57	M	Tongue
16	52	M	Tongue
17	47	M	Lower LIP+LT. buccal mucosa
18	48	F	Buccal mucosa
19	53	M	Mandible (R)
20	65	M	DL Supra gloti
21	61	M	Buccal mucosa
22	49	M	Tongue
23	62	M	BOT
24	47	M	R Maxilla
25	65	M	R Buccal mucosa
26	58	M	R Buccal mucosa + RMT
27	56	M	L Buccal mucosa
28	57	M	Buccal mucosa
29	50	M	L Buccal mucosa
30	55	M	Ca R
31	52	M	Buccal mucosa
32	54	M	Thyroid
33	54	M	Thyroid
34	62	M	H & N
35	37	M	GBS+RMT+ Buccal

			mucosa
36	56	M	H & N
37	45	M	Esophagus
38	42	M	H & N
39	42	M	H & N
40	45	F	H & N
41	45	F	H & N
42	50	F	H & N
43	50	F	H & N
44	42	M	H & N
45	42	M	H & N
46	57	M	H & N
47	77	M	H & N
48	77	M	H & N
49	41	F	H & N
50	42	F	H & N
51	70	F	H & N

Supplementary Table 3.1: Details of the primary and secondary antibodies used for Western blot

Name	Details	Dilution
Anti-GAPDH antibody	2118S, Cell Signaling Technology, USA	1:2000
Anti-p53 antibody	# BB-AB0100S, Bio Bharati Life Science, India	1:2000
Anti-p27 antibody	3686, Cell Signaling Technology, USA	1:2000
Anti-p21 antibody	2947, Cell Signaling Technology, USA	1:2000
Anti-p18 antibody	2896, Cell Signaling Technology, USA	1:2000
Anti-phospho-wee1 (Ser642)	4910, Cell Signaling Technology, USA	1:2000
Anti-cyclin D1 antibody	2978, Cell Signaling Technology, USA	1:2000
Anti-cyclin B1 antibody	12231, Cell Signaling Technology, USA	1:2000
Anti-cyclin E2 antibody	4132, Cell Signaling Technology, USA	1:2000
Anti-phospho- Akt (Ser473) antibody	4060S, Cell Signaling Technology, USA	1: 2000
Anti- Akt1 antibody	2938S, Cell Signaling Technology, USA	1: 2000
Anti-phospho- mTOR (Ser2448) antibody	5536T, Cell Signaling Technology, USA	1: 2000

Anti-mTOR antibody	2983T, Cell Signaling Technology, USA	1: 2000
Anti-phospho- S6 ribosomal protein (Ser235/236) antibody	4858T, Cell Signaling Technology, USA	1: 2000
Anti-S6 ribosomal protein antibody	2317S, Cell Signaling Technology, USA	1: 2000
Anti-p21 antibody	2947, Cell Signaling Technology, USA	1:2000
Anti-Caspase3 antibody	BB-AB0243S, Bio Bharati Life Science, India	1: 2000
Anti-Caspase9 antibody	BB-AB0245S, Bio Bharati Life Science, India	1: 2000
Anti-E-cadherin antibody	3195S, Cell Signaling Technology, USA	1: 2000
Anti-N-cadherin antibody	13116S, Cell Signaling Technology, USA	1: 2000
Anti-Bcl-2 antibody	Bio Bharati Life Science, India	1: 2000
Anti-LC3B antibody	2775S, Cell Signaling Technology, USA	1: 2000
Anti-COX-2 antibody	12282P, Cell Signaling Technology, USA	1: 2000
Anti-survivin antibody	2808BC, Cell Signaling Technology, USA	1: 2000
Anti-CXCR4 antibody	ab124824, abcam, Cambridge, USA	1: 2000
Anti-MMP-2 antibody	4022, Cell Signaling Technology, USA	1: 2000
Anti-MMP-9 antibody	13667P, Cell Signaling Technology, USA	1: 1500
Anti-VEGF-A antibody	ab46154, abcam, Cambridge, USA	1: 2000
Anti-rabbit secondary antibody	ab97080, abcam, Cambridge, USA	1: 6000
Anti-mouse secondary antibody	ab97040, abcam, Cambridge, USA	1: 6000

Figure legends:

1. Unnikrishnan J, Hegde M, Kumar A, **Girisa S**, Satpati P, Kunnumakkara AB. Biogenic Synthesis and Characterization of Ethyl Ferulate Gold Nanoparticle and its Efficacy against Triple-Negative Breast Cancer Cells. Current Nanomedicine. 2025 Feb;15(1):80-94.

2. Mehta K, Hegde M, **Girisa S**, Vishwa R, Alqahtani MS, Abbas M, Shakibaei M, Sethi G, Kunnumakkara AB. Targeting RTKs/nRTKs as promising therapeutic strategies for the treatment of triple-negative breast cancer: evidence from clinical trials. Mil Med Res. 2024 Dec 12;11(1):76. doi: 10.1186/s40779-024-00582-z. PMID: 39668367; PMCID: PMC11636053.

3. Kumar A, Parama D, Rana V, Daimary UD, **Girisa S**, Kunnumakkara AB. Personalized Medicine in Clinical Management of Breast Cancer: Where Do We Stand?. InMolecular Biomarkers for Cancer Diagnosis and Therapy 2024 Jun 30 (pp. 579-618). Singapore: Springer Nature Singapore.

4. Hegde M, **Girisa S**, Aswani BS, Alqahtani MS, Abbas M, Sethi G, Kunnumakkara AB. Harnessing potential role of gangliosides in immunomodulation and cancer therapeutics. Life Sci. 2024 Aug 15;351:122786. doi: 10.1016/j.lfs.2024.122786. Epub 2024 Jun 5. PMID: 38848944.

5. Vishwa R, BharathwajChetty B, **Girisa S**, Aswani BS, Alqahtani MS, Abbas M, Hegde M, Kunnumakkara AB. Lipid metabolism and its implications in tumor cell plasticity and drug resistance: what we learned thus far? Cancer Metastasis Rev. 2024 Mar;43(1):293-319. doi: 10.1007/s10555-024-10170-1. Epub 2024 Mar 5. PMID: 38438800.

6. Manickasamy MK, Jayaprakash S, **Girisa S**, Kumar A, Lam HY, Okina E, Eng H, Alqahtani MS, Abbas M, Sethi G, Kumar AP, Kunnumakkara AB. Delineating the role of nuclear receptors in colorectal cancer, a focused review. Discov Oncol. 2024 Feb 19;15(1):41. doi: 10.1007/s12672-023-00808-x. PMID: 38372868; PMCID: PMC10876515.

7. Sailo BL, Chauhan S, Hegde M, **Girisa S**, Alqahtani MS, Abbas M, Goel A, Sethi G, Kunnumakkara AB. Therapeutic potential of tocotrienols as chemosensitizers in cancer therapy. Phytother Res. 2024 Feb 14. doi: 10.1002/ptr.8131. Epub ahead of print. PMID: 38353331.

8. Unnikrishnan J, Hegde M, **Girisa S**, Satpati P, Kunnumakkara AB. Ellagic Acid-Gold Nanoparticle Complex as Anticancer Agent in Triple-Negative Breast Cancer Cells: Nano LIFE. 2024; 14(02):2450003.

9. Sailo BL, Liu L, Chauhan S, **Girisa S**, Hegde M, Liang L, Alqahtani MS, Abbas M, Sethi G, Kunnumakkara AB. Harnessing Sulforaphane Potential as a Chemosensitizing Agent: A Comprehensive Review. Cancers (Basel). 2024

Jan 5;16(2):244. doi: 10.3390/cancers16020244. PMID: 38254735; PMCID: PMC10814109.

10. Hegde M, **Girisa S**, Harsha C. Primary cell culture for organ-on-a-chip. InHuman Organs-on-a-Chip Technology 2024 Jan 1 (pp. 175-197). Academic Press.

11. Hegde M, **Girisa S**, Choudhary H. Human organs-on-a-chip for cancer modeling. InHuman Organs-on-a-Chip Technology 2024 Jan 1 (pp. 549-581). Academic Press.

12. Purohit S, **Girisa S**, Ochiai Y, Kunnumakkara AB, Sahoo L, Yanase E, Goud VV. Scirpusin B isolated from Passiflora edulis Var. flavicarpa attenuates carbohydrate digestive enzymes, pathogenic bacteria and oral squamous cell carcinoma. 3 Biotech. 2024 Jan;14(1):28. doi: 10.1007/s13205-023-03876-6. Epub 2024 Jan 2. PMID: 38173823; PMCID: PMC10758380.

13. Hegde M, Kumar A, **Girisa S**, Aswani BS, Vishwa R, Sethi G, Kunnumakkara AB. Nanoformulations of curcumin: An alliance for effective cancer therapeutics. Food Bioscience. 2023 Dec 1;56:103095.

14. **Girisa S,** Hegde M, Kunnumakkara AB. Molecular Mechanism of Spices and Their Active Constituents for the Prevention and Treatment of Diseases. InHandbook of Spices in India: 75 Years of Research and Development 2023 Oct 11 (pp. 695-753). Singapore: Springer Nature Singapore.

15. Hegde M, **Girisa S**, Devanarayanan TN, Alqahtani MS, Abbas M, Sethi G, Kunnumakkara AB. Network of extracellular traps in the pathogenesis of sterile chronic inflammatory diseases: Role of oxidative stress and potential clinical applications. Antioxid Redox Signal. 2023 Sep 19. doi: 10.1089/ars.2023.0329. Epub ahead of print. PMID: 37725535.

16. Hegde M, Kumar A, **Girisa S**, Alqahtani MS, Abbas M, Goel A, Hui KM, Sethi G, Kunnumakkara AB. Exosomal noncoding RNA-mediated spatiotemporal regulation of lipid metabolism: Implications in immune evasion and chronic inflammation. Cytokine Growth Factor Rev. 2023 Oct;73:114-134. doi: 10.1016/j.cytogfr.2023.06.001. Epub 2023 Jun 16. PMID: 37419767.

17. Hegde M, **Girisa S**, BharathwajChetty B, Vishwa R, Kunnumakkara AB. Curcumin Formulations for Better Bioavailability: What We Learned from Clinical Trials Thus Far? ACS Omega. 2023 Mar 13;8(12):10713-10746. doi: 10.1021/acsomega.2c07326. PMID: 37008131; PMCID: PMC10061533.

18. **Girisa S**, Kumar A, Kunnumakkara AB. Curcumin as a Chemopreventive Agent for Oral Submucous Fibrosis. Oral Submucous Fibrosis: A Guide to Diagnosis and Management. 2023 May 6:299-312.

19. Kumar A, **Girisa S**, Alqahtani MS, Abbas M, Hegde M, Sethi G, Kunnumakkara AB. Targeting Autophagy Using Long Non-Coding RNAs (LncRNAs): New Landscapes in the Arena of Cancer Therapeutics. Cells. 2023 Mar 6;12(5):810. doi: 10.3390/cells12050810. PMID: 36899946; PMCID: PMC10000689.

20. Kunnumakkara AB, Hegde M, Parama D, **Girisa S**, Kumar A, Daimary UD, Garodia P, Yenisetti SC, Oommen OV, Aggarwal BB. Role of Turmeric and Curcumin in Prevention and Treatment of Chronic Diseases: Lessons Learned from Clinical Trials. ACS Pharmacol Transl Sci. 2023 Mar 6;6(4):447-518. doi: 10.1021/acsptsci.2c00012. PMID: 37082752; PMCID: PMC10111629.

21. Brockmueller A, **Girisa S**, Kunnumakkara AB, Shakibaei M. Resveratrol Modulates Chemosensitisation to 5-FU via β1-Integrin/HIF-1α Axis in CRC Tumor Microenvironment. Int J Mol Sci. 2023 Mar 5;24(5):4988. doi: 10.3390/ijms24054988. PMID: 36902421; PMCID: PMC10003050.

22. Jose S, Devi SS, Sajeev A, **Girisa S**, Alqahtani MS, Abbas M, Alshammari A, Sethi G, Kunnumakkara AB. Repurposing FDA-approved drugs as FXR agonists: a structure based in silico pharmacological study. Biosci Rep. 2023 Mar 31;43(3):BSR20212791. doi: 10.1042/BSR20212791. PMID: 35348180; PMCID: PMC9977715.

23. Hegde M, **Girisa S**, Kunnumakkara AB. A compilation of bioinformatic approaches to identify novel downstream targets for the detection and prophylaxis of cancer. Adv Protein Chem Struct Biol. 2023;134:75-113. doi: 10.1016/bs.apcsb.2022.11.015. Epub 2023 Feb 18. PMID: 36858743.

24. Venkat R, Verma E, Daimary UD, Kumar A, **Girisa S**, Dutta U, Ahn KS, Kunnumakkara AB. The Journey of Resveratrol from Vineyards to Clinics. Cancer Invest. 2023 Feb;41(2):183-220. doi: 10.1080/07357907.2022.2115057. Epub 2022 Sep 26. PMID: 35993769.

25. Hegde M, Naliyadhara N, Unnikrishnan J, Alqahtani MS, Abbas M, **Girisa S**, Sethi G, Kunnumakkara AB. Nanoparticles in the diagnosis and treatment of cancer metastases: Current and future perspectives. Cancer Lett. 2023 Mar 1;556:216066. doi: 10.1016/j.canlet.2023.216066. Epub 2023 Jan 14. PMID: 36649823.

26. Brockmueller A, **Girisa S**, Motallebi M, Kunnumakkara AB, Shakibaei M. Calebin A targets the HIF-1α/NF-κB pathway to suppress colorectal cancer cell migration. Front Pharmacol. 2023 Jul 31;14:1203436. doi: 10.3389/fphar.2023.1203436. PMID: 37583906; PMCID: PMC10423823.

27. Hegde M, **Girisa S**, Naliyadhara N, Kumar A, Alqahtani MS, Abbas M, Mohan CD, Warrier S, Hui KM, Rangappa KS, Sethi G, Kunnumakkara AB.

Natural compounds targeting nuclear receptors for effective cancer therapy. Cancer Metastasis Rev. 2022 Dec 8. doi: 10.1007/s10555-022-10068-w. Epub ahead of print. PMID: 36482154.

28. Aswathy M, Vijayan A, Daimary UD, **Girisa S**, Radhakrishnan KV, Kunnumakkara AB. Betulinic acid: A natural promising anticancer drug, current situation, and future perspectives. J Biochem Mol Toxicol. 2022 Dec;36(12):e23206. doi: 10.1002/jbt.23206. Epub 2022 Sep 19. PMID: 36124371.

29. Jayaprakash S, Hegde M, BharathwajChetty B, **Girisa S**, Alqahtani MS, Abbas M, Sethi G, Kunnumakkara AB. Unraveling the Potential Role of NEDD4-like E3 Ligases in Cancer. Int J Mol Sci. 2022 Oct 16;23(20):12380. doi: 10.3390/ijms232012380. PMID: 36293239; PMCID: PMC9604169.

30. Jayaprakash S, Hegde M, **Girisa S**, Alqahtani MS, Abbas M, Lee EHC, Yap KC, Sethi G, Kumar AP, Kunnumakkara AB. Demystifying the Functional Role of Nuclear Receptors in Esophageal Cancer. Int J Mol Sci. 2022 Sep 19;23(18):10952. doi: 10.3390/ijms231810952. PMID: 36142861; PMCID: PMC9501100.

31. Sajeev A, Hegde M, **Girisa S**, Devanarayanan TN, Alqahtani MS, Abbas M, Sil SK, Sethi G, Chen JT, Kunnumakkara AB. Oroxylin A: A Promising Flavonoid for Prevention and Treatment of Chronic Diseases. Biomolecules. 2022 Aug 26;12(9):1185. doi: 10.3390/biom12091185. PMID: 36139025; PMCID: PMC9496116.

32. Sajeev A, Hegde M, Daimary UD, Kumar A, **Girisa S**, Sethi G, Kunnumakkara AB. Modulation of diverse oncogenic signaling pathways by oroxylin A: An important strategy for both cancer prevention and treatment. Phytomedicine. 2022 Oct;105:154369. doi: 10.1016/j.phymed.2022.154369. Epub 2022 Aug 1. PMID: 35985182.

33. Khatoon E, Parama D, Kumar A, Alqahtani MS, Abbas M, **Girisa S**, Sethi G, Kunnumakkara AB. Targeting PD-1/PD-L1 axis as new horizon for ovarian cancer therapy. Life Sci. 2022 Oct 1;306:120827. doi: 10.1016/j.lfs.2022.120827. Epub 2022 Jul 27. PMID: 35907493.

34. Paul T, Janakiraman I, Manikandan NA, Pakshirajan K, Pugazhenthi G, **Girisa S**, Kunnumakkara AB. Reuse Potential of Refinery Wastewater Treated Using a Two-Stage Submerged Membrane Bioreactor. Chemical Engineering & Technology. 2022 Jun;45(6):1017-26.

35. Hegde M, Daimary UD, **Girisa S**, Kumar A, Kunnumakkara AB. Tumor cell anabolism and host tissue catabolism-energetic inefficiency during cancer cachexia. Exp Biol Med (Maywood). 2022 May;247(9):713-733. doi:

10.1177/15353702221087962. Epub 2022 May 6. PMID: 35521962; PMCID: PMC9134760.

36. Parama D, **Girisa S**, Khatoon E, Kumar A, Alqahtani MS, Abbas M, Sethi G, Kunnumakkara AB. An overview of the pharmacological activities of scopoletin against different chronic diseases. Pharmacol Res. 2022 May;179:106202. doi: 10.1016/j.phrs.2022.106202. Epub 2022 Apr 1. PMID: 35378275.

37. Naliyadhara N, Kumar A, **Girisa S**, Daimary UD, Hegde M, Kunnumakkara AB. Pulsed electric field (PEF): Avant-garde extraction escalation technology in food industry. Trends in Food Science & Technology. 2022 Apr 1;122:238-55.

38. Bordoloi D, Harsha C, Padmavathi G, Banik K, Sailo BL, Roy NK, **Girisa S**, Thakur KK, Khwairakpam AD, Chinnathambi A, Alahmadi TA, Alharbi SA, Shakibaei M, Kunnumakkara AB. Loss of TIPE3 reduced the proliferation, survival and migration of lung cancer cells through inactivation of Akt/mTOR, NF-κB, and STAT-3 signaling cascades. Life Sci. 2022 Mar 15;293:120332. doi: 10.1016/j.lfs.2022.120332. Epub 2022 Jan 15. PMID: 35041835.

39. Devi Daimary U, **Girisa S**, Parama D, Verma E, Kumar A, Kunnumakkara AB. Embelin: A novel XIAP inhibitor for the prevention and treatment of chronic diseases. J Biochem Mol Toxicol. 2022 Feb;36(2):e22950. doi: 10.1002/jbt.22950. Epub 2021 Nov 29. PMID: 34842329.

40. Padmavathi G, Monisha J, Bordoloi D, Banik K, Roy NK, **Girisa S**, Singh AK, Longkumer I, Baruah MN, Kunnumakkara AB. Tumor necrosis factor-α induced protein 8 (TNFAIP8/TIPE) family is differentially expressed in oral cancer and regulates tumorigenesis through Akt/mTOR/STAT3 signaling cascade. Life Sci. 2021 Dec 15;287:120118. doi: 10.1016/j.lfs.2021.120118. Epub 2021 Nov 2. PMID: 34740574.

41. Rana V, Parama D, Khatoon E, **Girisa S**, Sethi G, Kunnumakkara AB. Reiterating the Emergence of Noncoding RNAs as Regulators of the Critical Hallmarks of Gall Bladder Cancer. Biomolecules. 2021 Dec 8;11(12):1847. doi: 10.3390/biom11121847. PMID: 34944491; PMCID: PMC8699045.

42. Kumar A, Harsha C, Parama D, **Girisa S**, Daimary UD, Mao X, Kunnumakkara AB. Current clinical developments in curcumin-based therapeutics for cancer and chronic diseases. Phytother Res. 2021 Dec;35(12):6768-6801. doi: 10.1002/ptr.7264. Epub 2021 Sep 8. PMID: 34498308.

43. **Girisa S**, Henamayee S, Parama D, Rana V, Dutta U, Kunnumakkara AB. Targeting Farnesoid X receptor (FXR) for developing novel therapeutics

against cancer. Mol Biomed. 2021 Jul 10;2(1):21. doi: 10.1186/s43556-021-00035-2. PMID: 35006466; PMCID: PMC8607382.

44. Verma E, Kumar A, Daimary UD, Parama D, **Girisa S**, Sethi G, Kunnumakkara AB. Potential of baicalein in the prevention and treatment of cancer: A scientometric analyses based review. Journal of Functional Foods. 2021 Nov 1;86:104660.

45. Kunnumakkara AB, Rana V, Parama D, Banik K, **Girisa S**, Henamayee S, Thakur KK, Dutta U, Garodia P, Gupta SC, Aggarwal BB. COVID-19, cytokines, inflammation, and spices: How are they related? Life Sci. 2021 Nov 1;284:119201. doi: 10.1016/j.lfs.2021.119201. Epub 2021 Feb 16. PMID: 33607159; PMCID: PMC7884924.

46. Bordoloi D, Padmavathi G, Banik K, Devi KA, Harsha C, **Girisa S**, Buhrmann C, Shakibaei M, Kunnumakkara AB. Human tumor necrosis factor alpha-induced protein eight-like 1 exhibited potent anti-tumor effect through modulation of proliferation, survival, migration and invasion of lung cancer cells. Mol Cell Biochem. 2021 Sep;476(9):3303-3318. doi: 10.1007/s11010-021-04060-1. Epub 2021 Apr 25. PMID: 33895911.

47. Gupta MK, Senthilkumar S, Chiranjivi AK, Banik K, **Girisa S**, Kunnumakkara AB, Dubey VK, Rangan L. Antioxidant, anti-tyrosinase and anti-inflammatory activities of 3, 5-dihydroxy-4′, 7-dimethoxyflavone isolated from the leaves of Alpinia nigra. Phytomedicine Plus. 2021 Aug 1;1(3):100097.

48. **Girisa S**, Saikia Q, Bordoloi D, Banik K, Monisha J, Daimary UD, Verma E, Ahn KS, Kunnumakkara AB. Xanthohumol from Hop: Hope for cancer prevention and treatment. IUBMB Life. 2021 Aug;73(8):1016-1044. doi: 10.1002/iub.2522. Epub 2021 Jul 6. PMID: 34170599.

49. **Girisa S**, Kumar A, Rana V, Parama D, Daimary UD, Warnakulasuriya S, Kumar AP, Kunnumakkara AB. From Simple Mouth Cavities to Complex Oral Mucosal Disorders-Curcuminoids as a Promising Therapeutic Approach. ACS Pharmacol Transl Sci. 2021 Mar 17;4(2):647-665. doi: 10.1021/acsptsci.1c00017. PMID: 33860191; PMCID: PMC8033761.

50. Ahmed SA, Parama D, Daimari E, **Girisa S**, Banik K, Harsha C, Dutta U, Kunnumakkara AB. Rationalizing the therapeutic potential of apigenin against cancer. Life Sci. 2021 Feb 15;267:118814. doi: 10.1016/j.lfs.2020.118814. Epub 2020 Dec 30. PMID: 33333052.

51. Rana V, Parama D, **Girisa S**, Harsha C, Kunnumakkara AB. Oxidative stress and inflammation. InAntioxidants and Functional Foods for Neurodegenerative Disorders 2021 Jan 7 (pp. 21-36). CRC Press.

52. Parama D, Rana V, **Girisa S**, Verma E, Daimary UD, Thakur KK, Kumar A, Kunnumakkara AB. The promising potential of piperlongumine as an emerging therapeutics for cancer. Explor Target Antitumor Ther. 2021;2(4):323-354. doi: 10.37349/etat.2021.00049. Epub 2021 Aug 30. PMID: 36046754; PMCID: PMC9400693.

53. **Girisa S**, Rana V, Parama D, Dutta U, Kunnumakkara AB. Differential roles of farnesoid X receptor (FXR) in modulating apoptosis in cancer cells. Adv Protein Chem Struct Biol. 2021;126:63-90. doi: 10.1016/bs.apcsb.2021.02.006. Epub 2021 Mar 31. PMID: 34090620.

54. Bordoloi D, Banik K, Vikkurthi R, Thakur KK, Padmavathi G, Sailo BL, **Girisa S**, Chinnathambi A, Alahmadi TA, Alharbi SA, Buhrmann C, Shakibaei M, Kunnumakkara AB. Inflection of Akt/mTOR/STAT-3 cascade in TNF-α induced protein 8 mediated human lung carcinogenesis. Life Sci. 2020 Dec 1;262:118475. doi: 10.1016/j.lfs.2020.118475. Epub 2020 Sep 22. PMID: 32976884.

55. Garodia P, **Girisa S**, Rana V, Kunnumakkara AB, Aggarwal BB. Lessons to be learnt from ayurveda: Nutraceuticals and cosmeceuticals from ayurveda herbs. InAyurveda in the new millennium 2020 Nov 10 (pp. 199-222). CRC Press.

56. Harsha C, Banik K, Ang HL, **Girisa S**, Vikkurthi R, Parama D, Rana V, Shabnam B, Khatoon E, Kumar AP, Kunnumakkara AB. Targeting AKT/mTOR in Oral Cancer: Mechanisms and Advances in Clinical Trials. Int J Mol Sci. 2020 May 6;21(9):3285. doi: 10.3390/ijms21093285. PMID: 32384682; PMCID: PMC7246494.

57. Banik K, Ranaware AM, Harsha C, Nitesh T, **Girisa S**, Deshpande V, Fan L, Nalawade SP, Sethi G, Kunnumakkara AB. Piceatannol: A natural stilbene for the prevention and treatment of cancer. Pharmacol Res. 2020 Mar;153:104635. doi: 10.1016/j.phrs.2020.104635. Epub 2020 Jan 8. PMID: 31926274.

58. **Girisa S**, Parama D, Harsha C, Banik K, Kunnumakkara AB. Potential of guggulsterone, a farnesoid X receptor antagonist, in the prevention and treatment of cancer. Explor Target Antitumor Ther. 2020;1(5):313-342. doi: 10.37349/etat.2020.00019. Epub 2020 Oct 30. PMID: 36046484; PMCID: PMC9400725.

59. Khwairakpam AD, Banik K, **Girisa S**, Shabnam B, Shakibaei M, Fan L, Arfuso F, Monisha J, Wang H, Mao X, Sethi G, Kunnumakkara AB. The vital role of ATP citrate lyase in chronic diseases. J Mol Med (Berl). 2020

Jan;98(1):71-95. doi: 10.1007/s00109-019-01863-0. Epub 2019 Dec 19. PMID: 31858156.

60. Kunnumakkara AB, Shabnam B, **Girisa S**, Harsha C, Banik K, Devi TB, Choudhury R, Sahu H, Parama D, Sailo BL, Thakur KK, Gupta SC, Aggarwal BB. Inflammation, NF-κB, and Chronic Diseases: How are They Linked? Crit Rev Immunol. 2020;40(1):1-39. doi: 10.1615/CritRevImmunol.2020033210. PMID: 32421977.

61. Sailo BL, Banik K, **Girisa S**, Bordoloi D, Fan L, Halim CE, Wang H, Kumar AP, Zheng D, Mao X, Sethi G, Kunnumakkara AB. FBXW7 in Cancer: What Has Been Unraveled Thus Far? Cancers (Basel). 2019 Feb 19;11(2):246. doi: 10.3390/cancers11020246. PMID: 30791487; PMCID: PMC6406609.

62. **Girisa S**, Shabnam B, Monisha J, Fan L, Halim CE, Arfuso F, Ahn KS, Sethi G, Kunnumakkara AB. Potential of Zerumbone as an Anti-Cancer Agent. Molecules. 2019 Feb 18;24(4):734. doi: 10.3390/molecules24040734. PMID: 30781671; PMCID: PMC6413012.

63. Singh YP, **Girisa S**, Banik K, Ghosh S, Swathi P, Deka M, Padmavathi G, Kotoky J, Sethi G, Fan L, Mao X. Potential application of zerumbone in the prevention and therapy of chronic human diseases. Journal of Functional Foods. 2019 Feb 1;53:248-58.

64. Kunnumakkara AB, Thakur KK, Rana V, Bora B, Banik K, Khatoon E, Sailo BL, Shabnam B, **Girisa S**, Gupta SC, Aggarwal BB. Upside and Downside of Tumor Necrosis Factor Blockers for Treatment of Immune/Inflammatory Diseases. Crit Rev Immunol. 2019;39(6):439-479. doi: 10.1615/CritRevImmunol.2020033205. PMID: 32421957.

65. Shabnam B, Padmavathi G, Banik K, **Girisa S**, Monisha J, Sethi G, Fan L, Wang L, Mao X, Kunnumakkara AB. Sorcin a Potential Molecular Target for Cancer Therapy. Transl Oncol. 2018 Dec;11(6):1379-1389. doi: 10.1016/j.tranon.2018.08.015. Epub 2018 Sep 11. PMID: 30216763; PMCID: PMC6134165.

1. Participated and delivered oral presentation at 'Emerging Approaches in Risk Analysis and Translational Aspects of Health and Environment (EARTH)' international convention held from November 27-30th, 2024 at CSIR-Indian Institute of Toxicology Research, Lucknow, India.
2. Participated in one-month online internship/workshop for 'Ensembl Browser and Multi-omics Data Analysis' held from August 01-23, 2024 hosted by Nextgenhelper.
3. Presented scientific poster at International Conference on Drug Discovery and Development (IC3D) 2024 held from 13-14th May 2024 in Penang, Malaysia.
4. Presented oral presentation at 6th International Conference on Nutraceuticals and Chronic Diseases (INCD)' from 22nd-24rd February 2024 at Panjab University Chandigarh, India.
5. Delivered an invited talk entitled "Studies on the role of Mortalin in oral cancer" in the Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore (NUS), on 7th November, 2023.
6. Presented scientific poster at Frontiers in Cancer Science 2023 international conference held from 6-8th November, 2023 in NUS, Singapore.
7. Participated and presented poster at '5th International Conference on Nutraceuticals and Chronic Diseases (INCD)' from 7th-9th October 2022 at University of Delhi, Delhi.
8. Attended international virtual conference on 'Frontiers in Health Sciences' organized by Cotton University, Guwahati from August 7th -8th, 2020.
9. Participated and presented poster at 'Fourth International Conference on Nutraceuticals and Chronic Diseases (INCD)' from 23-25th September 2019 at Indian Institute of Technology Guwahati (IITG)
10. Attended DHR-ICMR training workshop on 'Diagnosis in oncology using flow cytometry and real time PCR technology at North East Cancer Hospital and Research Institute (NECHRI), Guwahati, 2019.
11. Participated in Indo-Japan symposium on 'Recent Advances in Biomedical Research (RABR)' organized at Indian Institute of Technology Guwahati (IITG) 26th -27th March 2019
12. Participated in '20th Indo-US Flow Cytometry Symposium Cum Workshop on Applications of Flow Cytometry in Biotechnology' organized at Indian Institute of Technology Guwahati (IITG) from 13th -16th March 2019
13. Presented a poster at 'Research Conclave 2019, at Indian Institute of Technology Guwahati (IITG) held during 14th – 17th March, 2019.

14. Participated in a workshop on 'Drug Discovery Technology' held at Indian Institute of Technology (IIT) Delhi during February 19-20, 2019.

15. Participated in Recent Advances in Cancer Research (RACR) workshop at Indian Institute of Technology Guwahati (IITG), 2018

16. Participated in hands on training on "Quality Control of Biologicals" at National Institute of Biologicals, Noida, 2017.

1. Qualified JNU-CEEB, under Department of Biotechnology (DBT), Government of India, 2015
2. Qualified DBT JRF (Category II), 2017
3. Qualified GATE (Biotechnology), 2017
4. Qualified GATE (Life sciences), 2018
5. Qualified CSIR-NET, 2019
6. Selected for 6th Imaging international workshop organized by AIST, Tsukuba, Japan, under Sakura Science exchange program, 2019
7. Received best poster award in 'Recent Advances in Cancer Research' workshop organized at Indian Institute of Technology Guwahati (IITG) from 27th-28th March 2019.
8. Selected for 3 months training on 'Advanced training in Biotechnology under DBT-STAR Program' at AIST, Tsukuba, Japan
9. Selected for 2nd DAICENTER-SHIMADZU Analytics Workshop held during September 16-20, 2019 at Shimadzu Analytical India Pvt. Ltd, Mumbai
10. Received best poster award at 'Translational Cancer Research (TCR) conference at Banaras Hindu University (BHU), Varanasi held during 13th - 16th February 2020.
11. Received best poster award under young scientist session at 6th International Conference on Nutraceuticals and Chronic Diseases (INCD)' from 22nd-24rd February 2024 at Panjab University Chandigarh.