Understanding the Isoform-specific Role of Akt Kinase in the Development of Oral Squamous Cell Carcinoma (OSCC)

A thesis submitted for the degree of

Doctor of Philosophy

To

INDIAN INSTITUTE OF TECHNOLOGY

GUWAHATI

By

NAND KISHOR ROY

Department of Biotechnology

INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI

GUWAHATI 781039, ASSAM, INDIA

March 2018

To my Gurus and loving parents

For seeding the traits of righteousness

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

DECLARATION

I hereby declare that the contents of the research work described in this thesis titled **"Understanding the Isoform-specific Role of Akt Kinase in the Development of Oral Squamous Cell Carcinoma (OSCC)"**, 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 Dr. Ajaikumar B Kunnumakkara.

An honest effort has 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.

March, 2018

Nand Kishor Roy
Roll no. 10610605
Department of Biosciences and Bioengineering
IIT Guwahati

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

CERTIFICATE

This is to certify that the work described in the thesis titled **"Understanding the Isoform-specific Role of Akt Kinase in the Development of Oral Squamous Cell Carcinoma (OSCC)"**, submitted by Nand Kishor Roy (Roll no: 10610605) 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, India.

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

March, 2018

Dr. Ajaikumar B Kunnumakkara
Associate Professor
Cancer Biology Laboratory &
DBT-AIST Internationational Laboratory for Advanced Biomedicine
Department of Biosciences and Bioengineering
Indian Institute of Technology Guwahati
Guwahati, India

Acknowledgements

My heart fills with gratitude to acknowledge the people in my life for their constant support and motivation throughout without whom my Ph.D would not have been successful.

I am grateful to ***Dr. Ajaikumar B Kunnumakkara*** *for keeping his belief in me and my abilities to do good research. I thank him for his valuable suggestions during lab meetings. Without his support and guidance, it would have been impossible for me to complete the thesis work.*

I am deeply grateful to my DC chairperson ***Prof. Kannan Pakshirajan*** *for his valuable suggestions for my work. Also, I am thankful to my committee members* ***Dr. Manish Kumar, Dr Soumen Maiti*** *and* ***Dr Piruthvi Sukumar*** *for their critical inputs.*

I would like to thanks the past and present HoD of the department ***Prof. Arun Goyal*** *and* ***Prof. Venkata Veeranki Dasu*** *and* ***Prof. Kannan Pakshirajan*** *for making available departmental facilities.*

I am grateful to ***Staffs of the department, janitors*** *and* ***securities*** *for provide the assistance during my PhD*

I am thankful to ***CSIR-UGC*** *for providing me the fellowship for my PhD.*

I acknowledge the MHRD, DST and DBT for providing the facilities in laboratory.

I would always be grateful to special people like ***Souvika Didi, Ganesh Daiji, Suradip, Arghya, Kartikeya, Anuj Bhai*** *and* ***Shivali*** *who supported me, uplifted me and brought joy to my life.*

I am thankful to my lab members; ***Monisha, Devivasha, Padmavathi, Amrita, Bethsibie, Kishore, Harsha, Krishan, Nafisha, Subhrajyoti, Nabojit, Shabnam, Sana, Anand, Jai Prakash, Ajit, Shyamananda, Raju, and Amit*** *for their immeasurable help.*

I appreciate the assistance that I received from many trainees; ***Sudheshna, Anushmita, Doni, Tetam, Aso, Queen, Anubhuti, and Gayathri.***

I express my deep gratitude to all my ***teachers*** *for their effort that they put in me for imparting the knowledge and inspiring me with hope.*

I would be indebted to my ***parents (Gauri and Dinesh)*** *forever for their love and care and seeding the principles of hardwork and righteousness and making me*

what I am today. I thank my ***elder sister Subhadhra and my elder brother Bishambhar*** *for their unconditional love, support and care.*

I would like to express thanks to my friends, batchmates and seniors for all the fun, friendship and care - ***Mithu, Shomyadeep, Ankita, Tulsi, Sudeep, Manoj, Ankana, Anamika, Lyani, Arabinda, Dr. Naresh, Dr. Ratul, Dr. Ajoy, Dr. Manav, Sunita, Papori, Junjyoti, Krishna, Biju, Kabiraj, Adiraj, Vimal, Dharitri, Dr. Jaipraksh, Bibhas, Ankit, Yogendra, Rahul, Mahesh, Navodeep, and Rakesh.***

Last but not the least I would like to thank the ***LORD SHIVA*** *for choosing me and giving me strength to walk through this incredible journey.*

Nand Kishor Roy

CONTENTS

Chapter 1

Introduction and Review of Literature

1.1. INTRODUCTION

Oral cancer is one of the most common cancers in the world that lead to approximately 145,400 deaths in the year 2012. It is more prevalent in Melanesia, South-Central Asia, and Central and Eastern Europe, while in Western Africa and Eastern Asia its cases are quite rare (Torre et al 2015). The variation in the incidence and pattern of this cancer are the result of several factors like age of the population and regional lifestyle differences (Manoharan et al 2010). In India, approximately 70,000 people are diagnosed with oral cancer and it causes 48,000 deaths annually (Leemans et al 2011, Elango et al 2009). An age-adjusted rate of oral cancer in India is 20 per 100,000 population which is remarkably very high compared to other countries (Sankaranarayanan et al 2005). Therefore, World Health Organization (WHO) considers oral cancer as a significant public health challenge faced by the Indian population and based on the incidence it ranks number one among men and third among women. As per the statistics of Indian Council of Medical Research (ICMR), there will be a marked increase in the number of oral cancer patients in India in the coming years (ICMR 2009). As the overall and disease-free survival (DFS) rates of OSCC patients remains unchanged for the past few years because of high mortality and low cure rate, the development of novel markers for diagnosis and prognosis and novel targets for developing efficacious therapies against this disease become imperative (Bell et al 2007, Massano et al 2006, Mydlarz et al 2010). It is now well established that Akt/mTOR pathway is highly upregulated in oral cancer and leads to its development. The main risk factors of oral cancer include tobacco, alcohol, HPV etc are also known to induce Akt/mTOR pathway (West et al 2003, Neasta et al 2011, Surviladze et al 2013). This pathway consists of many proteins which interact like a

network and induce different functions such as cancer cell survival and proliferation, transcription and translation of genes, invasion, angiogenesis, metastases etc. Akt kinase is the main protein of this pathway and is known to induce tumorigenesis by affecting different hallmarks of cancer such as cell proliferation and survival, cell size and response to nutrient availability, intermediary metabolism, angiogenesis, and tissue invasion (Bellacosa et al 2005). It is now well established that Akt kinase exists in three different isoforms of Akt1, Akt2, and Akt3 and plays distinct functions in various cancers. However, the exact role of these different isoforms in the development of oral cancer is poorly understood. Therefore, the present study was aimed at evaluating the role of different isoforms of Akt in the pathogenesis of oral cancer and to develop isoform-specific inhibitors for the treatment of this disease.

1.2. REVIEW OF LITERATURE

Oral cancer can be defined as the cancers of oral cavity and oropharynx. The oral cavity consists of lips, buccal mucosa, gingiva, hard palate, the floor of the mouth and anterior 2/3s of the tongue, while oropharynx involves posterior 1/3 of the tongue, soft palate, tonsils and posterior pharyngeal wall (Fig 1.1) (Neville et al 2009). Based on epidemiological and clinicopathological factors, "oral cancer" can be classified into three categories: carcinomas of the oral cavity proper, carcinomas of the lip vermilion, and carcinomas arising in the oropharynx (Brad and Terry 2002). The frequency of oral cancer is very high compared to all other head and neck cancers, of which 90% are oral squamous cell carcinoma of the oral cavity followed by adenocarcinomas and rarely other types of malignant tumors.

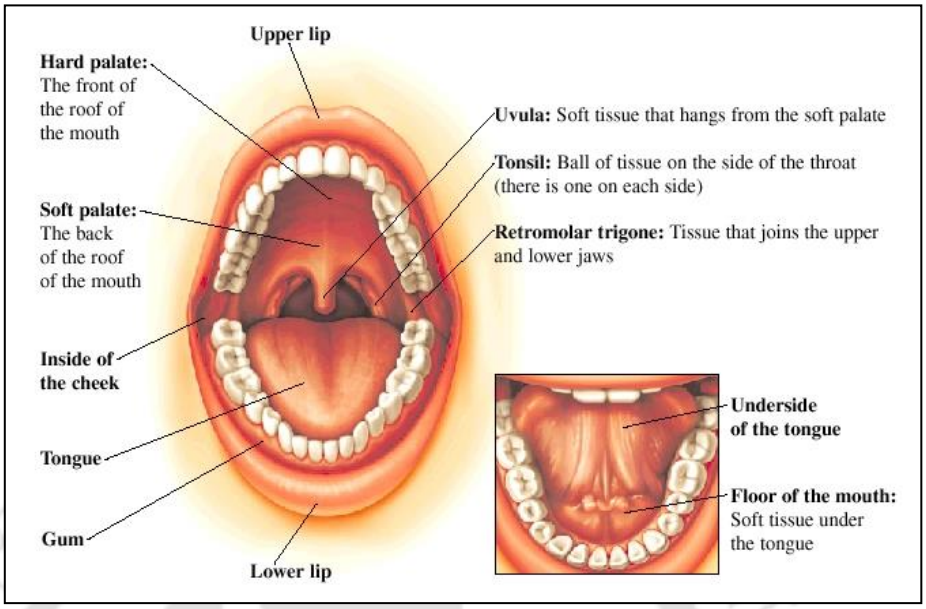


Figure 1.1: Different Regions of oral cavity.
(http://www.uofmmedicalcenter.org/healthlibrary/Article/84505)

1.2.1. Etiology

Development of oral cancer is a multi-stage process and it is believed that it would take a minimum of 10-15 years to develop oral cancer from premalignant lesions. There are a plethora of factors associated with its development that function over time and is dependent on each person's unique response to these factors. Though, it is also true that patients without apparent risk factors can develop oral cancer and premalignant lesions (Laronde et al 2008, Blot et al 1988). Among the risk factors various patterns of tobacco use, alcohol consumption, betel quid and areca nut chewing, viruses and other factors like genetic susceptibility, diet, immunosuppression etc. have their implications in oral cancer either alone or in synergistically along with other factors (Fig 1.2).

1.2.1.1. Tobacco

The chewing of tobacco with betel quid and cigarette smoking with heavy alcohol intake are chief risk factors linked with oral neoplasia (Neville et al 2009, Franceschi et al

2000). Approximately 2500 distinct compounds are reported in tobacco, of which 300 are known to be carcinogenic. Tobacco-specific-nitrosamines (TSNA) such as N nitrosonornicotine (NNN), 4[methylnitosoamino]-1-[3-6-pyridyl]-1-butanone (NNK) etc are accepted as most significant carcinogens in tobacco. Other carcinogenic compounds include polycyclic aromatic hydrocarbons (PAH), α-particle emitting ^{210}Po (Polonium), trace metals, carbon monoxide, hydrogen cyanide, and phenols. In addition, reactive oxygen species (ROS) generated during chewing of tobacco is associated with carcinogenesis. Tobacco smoke contains pro-carcinogens like benzo[a]pyrene (BAP), which can be metabolized by oxidizing enzymes, notably cytochrome P450 into reactive carcinogenic intermediates thus can take part in the development of oral cancer (Jane et al 2007, Dept. of Health and Human Services 2010).

1.2.1.2. Alcohol

Excessive alcohol consumption is considered as an important causative factor for oral cancer. The person who consumes 50 or more grams of alcohol per day is two to three times at a greater risk of developing these cancers than non drinkers (Ogden 2005, Baan et al 2007). It can act both independently as well as synergistically with smoking. It has been observed that alcohol increases the mucosal permeability to other toxins and carcinogens. Acetaldehyde, the first metabolite of alcohol is a mutagenic and carcinogenic agent which causes cellular damage to the oral epithelial cells. In addition, alcohol disrupts the normal salivary gland function by reducing the clearance of locally acting carcinogens. All these processes can potentiate oral carcinogenesis (Hashibe et al 2009, Boffetta and Hashibe 2006).

1.2.1.3. Betel quid and areca nut

Betel quid chewing is one of the main risk factors for oral cancer especially in South and Southeast Asian countries. Oral premalignancies are a very common phenomenon in betel quid chewers of which about 10% undergoes malignant transformation (Chiba 2001). The habit of betel quid chewing with areca nut and tobacco exposes a person to a relatively higher risk of oral cancer ranging from 8-15 times as compared to 1-4 times associated with quid without tobacco (Manjari et al 1996). The production of ROS occurs in the chewer's saliva under alkaline condition by auto-oxidation of areca nut (AN) polyphenols. These ROS can initiate tumor induction by causing gene mutation and genotoxicity. Also, it can affect salivary gland proteins and oral mucosa which can disrupt the structural integrity of oral mucosa and thus may assist in the penetration of other betel quid ingredients and environmental toxicants. The AN-specific nitrosamines produced by nitrosation of areca alkaloids in the saliva of betel quid chewers are mutagenic, genotoxic and can even initiate tumor in animal models (Jeng et al 2001).

1.2.1.4. Viruses

The association of Human papillomavirus (HPV) in the development of oral cancer is well-known (Smith et al 1998, Herrero et al 2003). The involvement of HPV in the process of carcinogenesis was first reported in cervical cancer and it was found that HPV type 16 and 18 were present in most of the cases (90%) (Walboomers et al 1999). HPV codes for oncoproteins E6 and E7 which are known to interact with tumor suppressor genes Rb and p53, and thus if any alterations or mutations in these proteins can initiate carcinogenesis (Shah 1998). Apart from HPV, Herpes simplex virus (HSV) and Epstein-

Barr virus (EBV) are also known to cause oral cancer (Shimakage et al 2002, Kassim and Daley 1988).

1.2.1.5. Other factors

Immunodeficiency is an important factor which has been implicated in oral cancer, where immunity of patients depletes (39, 40). Thirty to forty percent of global cancer cases are linked to unhealthy diets, lack of physical activity and obesity. Lack of fruits in diet, non-starchy vegetables and foods containing carotenoids are associated with oral cancer with some evidence, and 10–15% of cases are attributable to low fruit and vegetable intake. (Petti 2009, Wanebo et al 1975, Jenkins et al 1997). Also, some studies on genetic polymorphisms of genes like CYP1A1, GSTM1, and GSTT1 reveal their importance in OSCC which codes for important xenobiotic enzymes (Zaini et al 2010, Shukla et al 2012).

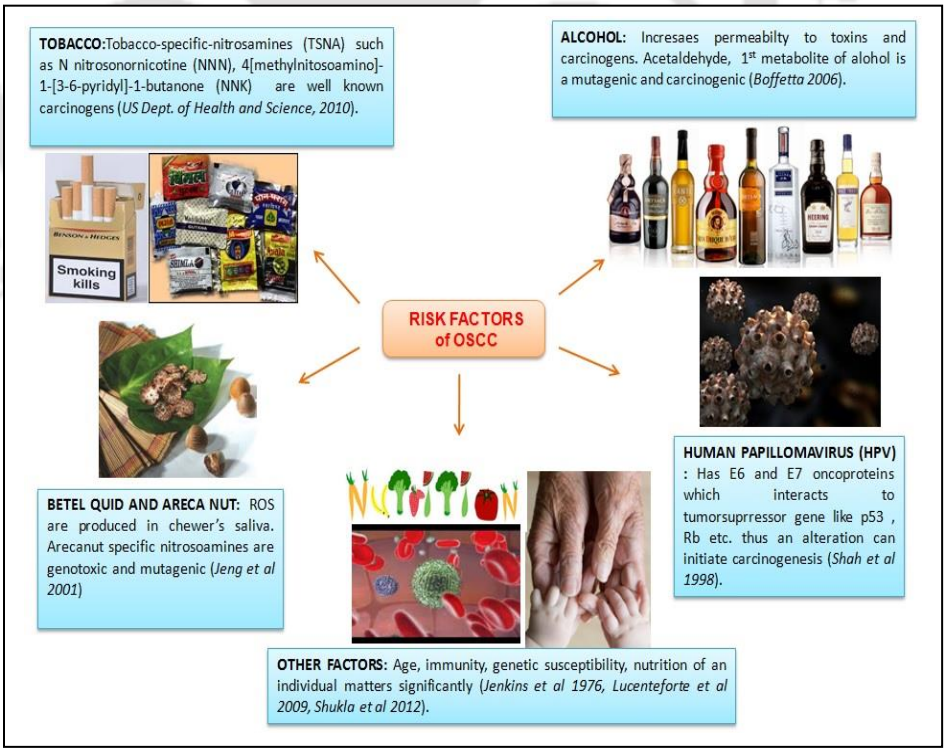


Figure 1.2: Risk factors associated with the development of OSCC.

1.2.2. Clinical features of OSCC

Tumorigenesis is a multi-step process and it involves the step-wise transition from pre-malignant lesions to the metastatic tumor phenotype. Different genomic alterations gradually accumulate to facilitate this transition into malignant tumors. Oral cancer follows the same steps from benign hyperplasia, to dysplasia, to carcinoma in situ and advanced cancer (Califano et al 1996).

1.2.2.1. Premalignant lesions

Premalignant lesions are asymptomatic morphologically altered tissues having a higher risk of attaining cancer than the normal tissues (Gillenwater et al 2006). ***Leukoplakia****:* Clinically, leukoplakia can be defined as a white keratotic plaque which cannot be removed by manual interference (Gupta 1989). This term was first used by Schwimmer in 1877 for a white lesion of the tongue which probably was a syphilitic glossitis (Schwimmer 1877). Histologically, it consists of hyperkeratosis which is thickening of the stratum corneum, parakeratosis that is increased number of nucleated cells near the surface and acanthosis refers to the elongation of rete pegs into the submucosa (Mehta and Hamner 1993).The chances of malignant transformation of oral leukoplakia range from 0.13 to 17.5% (Teruo et al 2006). ***Dysplasia****:* Dysplasia term was first introduced by Reagon and Hamonic in 1956 for the cells exfoliated from lesions of the uterine cervix (Reagan and Hamonic 1956). It is being used for varying degrees of cytologic atypia such as hyperchromatism, increased nuclear size, pleomorphism, dyskeratosis, and increased or abnormal mitotic figures (Bouquot et al 2006). Traditionally, dysplasia is divided into three division of mild, moderate and severe dysplasia. Mild dysplasia is an atypia limited to basilar and parabasilar keratinocytes while moderate dysplasia becomes extended in

the middle one-third of the epithelium. The terms severe dysplasia and carcinoma in situ are applied to hyperproliferation of cells expanding up to the surface layer (Speight 2007). The rate of oral epithelial dysplasia malignant transformation is reported to be more than 7% (Dost et al 2014, Ho et al 2009). ***Erythroplakia****:* The term erythroplakia was originally used by Queyrat for describing a red, precancerous lesion of the penis. It refers to a red patch that cannot be attributed clinically or pathologically as any other condition (Queyrat 1911). When erythroplakias exists along with white foci, they are known as speckled leukoplakias or erythroleukoplakias (Suter et al 2008). Though, the rate of occurrence of leukoplakia is 13 times more than but it is observed that OSCC cases are more frequent in erythroplakia cases (Lapthanasupkul 2007).

Oral lichen planus (OLP)*:* It is a term used for chronic inflammatory disease of unknown etiology. The different characteristics of OLP are white papules, white plaques, erythema, erosions or blisters affecting mostly the buccal mucosa, tongue and gingiva (Sugerman and Savage 2002). Risk of malignant transformation of OLP is reported to be around 1% (Fang et al 2009). ***Oral submucous fibrosis (OSMF)****:* It is a premalignant condition arises due to an extensive habit of chewing areca- nut with or without tobacco. In OSMF mucosal rigidity of varying intensity, reduction in the vasculature, atrophy of surface epithelium and dysphagia condition occurs (Karemore and Karemore 2011). In India, malignant transformation rate of OSMF is found to be 7.6% and worldwide its rate ranges from 4-13% (Rajalalitha and Vali 2005).

1.2.2.2. Squamous cell carcinoma (SCC)

Early squamous cell carcinoma commonly appeared as a white patch (leukoplakia), red patch (erythroplakia), or a mixed red and white lesion (erythroleukoplakia) but gradually

superficial ulceration of the mucosal surface may develop. These lesions can grow to form an exophytic mass with a fungating or papillary surface or endophytic growth pattern can be followed to form a depressed, ulcerated surface with a raised, rolled border (Neville et al 2002). Pain cannot be considered as a reliable indicator of malignancy because many early oral cancers are asymptomatic or may present only minor discomfort though advanced carcinomas are most often painful. The tongue is the most frequent site affected with intraoral carcinoma accounting for around 40% of all OSCC (Tanaka et al 2011). Many different forms of SCC are present of which two are most prevalent that is basaloid squamous cell carcinoma and verrucous carcinoma. Basaloid SCC is an aggressive type of SCC and it involves basaloid cells which are arranged in nests or cords with pseudo-glandular spaces and a high mitotic rate. Only 5% of all oral cavity carcinomas are verrucous carcinoma. It is described as a whitish, warty, bulky cauliflower-like growth, having a broad base. It affects buccal mucosa mostly and has a better prognosis than SCC and considered as low-grade malignancy (Das and Nagpal 2002).

1.2.3. Staging of oral cancer

The staging of oral cancer is a prerequisite to frame the proper treatment and prognosis. The TNM system is the most widely used cancer staging system. The T refers to the size and extent of the main tumor. The main tumor is usually called the primary tumor. The N refers to the number of nearby lymph nodes that have cancer. The M refers to the metastasis of cancer (Table1.1) (Neville and Day 2002).

TNM Staging of Oral Cancer	
Primary Tumor (T)	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor 2 cm or less in greatest dimension
T2	Tumor more than 2 cm but not more than 4 cm in greatest dimension
T3	Tumor more than 4 cm in greatest dimension
T4	Tumor invades adjacent structures (e.g., through cortical bone, into maxillary sinus, skin, pterygoid muscle, deep muscle of tongue)
Nodal Involvement (N)	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in a single ipsilateral lymph node, 3 cm or less in greatest dimension
N2	Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension; or in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension; or in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension
N2a	Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension
N2b	Metastasis in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension
N2c	Metastasis in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension
N3	Metastasis in a lymph node more than 6 cm in greatest dimension
Distant Metastasis (M)	
MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis
Stage Grouping	
Stage 0	Tis N0 M0
Stage I	T1 N0 M0
Stage II	T2 N0 M0
Stage III	T3 N0 M0; T1 or T2 or T3 N1 M0
Stage IV	Any T4 lesion, or Any N2 or N3 lesions, or Any M1 lesion

Modified from AJCC Manual for Staging of Cancer, 1997, Ed: Fleming ID, et al. Lippincott-Raven Publishers, Philadelphia, PA.

Table 1.1: TNM staging system for the oral cavity (CA Cancer J Clin 2002; 52:195-215).

1.2.4. Molecular alterations in OSCC

The process of oral squamous carcinogenesis is a multifaceted process that occurs when squamous epithelium is affected by several genetic alterations under the influence of environmental factors such as tobacco, alcohol, chronic inflammation, and viral infection. (Fig 1.3) (Choi and Myers 2008, Tanaka and Ishigamori 2011). Field cancerization is a theory proposed for oral carcinogenesis which suggests that the total area of oral epithelium is at higher risk of developing malignant lesions due to the accumulation of genetic alterations of oncogenes and tumor suppressor genes as they are more prone to carcinogenic factors (Slaughter et al 1953). Alterations in the tumor suppressor genes by genetic events like mutation, loss of heterozygosity, or deletion, or by epigenetic

modifications such as DNA methylation or chromatin remodelling can initiate the process of carcinogenesis. The activation of oncogenes occurs through overexpression due to gene amplification, increased transcription, or changes in structure due to mutations that result in increased transforming activity (Choi and Myers 2008).

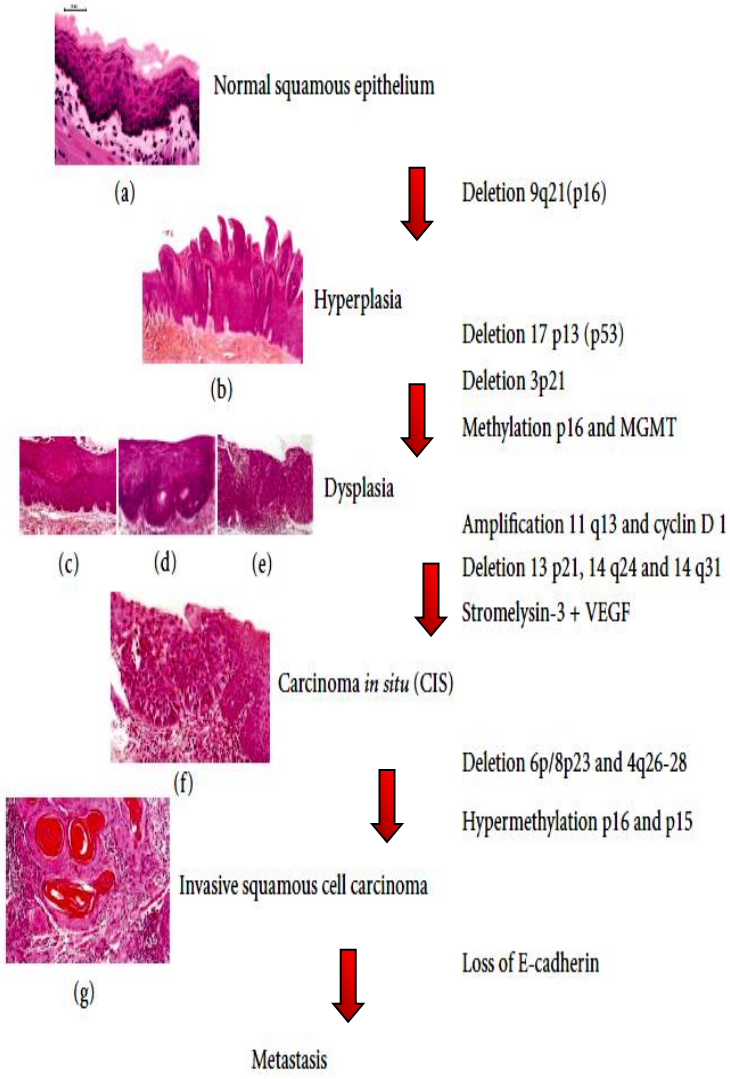


Figure 1.3: Natural history and genetic alterations of oral carcinogenesis; (a), Normal oral mucosa, (b) papillary hyperplasia, (c) mild dysplasia, (d) moderate dysplasia, (e) severe dysplasia, (f) carcinoma in situ, and (g) invasive squamous cell carcinoma (well differentiated) (Journal of Oncology Volume 2011, Article ID 603740).

Numerous studies have led to the identification of specific genetic alterations in oral carcinomas and in premalignant lesions of the oral cavity. Bockmuhl and colleagues have reported deletions of chromosome 3p, 5q, and 9p with 3q gain in well differentiated tumors, whereas in poorly differentiated tumours deletions of 4q, 8p, 11q, 13q, 18q, and 21q and gains in 1p, 11q, 13, 19, and 22q were identified, thus suggesting an association with tumor progression by using comparative genomic hybridization of primary head and neck carcinomas. The marked improvement in the expansion of molecular techniques, such as microsatellite assays and restriction fragment length polymorphism have resulted in thorough knowledge about the oral carcinogenesis process. It has been shown that allelic imbalance of chromosomal 9p is the most common chromosomal arm loss in head and neck squamous cell carcinoma (Williams et al 2000). The neoplastic transformation takes many years to ultimately develop into OSCC. *p53* is one of the most important tumor suppressor genes which have been associated with many cancers. In oral cancer, inactivation of *p53* has been reported and it was found that different forms of tobacco consumptions were responsible for causing mutation in the gene (Tanaka and Ishigamori 2011, Brennan et al 1995, Lazarus et al 1998). Other tumor suppressor genes such as *doc-1*, *pRb*, *pR6*, *p16*, *p21*, *mdm2*, and *APC* are found to be important for the pathogenesis of oral cancer (Williams et al 2000). The proto-oncogenes are likely to become oncogenes, by the gain of functions mutations and may involve in the initiation and progression of oral cancer. The mechanism of point mutations and DNA rearrangements can lead to activation of cellular oncogenes. Any deregulation in the expression of proto-oncogenes such as epidermal growth factor receptor (EGFR1 c-erb 1) members of the ras family, *c-myc*, *int-2*, *hst-1*, *PRAD-l*, and *bel* can contribute in the process of oral neoplasia

(Williams et al 2000, Jurel et al 2014, Bishop 1991). In addition to these, other molecular mediators such as *COX-2*, Thrombospondin 1 (*TSP1*), human trophoblast cell-surface antigen (*TROP2*), epithelial adhesion molecule (*EpCAM*), ras gene family (H-*ras*, *K-ras* and *N-ras*) etc are known play important role in the pathogenesis of oral cancer (Williams et al 2000, Jurel et al 2014, Bishop 1991, Fong et al 2008, Sen and Carnelio 2016, Castellano and Santos 2011).

1.2.5. Therapeutic approaches

Treatment of OSCC is a multidisciplinary task. The prime objective is to eradicate cancer and prevents its recurrence by restoring the form and function of the affected parts. The different pre-considerations are made before opting for the treatment module which includes the characteristics of carcinoma (specific site affected, clinical size, the extent of local invasion, histopathological features, regional lymph node involvement and distant metastasis), patient's age, health condition, a history of previously treated oral SCC and addictions (Shah and Gil 2009).

There are different methods of treatment available, most common among them are surgical resection, radiotherapy, and chemotherapy. These are applied alone or in combination with other depending on the cancer status of the patient.

1.2.5.1. Surgical resection

Surgery is preferred for small accessible OSCCs (Stage I and Stage II). Tumors approaching the gingiva need to be removed with the gingiva and periosteum. It has been observed that rate of occult cervical metastasis is very high (10%-26%) so alternative treatment is also required (Alvi 1996). Reconstructive surgery is usually preferred after resection of the primary tumor to restore oral function and cosmetic appearance. The

most suitable reconstruction method is dictated by several aspects, such as characteristics of the primary site and defects, the general medical condition and social history of the patient, the prognosis, and the expertise of the surgeon (Omura 2014).

1.2.5.2. Radiotherapy

Radiotherapy employs the high-energy x-rays or particles to damage the cancer cells or slow their growth rate. In oral cancer, radiation therapy plays a significant role in the treatment in early stages or combined with other therapeutic options such as surgery and/or chemotherapy in advanced stages. The process of delivering a dose of radiation over time is known as fractionation (Budach et al 2006, Awwad et al 2002). External beam radiotherapy (EBRT) alone or in combination with chemotherapy is generally utilized in three conditions that is adjuvant to primary surgery to increase the loco-regional control (LRC) for cases having unfavorable pathological characteristics, the primary treatment for cases unable to endure or unfit for surgery, and salvage treatment in the constant or recurrent disease conditions. In comparison conventional radiotherapy, Brachytherapy is an another options which provides a high localized dose of radiation, with rapid fall-off and short overall treatment time. It has been used in the treatment of carcinoma of the lip, tongue, floor of the mouth, oral mucosa, base of the tongue, tonsillar region, soft palate, and nasopharynx (Huang 2013, Yamazak et al 2012).

1.2.5.3. Chemotherapy

In the past, chemotherapy was mainly applied as a palliative treatment for oral cancer but the discovery of new drugs has made it a significant curative treatment for advanced oral cancer. In chemotherapy, synthetic or biological agents are used to destroy dividing abnormal cancer cells in order to manage spread and metastasis. Various agents like

retinoids, α- tocopherol, α-interferon, and COX-2 inhibitors are employed for this purpose. The common classes of chemotherapeutic agents comprise of platinum compounds (cisplatin and carboplatin), antimetabolites (methotrexate and 5-fluorouracil), taxanes (docetaxel), plant alkaloids, hydroxyurea, anthracyclines, and most recently taxoids. 5-fluorouracil in combination with docetaxel and cisplatin has been proven to be efficacious in induction therapy whereas the platinum derivative cisplatin alone is used for induction therapy (Fotedar et al 2013, Vermorken and Specenier 2010, Prelec and Laronde 2014). The recent period has observed more focused research on molecules to be developed as an agent for targeted therapies. Cetuximab is one such example of targeted therapy which targets the epidermal growth factor receptor (EGFR) (Hamakawa et al 2008, Prelec and Laronde 2014). Different variations of chemotherapy such as neoadjuvant or induction chemotherapy are used during the course of treatment (Jain et al 2008).

1.2.6. Treatment-associated complications

Several complications are often associated with the available treatment methods, notable among these are chemoresistance, tumor recurrence and side effects caused by treatment modalities.

1.2.6.1. Chemoresistance

Chemotherapy is one of the most important strategies used to combat cancer. But it also fails to completely eliminate the cancer cells due to the factor, known as chemoresistance (Longley and Johnston 2005). Tumors can be inherently resistant to chemotherapeutic agents or it may be acquired during the course of treatment. Moreover, in the process of attaining resistance, the tumor may become resistant to the range of other

chemotherapeutic agents which can limit the treatment and can cause metastasis in 90% of patients. Different factors are attributed for providing the resistance; it includes increased drug efflux, drug activation and inactivation, alterations in drug target, DNA methylation, processing of drug-induced damage, and evasion of apoptosis (Wilson et al 2006). In many cases of OSCCs, less responsiveness to common cytotoxic drugs has been observed due to the mechanisms that either restrict the transport of these agents into the cells or obstruct with their intracellular molecular targets (Warnakulasuriya and Johnson 1996).

1.2.6.2. Tumor relapse

Tumor relapse has become a major hurdle in treating the oral cancer patients. It is observed that 20%-30% cases of resection of OSCC with adequate, wider than 5 mm, tumor-free margins proved through histopathological examination can develop local or contiguous regional "recurrence". This may be either due to carcinomatous keratinocytes left at the margins of the surgical wound or because of the large field of precancerized epithelium consisting of precancerous keratinocytes at different stages of transformation from which the primary carcinoma developed which were not removed by surgical intervention. Epithelium from a field of precancerization may appear normal microscopically but can have cytogenetic alterations like loss of heterozygosity and p53 mutations, or epigenetic changes in methylations of certain promoters of tumor-suppressor genes and DNA repair genes. Further accumulation of genetic alterations either in the keratinocytes in the dysplastic epithelium or the genetically transformed keratinocytes may become cancerous and can form a new field of carcinoma close to the

resected primary carcinoma (Braakhuis et al 2002, Vries et al 1986, Braakhuis et al 2010, Levi et al 2006, Gallo and Bianchi 1995, Goldenberg et al 2004).

1.2.6.3. Side effects

Radiotherapy and chemotherapy are mostly used for the treatment of oral cancer. But these two methods have many complications of side effects associated with it which can cause patient morbidity and mortality. Several difficulties which arise with chemotherapy and/or radiation therapy are mucositis (stomatitis), xerostomia (dry mouth), bacterial, fungal, or viral infection (particularly in neutropenic patients), dental caries, loss of taste, osteoradionecrosis, odynodysphagia, dyseugia and subsequent dehydration, and malnutrition (Dose 1995, Zlotolow 1998, Bitran et al 1996).

Considering all the complications and drawbacks associated with the already available diagnostic and therapeutic approaches for oral cancer it becomes imperative to focus the research on finding the novel targets which can help in prognosis as well as in developing efficacious chemotherapeutic agents. Understanding the molecular profile of OSCC is an important step to explore the novel biomarker for the treatment and prognosis. Akt or protein kinase B (PKB) is one such protein of the PI3K/Akt/mTOR pathway which can be developed as a biomarker of prognostic and therapeutic importance. Activation of PI3K/Akt/mTOR pathway has been observed in various cancers and Akt kinase of this pathway is known to regulate different cellular processes such as cell proliferation and survival, cell size and response to nutrient availability, tissue invasion, and angiogenesis. Several oncoprotein and tumor suppressor genes are found to be involved in cell signaling/metabolic regulation and interact with the Akt signal transduction pathway and can initiate the process of carcinogenesis (Altomare and Testa 2005).

1.2.7. PI3K/Akt/ mTOR pathway

The PI3K/Akt/mTOR pathway is known to be an extremely conserved pathway that is firmly regulated by a multistep process. Class I phosphatidylinositol 3-kinase (PI3K) can trigger the Akt pathway by getting activated from stimulated receptor tyrosine kinases (RTKs) either directly through binding or by tyrosine phosphorylated scaffolding adaptors like insulin receptor substrate (IRS) proteins. Activated PI3K converts its catalytic domain of phosphatidylinositol (3,4)-bisphosphate (PIP2) lipids to phosphatidylinositol (3,4,5)-trisphosphate (PIP3). PIP3 binds to Akt at the plasma membrane to let PDK1 access and phosphorylate T308 in the "activation loop", which can result in partial activation of Akt. This modified Akt has the potential to activate mTORC1 complex mTOR complex 1 (mTORC1) via multisite phosphorylation of TSC2 within the TSC1-TSC2 complex which obstructs the ability of TSC2 to act as GTPase-activating protein (GAP) for Rheb, thus Rheb-GTP accumulates. This Rheb-GTP activates mTORC1. Partially activated Akt can also inactivate proline-rich Akt substrate of 40 kDa (PRAS40) which inhibits mTORC1. mTORC1 substrates consist of eukaryotic translation initiation factor 4E binding protein 1 (4EBP1), and ribosomal protein S6 kinase, 70 kDa, polypeptide 1 (S6K1). S6K1 subsequently phosphorylates the ribosomal protein S6 (S6/RPS6) to promote protein synthesis and cellular proliferation. Full activation of Akt requires a phosphorylation at S473 in the carboxy-terminal hydrophobic motif either by mTORC2 complex or by DNA-PK. Fully activated Akt can initiate a series of substrate-specific phosphorylation events both in the cytoplasm and nucleus, including inhibitory phosphorylation of the pro-apoptotic FOXO proteins. Thereby, it can control many cellular functions such as angiogenesis, metabolism, growth, proliferation,

survival, protein synthesis, transcription, and apoptosis. Dephosphorylation of T308 and S473 by PP2A and PHLPP1/2 respectively and also the conversion of PIP3 to PIP2 by PTEN can halt the Akt signaling (Fig 1.4) (Hemmings and Restuccia 2012, Brendan et al 2007).

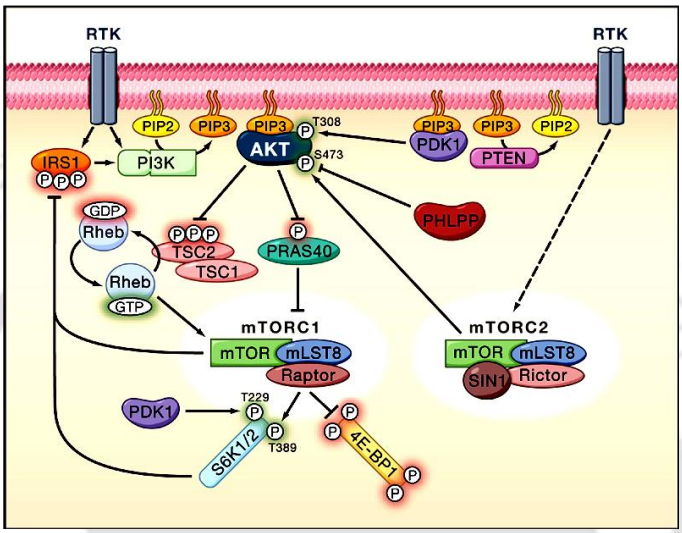


Figure 1.4: PKB/Akt activation downstream of RTKs via the P13K pathway.

1.2.8. Akt/ protein kinase B (PKB)

Akt, a conserved serine/threonine kinase is an important regulatory component of PI3K/Akt/mTOR pathway that transduces signals from growth factors and oncogenes to downstream targets regulating diverse signaling cascades important in both normal cellular physiology and various disease states (Fig 1.5). Akt signaling controls cell proliferation and survival, cell growth (size), glucose metabolism, cell motility and angiogenesis. Any alterations of these processes can lead to cancer, and different reports indicate frequent hyperactivation of Akt signaling in many human cancers. Different oncoproteins and tumor suppressors traverse through the Akt signal transduction pathway and are activated or inactivated, respectively, in cancer (Testa and Tsichlis 2005, Martini et al 2014).

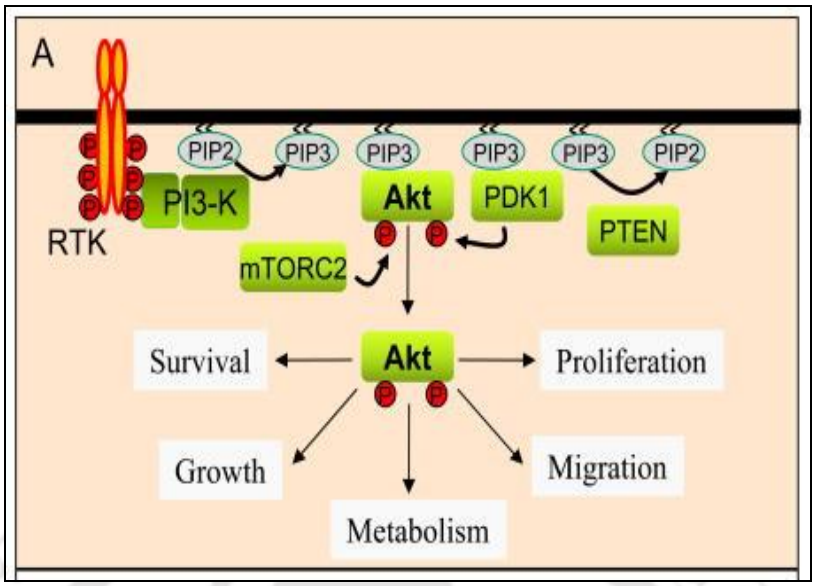


Figure 1.5: Growth factor mediated Akt activation. In response to numerous growth factors and cytokines Akt is activated downstream of PI3-kinase following a multistep mechanism. Activated PI3-kinase converts PIP2 into PIP3 providing sites of recruitment at the plasma membrane of proteins containing PH domains, including Akt and PDK1 kinases. Upon translocation to the plasma membrane Akt is phosphoryalated by PDK1 within the hydrophobic motif, which renders Akt catalytically active. Activated Akt, through the phosphorylation of numerous downstream targets located throughout the cell, regulates a wide array of cellular functions.

Akt kinase has a pleckstrin-homology (PH) domain which gets activated in response to growth factors by the involvement of phosphoinositide 3-OH kinase. It was first identified by three groups independently in the year 1991. This 60 kDa kinase was identified due to their homology with protein kinase A (68%) and protein kinase C (73%) and accordingly, it was named as protein kinase B. Also, this kinase was recognized as the product of the oncogene *v-akt* of the acutely transforming retrovirus AKT8 affecting the rodent T-cell lymphoma. This retroviral encodes a fusion protein of cellular Akt and viral Gag structural protein (Coffer and Woodgett 1991, Jones et al 1991, Bellacosa et al 1991).

1.2.9. The Akt family

Akt family includes three closely related cellular isoforms present in the mammalian cells which are known as Akt1, Akt2, and Akt3 (Jones et al 1991, Altomare et al 1995, Brodbeck et al 1999, Nakatani 1999). These three genes share a sequence identity of more than 85% and have the same structural organization. At the N terminal domain, PH domain is present comprising of around 100 amino acids which bind to the phospholipids. Catalytic domain follows the PH domain linked by a short stretch of a glycine-rich region. The C-terminal region contains the putative regulatory domain of 70 amino acids. The V-Akt has an additional truncated region of viral oncoprotein Gag fused with the full-length Akt1 protein through a short untranslated region. The PH domain and the conserved serine and threonine residues are vital for Akt activation. Moreover, the distance between two phosphorylated residues (approx. 160-170 amino acids) is kept conserved in these protein kinases. Serum- and glucocorticoid-inducible kinase (SGK) is found to be closer to Akt. It shows great sequence homology to Akt's catalytic domain and owns residues equivalent to T308 and S473 of Akt1 but are deficient in PH domain (Fig 1.6) (Kandel and Hay 1999). These isoforms of Akt are ubiquitously expressed in mammals but their expression level varies among different tissues. In most of the tissues, Akt1 is predominantly present whereas Akt2 expression is found in insulin-responsive tissues such as skeletal muscle, heart, liver, and kidney indicating their importance in insulin signaling. This is further supported by the fact that the insulin-responsive tissues of developing embryos notably liver, brown fat and skeletal muscle have the highest expression of Akt2. Akt3 have a very limited pattern of expression observed only in testis and brain and low levels in the adult pancreas, heart, and kidney. It is not necessary to

conclude about the Akt's activities by their expression profile as it is observed that varied level of kinase activities possessed by different isoforms during differentiation that cannot be correlated with their expression level (Kandel and Hay 1999).

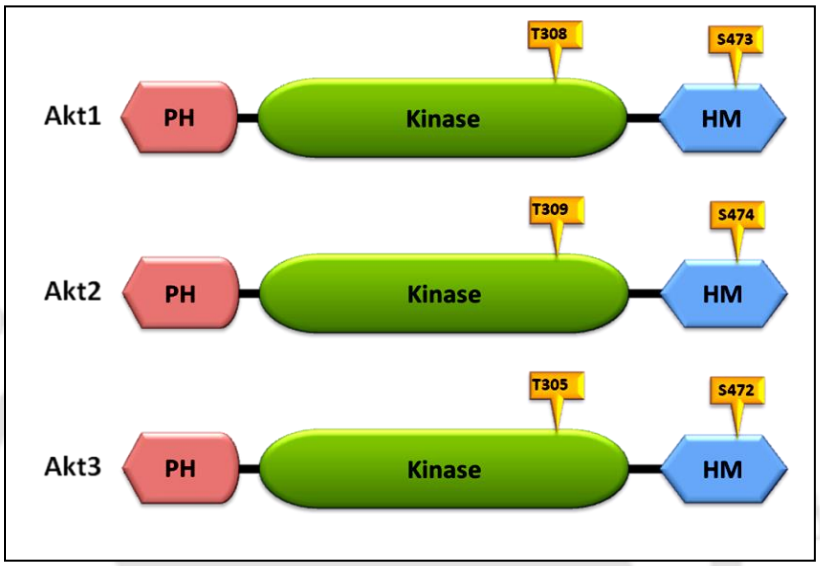


Figure 1.6: Structural organization of the three major Akt/PKB isoforms showing its domain structure: PH (Plekstrin homology) domain functions as phosphoinositide-binding modules. Kinase domain act as a catalytic zone where phosphorylation site; Threonine (T) is present in the activation loop. The HM (hydrophobic motif) has another phosphorylating site; Serine (S).

AKT isoforms	**Akt 1**	**Akt2**	**Akt3**
Chromosome location	14q32	19q13	1q44
Gene size	Approx. 26.4 Kb	Approx. 54.4 Kb	Approx. 35.4 Kb
No. of exons	14	14	14
Coding sequence length	1443 bp	1445 bp	1440 bp
Protein molecular weight	55.6 kDa	55.7 kDa	55.8 kDa
No. of amino acids	480	481	479
Site of expression	Predominantly present in most of the tissues such as brain, heart, lung etc	Insulin responsive tissues such as normal brown fat, skeletal tissue, and liver	Limited to kidney, brain, embryonic heart, and testis
References	Etro et al 2010	Altomare et al 2008	Cheung et al 2008

Table 1.2: Physical parameters of Akt isoforms and their site of expression.

1.2.10. Physiological role of Akt kinase isoforms in normal cells

Despite the similarity in the amino acid sequence in the three Akt isoforms, they have unique modes of activation and many distinct functions (Santi et al 2011). Akt1 kinase isoform has been found to play the significant role in many signaling cascades. It is known to mediate the posterior 5' Hoxc gene expression via epigenetic modification and in part through direct binding to the promoter region in mouse embryonic fibroblast cells (Kong et al 2014). It participates in myogenic differentiation and NF-κB activation which is considered important for oncogenic cell growth by Ki-Ras (Sumitani et al 2002, Kwon et al 2008). In addition, its activation increases HIF-1A expression through enhanced protein translation with the help of mTOR-independent pathway (Pore et al 2006). Besides, it also regulates physiological cardiac hypertrophy while antagonizing pathological hypertrophy (DeBosch et al 2006). Moreover, it is found to be the main Akt isoform that controls apoptosis in limiting cytokine concentrations (Green et al 2013). Also, it is known to play a significant role in inhibiting autophagy via reduced UVRAG (UV radiation resistance-associated gene) expression and assists in the IFN-β transcription downstream of TLR3 activation (Yang et al 2013, Gantner et al 2012). Akt2 is another important member of serine/threonine-protein kinases that are central to several important functions of a cell. It is known to increase the expression of actin-bundling protein palladin (Chin and Toker 2010, Jensen et al 2010). It is a key regulator involved in glucose uptake and metabolism. It also takes part in survival and differentiation of different types of cells such as bone cells, myoblasts, and thymocytes (Mukherjee et al 2010, McCurdy et al 2005, Bouzakri et al 2006, Lee et al 2011, Zhang et al 2011, Muslin 2011, Juntilla et al 2007). Akt3 kinase shares 83.6% with Akt1 and 78%

with Akt2 sequence identity at the protein level (Cheung et al 2008). It plays a very important role in determining the normal brain and other organ sizes. Also, it is involved in glucose homeostasis and growth deficiencies (Easton et al 2005, Dummler et al 2006). It is believed to be the important coordinator of mitochondrial biogenesis (Wright et al 2008). In addition, it also participates in platelet activation and thrombosis (O'Brien et al 2011).

1.2.11. Akt kinase isoform-specific genetic alterations in different cancers

Recent studies have shown the relevance of isoform-specific involvement of Akt kinases in different cancers. The functions of these isoforms are found to be non-overlapping that are responsible for their functional-diversity (Gonzalez et al 2009, Xu et al 2004). Despite their high homology, Akt isoforms regulate distinct physiological functions which might be attributed to distinct tissue distribution of the Akt isoforms, differential activation of the Akt isoforms by extracellular stimuli (that is, cues like the amplitude or timing of PI3 kinase activity triggered by different stimuli could be translated into differential activation/regulation of the Akt isoforms), distinct intrinsic catalytic activity of the Akt isoforms to phosphorylate substrates, and cell context-specific factors (Fig 1.7).

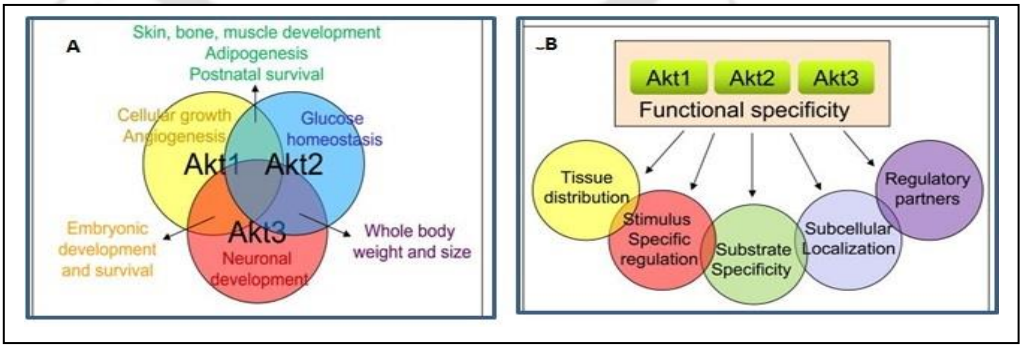


Figure 1.7: Functions of Akt isoforms. A. Overlapping and specific functions of the Akt family members. Summarized are common and distinct Akt isoforms elucidated from the phenotypic analysis of single and double Akt isoform knockout mice. B. Potential mechanisms dictating Akt isoform functional specificity.

Examples of this mode of regulation include isoform-specific subcellular compartmentalization that determines access to substrates and/or specific adaptor proteins that confer specificity to substrate selection. The pioneering report of Akt kinase involvement in cancer came through the studies of Staal group in the year 1987 where they have found the amplification of Akt1 in a single case of gastric carcinoma. Subsequently, in the year 1992, amplification and overexpression of Akt2 was observed in a subset of ovarian carcinoma (Staal et al 1987, Cheng et al 1992). In the ensuing period, this notion has been solidified and extended to various other cancers and at present approximately 30% of human cancers are associated with alterations of Akt kinase pathway (Table 1.3) (Pal and Mandal 2012). Different genetic alterations such as overexpression, amplification, and mutations have been observed in different cancers.

1.2.11.1. Overexpression and amplification of Akt isoforms in different cancers

The Akt isoforms alone or along with other isoforms are overexpressed and participate in the different process of carcinogenesis in many cancers. The overexpression of Akt1 has been reported in OSCC, hepatocellular carcinoma (HCC), and thyroid cancer (Nakashiro et al 2015, Wang et al 2013, Krześlak et al 2011). Akt2 isoform was observed to be overexpressed in cancers such as OSCC, Laryngeal squamous cell carcinoma (LSCC), estrogen receptor α (ERα) positive breast carcinomas, lung adenocarcinoma and squamous carcinoma, HCC, osteosarcoma, and colorectal cancer (Iamaroon and Krisanaprakornkit 2009, Chen et al 2009, Sun et al 2001, Wang et al 2006, Pu et al 2010, HCC Miao et al 2011, Xu et al 2004, Zhu et al 2014, Rychahou et al 2008). Akt3 was found to be upregulated in estrogen receptor-deficient breast cancer cell lines, melanoma, and ovarian cancer (Nakatani et al 1999, Stahl et al 2004, Cristiano et al 2006). In breast

cancer and ovarian cancer, both Akt1 and Akt2 were found to be overexpressed (Stål et al 2003, Noske et al 2007). All the three isoforms of Akt has been found to be overexpressed in salivary gland cancer cells (Hara et al 2008). Amplification is known to increase the number of copies which can lead to increase in the mRNA level and in turn protein. The Akt1 amplification was reported in prostate cancer specimens (Kirkegaard et al 2010). In lung cancer, amplification of Akt and Akt2 gene was observed through polysomy of the chromosomal segment (Dobashi et al 2012). The amplification of Akt2 gene has been observed in cancers such as ovarian, prostate, and pancreatic cancer (Cheng et al 1992, Nakayama et al 2006, Shukla et al 2007, Cheng et al 1996, Ruggeri et al 1998, Altomare et al 2002). The amplification of Akt3 isoform has been reported in the case of breast cancer (Kirkegaard et al 2010).

The prognostic importance of Akt kinases generally has been well recognized in different cancers, but focused reports of Akt isoform-specific associations are limited in number (Table 1.4) (Valkov et al 2011, Prijić et al 2014, Pickhard et al 2014). In case of HCC, one of the studies has suggested the importance of Akt1 in cancer prognosis. Although, another study led by Zhang et al 2014 have shown the importance of all the Akt isoforms in determining the prognosis of the disease (Xu et al 2004, Zhang et al 2014). The p-Akt1 (phosphorylated Akt) has been associated with prognosis of different cancers such as pancreatic cancer, intrahepatic cholangiocarcinomas, lung cancer, and breast cancer (Liu et al 2010, Lee et al 2012, Shi et al 2011, Liu et al 2012). In a recent study, a co-heightened EGFR/Akt1 gene copy number has been associated with worse clinical outcome (Li et al 2015). In addition to Akt1, Akt2 expression has also been found to have prognostic impact in various cancers such as NSCLC, breast cancer, meningiomas and

osteosarcoma where its expression has been linked with different clinicopathological conditions and available treatment outcomes (Miao et al 2011, Fohlin et al 2013, Wang et al 2014, Zhu et al 2014).

Type of cancer	***In vitro / In vivo/* Clinical**	**Status of Akt Isoforms**	**Reference**
Breast cancer	*In vitro*	Upregulation of Akt3	Nakatani et al 1999
Breast cancer	Clinical	Activation of Akt2	Sun et al 2001
Breast cancer	*In vitro*	Up regulation of Akt2	Bacus et al 2002
Breast cancer	Clinical	Overexpression of Akt1 and Akt2	Stal et al 2003
Breast cancer	Clinical	E17K mutation in Akt1	Carpten et al 2007
Breast cancer	Clincal	E17K mutation in Akt1	Stemke-Hale et al 2008
Breast cancer	Clinical	E17K mutation in Akt1	Bleeker et al 2008
Breast cancer	Clincal	E17K mutation in Akt1	Arnedos et al 2012
Breast cancer	Clincal	Amplification of Akt3 and deletions of Akt1 and Akt2	Kirkegaard et al 2010
Colorectal cancer	Clinical	High expression of Akt1 and Akt2	Rychahou et al 2008
Colorectal cancer	Clinical	E17K mutation in Akt1	Bleeker et al 2008
Glioma	*In vitro*/Clinical	Increased expression of Akt2	Wang et al 2010
Glioma	*In vitro*/Clinical	Higher expression of Akt2	Mure et al 2010
HNSCC	Clinical	E17K mutation in Akt1	McBride et al 2014
HCC	Clinical	High expression of Akt2	Xu et al 2004
HCC	*In vitro*/Clinical	Constitutive expression of Akt1	Wang et al 2013
LSCC	Clinical	Positive rate of Akt2 expression	Chen et al 2009
Lung carcinoma	Clinical	Amplification and high-level polysomy of Akt1 and Akt2	Dobashi Y et al 2012
Lung carcinoma	Clinical	E17K mutation in Akt1	Do et al 2008
Lung carcinoma	Clinical	E17K mutation in Akt1	Malanga et al 2008
Lung carcinoma	Clinical	E17K mutation in Akt1	Bleeker et al 2008
NSCLC	Clinical	Higher expression of Akt2	Wang et al 2006
NSCLC	Clinical	Positive rate of Akt2 expression	Pu et al 2010
NSCLC	Clinical	Positive rate of Akt2 expression	Miao et al 2011
NSCLC	Clinical	G1112A, R371H, L861Q and E542Q in Akt2	Sasaki et al 2008
NSCLC	Clinical	R371H mutation in Akt2	Sasaki et al 2010
NSCLC	Clinical	E17K mutation in Akt1	Do et al 2010
Melanoma	*In vitro*/Clinical	Elevated expression of Akt3	Stahl et al 2004
Melanoma	Clinical	Mutation of Akt3	Davies et al 2008
OSCC	*In vitro*	Constitutive expression of Akt1, Overexpression of Akt2 and minimal expression of Akt3	Iamaroon and Krisanaprakornkit 2009
Ovarian cancer	*In vitro*/Clinical	Amplification and overexpression of Akt2	Cheng et al 1992
Ovarian cancer	Clinical	Activation of Akt2	Yuan et al 2000
Ovarian cancer	Clinical	Amplification of Akt2	Nakayama et al2006
Ovarian cancer	*In vitro*	Expression of Akt1 and Akt2	Noske et al 2007
Ovarian cancer	*In vitro*/Clinical	High expression of Akt3	Cristiano et al 2006
Pancreatic cancer	*In vitro*/Clinical	Amplification of Akt2	Cheng et al 1996
Pancreatic cancer	Clinical	Amplification and overexpression of Akt2	Ruggeri et al 1998
Pancreatic cancer	Clinical	Activation of Akt2	Altomare et al 2002

Pancreatic cancer	Clinical	Positive expression of p-Akt1	Liu et al 2010
Prostate cancer	Clinical	Amplification of Akt1	Kirkegaard et al 2010
Prostate cancer	Clinical	E17K mutation in Akt1	Boormans et al 2008
Prostate cancer	Clinical	E17K mutation in Akt1	Boormans et al 2010
Prostate cancer	*In vitro*	Upregulation of Akt3	Nakatani et al 1999
Salivary gland carcinoma	*In vitro*/Clinical	Overexpression of Akt1, Akt2 and Akt3	Hara et al 2008
Thyroid neoplasms	Clinical	Overexpression of Akt1	Krześlaket al 2011

Table 1.3: Genetic alterations of Akt isoforms in different cancers (Roy et al 2017).

1.2.11.2. Mutations of Akt isoforms in different cancers

Mutations of Akt isoforms have been observed in approximately 3-5% of cancers. E17K accounts for 36% of Akt1 mutations in cBioPortal but is less prevalent in Akt2 and Akt3. The maximum portion of Akt1-3 mutations is spread across the coding sequence at low frequencies. The most common activating Akt mutations, including Akt1 E17K, L52R, and Q79K don't have any impact on sensitivity or resistance to Akt inhibitors. However, Akt1 D323H and Akt2 W80C mutants have been reported to be relatively resistant to the allosteric Akt inhibitor MK-2206, but not an ATP-competitive inhibitor (Kyung et al 2016). The E17K mutation has been observed in various cancers such as HNSCC, breast cancer, lung cancer, prostate cancer, and melanoma. It is responsible for the substitution of glutamic acid to lysine at 17^{th} amino acid in the lipid-binding pocket of Akt1 has been detected in human breast cancer. This mutation was responsible for altering the electrostatic interactions and activating Akt1 in a PI3K-independent manner which was capable of transforming rodent cells *in vitro* and could induce leukemia in mice. Also, the Q79K mutation was reported in the case of melanoma cancer cells. In Akt2 isoform, the R371H mutation has been reported in HNSCC. In melanoma cancer cells E17K mutation was observed in Akt1 and Akt3 isoform (Carpten et al 2007, Bleeker et al 2008, Stemke-Hale et al 2008, Arnedos et al 2012, Rudolph et al 2016, McBride et al 2014, Do et al

2008, Malanga et al 2008, Do et al 2010, Sasaki et al 2008, Kirkegaard et al 2010, Boormans et al 2008, Davies et al 2008, Shi et al 2014).

Akt Isoforms	Cancer	Alteration correlated to prognosis	Remark	References
Akt1	Breast cancer	Akt1 gene copy number	Co-heightened EGFR/Akt1 gene copy numbers alterations had a worse outcome	Li et al 2015
Akt1	Breast cancer	p-Akt1 expression	Associated with poor DFS and OS	Liu et al 2012
Akt1	HCC	Upregulation of Akt1, Akt2 and Akt3 proteins	Associated with tumor aggressiveness and poor prognosis	Zhang et al 2014
Akt1	ICC	Aberrant expressions of p-Akt1	Associated with a favorable prognosis	Lee et al 2012
Akt1	NSCLC	Positive expression of PAkt-1	pERK1/2 and pAkt-1 can act as independent prognostic biomarkers for predicting RFS	Shi et al 2011
Akt1	Pancreatic cancer	Positive expression of pAkt-1	Associated with poor DFS and OS	Liu et al 2010
Akt2	Breast cancer	High Akt2 expression increased with higher oestrogen receptor levels	Prognostic value of Akt2	Fohlin et al 2013
Akt2	HCC	Upregulation of Akt1, Akt2 and Akt3	Associated with tumor aggressiveness and poor prognosis	Zhang et al 2014
Akt2	HCC	High Akt2 expression	Akt2 was an independent cancer relapse prognostic marker	Xu et al 2004
Akt2	Meningiomas	High Akt2 expression	High levels of Akt2 correlated with high rates of tumor recurrence	Wang et al 2014
Akt2	NSCLC	Positive rate of Akt2 expression	Significantly correlated with the PFS and OS	Miao et al 2011
Akt2 Akt2	Osteosarcoma	Elevated expression of	Associated with aggressive clinical behavior and poor outcome	Zhu et al 2014
Akt3	HCC	Upregulation of Akt1, Akt2 and Akt3	Associated with tumor aggressiveness and poor prognosis	Zhang et al 2014

Table 1.4: Prognostic implications of Akt isoforms in different cancers (Roy et al 2017).

1.2.12. Role of Akt kinase isoforms in cancer cells

Akt isoforms are known to play the central role in regulating pathways important for several cellular processes such as transcription and translation of genes, cell proliferation and survival, glucose metabolism, genome stability, angiogenesis, and metastasis (Fig 1.8). All these cellular processes are found to be important for the pathogenesis of cancer.

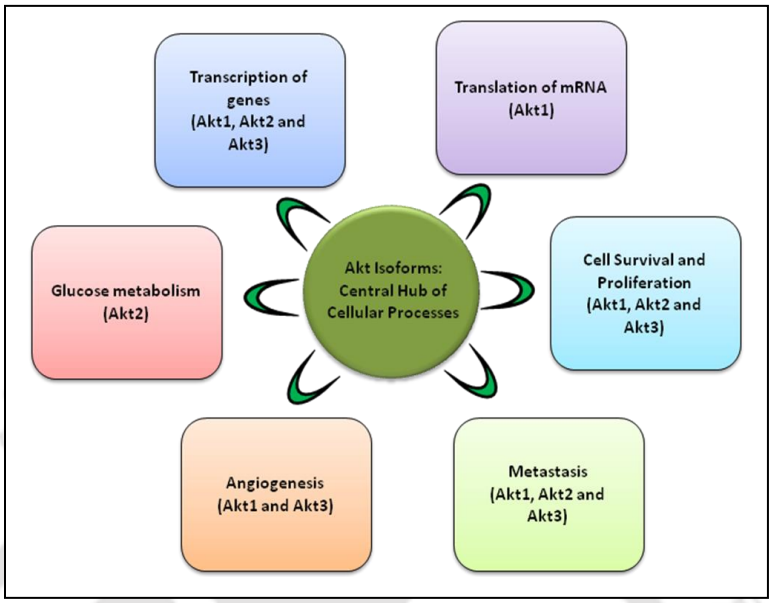


Figure 1.8: Diagrammatic representation of critical role of Akt isoforms in diverse cellular processes (Roy et al 2017).

1.2.12.1. Role of Akt isoforms in cancer cell survival and proliferation

The role of Akt1 in cancer cell survival and proliferation has been observed in different cancers such as mesothelioma, breast cancer, lung cancer, glioma, HCC, ovarian cancer, and prostate cancer (Menges et al 2012, Riggio et al 2017, Hollander et al 2011[a], Mure et al 2010, Jung et al 2013, Shearn et al 2012, Linnerth-Petrikb et al 2016, Virtakoivua et al 2012). The positive expression of Akt2 isoform has been found to be important for the survival and proliferation of cancer cells of breast cancer, neuroblastoma, HCC, and prostate cancer (Bacus et al 2002, Qiao et al 2008, Foley et al 2010, Wang et al 2014, Virtakoivua et al 2012). The significance of Akt3 isoform has been observed in survival and proliferation of lung cancer, prostate cancer, and ovarian cancer cells (Lee et al 2011, Lin et al 2015, Cristiano et al 2006).

1.2.12.2. Role of Akt isoforms in epithelial-mesenchymal transition (EMT), invasion, and metastases

The role of Akt1 has been correlated with the invasion and metastases of glioma, thyroid cancer and melanoma (Mure et al 2010, Jung et al 2013, Krześlak et al 2011, Saji et al 2011). Expression of Akt2 plays a significant role in the invasion of cancer cells such as hepatocellular, glioma, neuroblastoma, breast cancer, LSCC, and papillary thyroid carcinoma (Qiao et al 2008, Foley et al 2010, Xu et al 2004, Wang et al 2004, Pu et al 2004, Pu et al 2006, Zhang et al 2009, Qiao et al 2008, Foley et al 2010, Riggio et al 2017, Chen et al 2009, Zhou et al 2017, Arboleda et al 2003). Akt2 plays a key role in EMT by interacting with Snail1 in the E-cadherin promoter region. (Villagrasa et al 2012). Recently, it was found that Akt2 is involved in colorectal cancer cells metastasis through regulation of metastasis suppressor 1 (MTSS1) (Agarwal et al 2017). However, few reports of involvement of both Akt1 and Akt2 in metastases of colorectal cancer cells are also known (Ericson et al 2010, Häggblad et al 2017). The expression of Akt3 has been found to be important for invasion and metastases of NSCLC and thyroid cancer cells (Lee et al 2011, Boufraqech et al 2014, Zhuang et al 2017, Lin et al 2017, Sui et al 2017).

1.2.12.3. Role of Akt isoforms in angiogenesis

Role of Akt1 has been observed in the process of angiogenesis of endothelial cells (Lee et al 2014, Phung et al 2015). In ovarian cancer, Akt3 was found to induce the secretion of VEGF which in turn affected the vascular growth in a tumor of a xenograft mouse model (Liby et al 2012). However, a contrasting observation was made in endothelial tumor cell

where Akt3 was found to inhibit the vascular tumor endothelial cell growth (Phung et al 2015).

1.2.13. Role of Akt isoforms in chemo and radioresistance of cancer cells

Chemotherapy and radiotherapy remain the standard methods for the treatment but often the patients experience tumor recurrence due to the development of resistance in cancer cells. Development of resistance is a complex process and is regulated by many signaling pathways (Kim et al 2015, Abdullah and Kai-Hua 2013, Chuthapisith 2011, Hittelman 2010). Akt kinase pathway is one of the important pathways involved in chemoresistance and radioresistance of various cancers (Shimura et al 2012). However, only limited number of studies on Akt isoform-specific associations with the development of chemoresistance and/or radioresistance has been reported. Constitutive activation of Akt1 has been shown to prevent the mammary epithelial cells from anoikis and suppressed chemotherapy-induced apoptosis (Schmidt et al 2002). The activation of the Akt1 kinase in radioresistance has been linked to different cancer lines by inhibition of PP1alpha (Eke et al 2010). Moreover, upregulation of Akt3 confers resistance to the Akt inhibitor MK2206 in breast cancer (Stottrup et al 2016). Overexpression of Akt2 has been associated with chemoresistance against cisplatin in ovarian cancer, docetaxel in both ovarian and breast cancer, VM-26 in glioma, and erlotinib in pancreatic cancer (Yuan et al 2003, Xing et al 2008, Cui et al 2012, Banno et al 2017). In case of colon cancer, Akt2 overexpression has been linked to proliferation, apoptosis, and development of chemoresistance (Ding et al 2015, Li et al 2011). Though, for developing radioresistance in colon cancer cells both Akt1 and Akt2 are efficiently involved through nonhomologous end joining pathway (Sahlberg et al 2014). In case of uterine cancer, the

participation of both Akt2 and Akt3 was indicated in inducing chemoresistance to cisplatin (Gagnon et al 2004). In endometrial cancer, both Akt1 and Akt2 has been implicated in the development of chemoresistance against cisplatin and doxorubicin (Girouard et al 2013).

1.2.14. Recent advances in therapeutics for specific targeting of Akt isoforms

Targeting of Akt kinase has led to significant advances in the field of cancer therapeutics and at present many Akt inhibitors are under pre-clinical and clinical trials. However, miltefosine is the only drug that has been approved by FDA. Depending on the mechanism of action, Akt inhibitors are majorly divided into six main classes which include ATP- competitive inhibitors of Akt, lipid-based Akt inhibitors (act by preventing the generation of PIP3 by PI3K), pseudosubstrate inhibitors, allosteric inhibitors of Akt kinase domain, antibodies and compounds that interact with the PH domain of Akt. Also, other molecules such as antisense oligonucleotides are found to be significant in targeting Akt kinase (Bhutani et al 2013). Nevertheless, isoform-specific roles of Akt indicated the specific targeting is necessary for more precise cancer treatment. Albeit not much has been achieved in targeting them more specifically and only limited number of reports are available so far. Utilization of antisense oligonucleotides such as siRNAs, shRNAs, and microRNAs for specific targeting in different cancers has been tried so far. Their application was found to either reduce the proliferation of cancer cells or increases the sensitivity of drugs when used in combination (Nishikawa et al 2015, Tei et al 2015, Chen et al 2014, Cai et al 2013, Chinet al 2014[a]). Though, in some of the studies, pharmacological inhibitors have been used for specific Akt isoforms targeting. The 2,3,5-trisubstituted pyridine derivatives have been shown to be an allosteric Akt1 and Akt2

inhibitors and displayed selectivity over the Akt3 isozyme. Moreover, on substitution with a heterocycle in the 5 position, it became more potent for Akt2 than Akt1 isoform (Hartnett et al 2008). In another study, hyperforin (natural phloroglucinol compound) was reported to inhibit Akt1 kinase activity and promote caspase-mediated apoptosis (Merhi et al 2011). Recently, a protein-targeting strategy has been applied to develop a selective inhibitor of the E17K point mutation of Akt1. In this study, it was found that azide presenting peptides can selectively block the E17K Akt1 interaction with its PIP3 (phosphatidylinositol (3,4,5)-trisphosphate) substrate (Deyle et al 2015). Until now, a handful of studies have been conducted in specific targeting of Akt isoforms and this process can be accelerated by categorizing the already available Akt inhibitors based on their affinity towards the isoforms and recommend it to patients on cancer to cancer basis depending on the active Akt isoform involved.

1.3. IMPORTANCE OF THE STUDY

Cancer is known to be a multifactorial disease and there are some key players involved in signal transduction pathways of tumor progression and development which are an utmost requirement. Akt is one such kinase whose functions are proved to be crucial for the pathogenesis of cancer. Some level of preliminary work has been accomplished regarding its involvement in the development of OSCC, although the studies related to Akt-isoforms specific associations are found to be limited. Decoding the molecular network of Akt isoforms in OSCC can provide us the specific target against which appropriate therapeutic modalities can be developed without affecting the other molecular mediators. Also, these investigations can help us in revealing the potential of Akt isoforms as a prognostic marker in the development of OSCC, since many reports are available where

Akt expressions have been correlated with grading and staging of cancer and even the treatment outcome.

Based on these scopes the objectives of the study were framed as:

1. To determine the expressional and mutational status of Akt isoforms in (Akt1, Akt2, and Akt3) in different stages of OSCC of patients' samples.

2.To check the effect of pure Tobacco extract (TE) and other commercially available tobacco components on OSCC cells in relation to Akt isoforms and to establish the role of different Akt isoforms in the development of OSCC and to examine.

3. To demarcate the general Akt kinase inhibitors into Akt-isoform Specific Inhibitors based on docking studies.

Chapter 2

Expression and Mutational Analysis of Akt Isoforms in Oral Cancer

2.1. INTRODUCTION

As discussed in the earlier chapter, OSCC is the most frequent malignant tumor of the head and neck, and in the recent years, its incidence has increased dramatically. Although, till date, several advancements has been made in chemotherapy, radiotherapy, and targeted therapy, the prognosis of OSCC is relatively poor as a result of aggressive local invasion and metastasis, leading to recurrence. As a consequence, OSCC still remains a challenging disease to treat (Gareth 2009, Wang et al 2013, Bagan et al 2008). According to WHO, the oral cancer patient has very poor five-year survival rate of 45% if diagnosed at late stages. However, if oral cancer is detected at early stages of development, it has a high survival rate of 80 to 90%. Although, the lack of public awareness and advance screening methods are responsible for poor prognosis and low survival rate (Liu et al 2006, Wang et al 2014, Ehsan et al 2012). It is now well established that Akt isoforms are overexpressed in different cancers and their expression varies significantly in different cancers. Moreover, their active involvement has been observed in radio and chemoresistance of cancer cells. In addition, their potential as predictive as well as prognosis biomarkers of the disease is highly promising. However, till date very limited studies have been carried out to evaluate the expression of Akt isoforms in oral cancer; the expression of these isoforms in different stages, cytological types, and organs of oral cancer. Therefore, the present study was an attempt to evaluate the expression of Akt isoforms in oral cancer. Moreover, as per the preceding chapter, it was observed that aberrant activation of Akt kinase is present in different cancers and it can occur by various mechanisms such as amplifications, overexpression, mutations in Akt genes, and/or alterations in Akt upstream regulators (Roy et al 2017, Gonzalez and

McGraw 2009). Akt isoforms are known to play specific roles and even opposing functions in different cancers as suggested by many studies (Toker 2012, Veeriah 2012, Phung et al 2015, Linnerth-Petrik et al 2014, Riggio et al 2017, Linnerth-Petrik et al 2016). Keeping in mind the fact that genetic alterations present in Akt isoforms may have different implications and functional consequences in different cancers, it becomes imperative to explore these variations in oral cancer exclusively. In the present work, we have analysed the expression of Akt isoforms in human oral tumor samples as well as in normal tissues samples collected from different organs of patients such as nose, larynx, and tongue of different stages, grades and degree of differentiation from both male and female of different ages. Moreover, apart from analysing the expressional status of Akt isoforms in oral cancer, mutational status of these isoforms in cancer patients was evaluated from "The Cancer Genome Atlas (TCGA)" data portal. Additionally, the correlation between genetic alterations of the Akt isoforms with the progression free survival (PFS) and disease free survival (DFS) has been examined.

2.2. MATERIALS AND METHODS

2.2.1. Tissue microarray (TMA)

TMA slides were used for analyzing the expression of Akt isoforms in oral cancer tissues. The slides consisted of oral cancer tissues of 80 different patient samples of different age groups, genders and were from organs such as tongue, cheek, gingiva, lip, and palate. The slides contain 28 cases of squamous cell carcinoma, 4 adenocarcinoma, 8 mucoepidermoid carcinoma, 2 basal cell carcinoma, 4 metastatic carcinoma, 8 adamantinoma, 6 hyperplasia, 5 each of adjacent tissue, inflammation, adjacent normal tissue and normal tissue, single core per case. The tissue microarray slides were obtained

from US biomax company (OR802) (Fig 2.1A). The core diameter was 1.5 mm with a thickness of 5μM.

TMA Details:

Oral cavity disease spectrum (oral cavity cancer progression) tissue array, 79 cases/80 cores

Name: OR802
Panel: Oral cavity disease spectrum (oral cavity carcinoma progression) tissue microarray
Cases: 79
Cores: 80
Diameter: 1.5mm
Rows: 8
Columns: 10

Position	Age	Sex	Organ	Pathology diagnosis	TNM	Stage
A1[a]	40	M	Gingiva	Squamous cell carcinoma	T4N0M0	IV
A2[a]	47	F	Tongue	Squamous cell carcinoma	T1N0M0	I
A3[a]	81	M	Lip	Squamous cell carcinoma	T2N0M0	II
A4[a]	57	M	Tongue	Squamous cell carcinoma	T1N0M0	I
A5[a]	52	F	Lip	Squamous cell carcinoma	T1N0M0	I
A6[a]	53	M	Cheek	Squamous cell carcinoma	T2N0M0	II
A7[a]	62	F	Cheek	Squamous cell carcinoma	T1N0M0	I
A8[a]	48	M	Base of tongue	Squamous cell carcinoma	T2N0M0	II
A9[a]	68	M	Right palate	Squamous cell carcinoma	T2N0M0	II
A10[a]	56	F	Cheek	Squamous cell carcinoma	T2N0M0	II
B1[a]	79	M	Cheek	Squamous cell carcinoma	T2N0M0	II
B2[a]	60	M	Gingiva	Squamous cell carcinoma	T1N0M0	I
B3[a]	55	M	Cheek	Squamous cell carcinoma	T1N0M0	I
B4[a]	66	M	Tongue	Squamous cell carcinoma	T1N0M0	I
B5[a]	46	F	Tongue	Squamous cell carcinoma	T1N0M1	IV
B6[a]	39	F	Tongue	Squamous cell carcinoma	T1N0M0	I
B7[a]	78	M	Tongue	Squamous cell carcinoma	T2N0M0	II
B8[a]	78	F	Lip	Squamous cell carcinoma	T1N0M1	IV
B9[a]	54	F	Lip	Squamous cell carcinoma	T1N0M1	IV
B10[a]	75	F	Lip	Squamous cell carcinoma	T1N0M1	IV
C1[a]	60	M	Tongue	Squamous cell carcinoma	T1N0M0	I
C2[a]	73	M	Lip	Squamous cell carcinoma	T1N0M0	I
C3[a]	60	M	Gingiva	Squamous cell carcinoma	T1N0M0	I
C4[a]	78	M	Lip	Squamous cell carcinoma	T1N0M0	I
C5[a]	55	M	Gingiva	Squamous cell carcinoma	T1N0M0	I
C6[a]	47	M	Lower mandible	Squamous cell carcinoma	T2N0M0	II
C7[a]	41	M	Dental alveoli	Squamous cell carcinoma	T1N0M0	I

C8[a]	60	M	Tongue	Squamous cell carcinoma	T2N0M0	II
C9[a]	40	F	Palate	Adenoid cystic carcinoma	T1N0M0	I
C10[a]	45	M	Left lower mandible	Adenoid cystic carcinoma (sparse)	T1N0M0	I
D1[a]	64	M	Palate	Adenoid cystic carcinoma	T2N0M0	II
D2[a]	66	M	Parotid gland	Acinic cell carcinoma	T2N0M0	II
D3[a]	71	M	Mouth floor	Mucoepidermoid carcinoma	T1N0M0	I
D4[a]	57	M	Palate	Mucoepidermoid carcinoma	T2N0M0	II
D5[a]	50	F	Cheek	Mucoepidermoid carcinoma	T2N0M0	II
D6[a]	57	M	Upper lip	Mucoepidermoid carcinoma (skeletal muscle and blood vessel)	T1N0M0	I
D7[a]	48	F	Right lower mandible	Mucoepidermoid carcinoma	T1N0M0	I
D8[a]	55	M	Gingiva	Mucoepidermoid carcinoma	T1N0M0	I
D9[a]	60	M	Right lower mandible	Mucoepidermoid carcinoma	T3N0M0	III
D10[a]	50	M	Root of tongue	Mucoepidermoid carcinoma (sparse)	T1N0M0	I
E1[a]	79	F	Lip	Basal cell carcinoma (sparse)	T2N0M0	II
E2[a]	48	F	Lip	Basal cell carcinoma	T2N0M0	II
E3[b]	70	F	Lymph node	Metastatic squamous cell carcinoma of neck from cheek	-	-
E4[b]	79	M	Lymph node	Metastatic squamous cell carcinoma of neck from tongue	-	-
E5[b]	59	F	Lymph node	Metastatic squamous cell carcinoma of neck from mandible	-	-
E6[b]	40	F	Lymph node	Metastatic mucoepidermoid carcinoma of neck from mandible	-	-
E7[e]	11	M	Mandible	Adamantinoma	-	-
E8[e]	28	M	Left mandible	Adamantinoma	-	-
E9[e]	51	M	Right mandible	Adamantinoma	-	-
E10[e]	64	M	Mandible	Adamantinoma (fibrous tissue and blood vessel)	-	-
F1[e]	37	F	Mandible	Adamantinoma	-	-
F2[e]	40	M	Lower mandible	Adamantinoma	-	-
F3[e]	47	F	Mandible	Adamantinoma	-	-
F4[e]	70	F	Right jaw bones	Adamantinoma	-	-
F5[f]	67	M	Lip	Hyperplasia of squamous epithelium	-	-
F6[f]	40	M	Lip	Mild atypical hyperplasia of squamous epithelium	-	-
F7[f]	82	M	Lip	Hyperplasia of squamous epithelium	-	-
F8[f]	46	M	Tongue	Hyperplasia of squamous epithelium (skeletal muscle	-	-

				and blood vessel)		
F9[f]	60	F	Tongue	Hyperplasia of squamous epithelium	-	-
F10[f]	3	F	Tongue	Hyperplasia of squamous epithelium	-	-
G1[g]	68	M	Parotid gland	Cancer adjacent tissue (with squamous cell carcioma sparse)	-	-
G2[g]	53	F	Tongue	Cancer adjacent tissue	-	-
G3[g]	53	M	Tongue	Cancer adjacent tissue (hyperplasia of squamous epithelium)	-	-
G4[g]	70	M	Parotid gland	Cancer adjacent tissue (with mucoepidermoid carcinoma)	-	-
G5[g]	63	M	Lip	Cancer adjacent tissue (chronic inflammation of fibrous tissue and blood vessel)	-	-
G6[h]	43	M	Sub maxillary gland	Chronic submaxillaritis	-	-
G7[h]	23	F	Parotid gland	Chronic parotitis	-	-
G8[h]	66	F	Parotid gland	Chronic parotitis	-	-
G9[h]	40	F	Right cheek	Chronic inflammation of mucosa	-	-
G10[h]	75	F	Lower lip	Chronic inflammation of mucosa of No. 20	-	-
H1[d]	48	F	Salivary gland	Cancer adjacent normal salivary gland tissue	-	-
H2[d]	48	F	Salivary gland	Cancer adjacent normal salivary gland tissue	-	-
H3[d]	37	M	Salivary gland	Cancer adjacent normal salivary gland tissue	-	-
H4[d]	63	M	Salivary gland	Cancer adjacent normal salivary gland tissue	-	-
H5[d]	56	M	Salivary gland	Cancer adjacent normal salivary gland tissue	-	-
H6[c]	42	F	Tongue	Normal tongue tissue	-	-
H7[c]	38	F	Salivary gland	Normal salivary gland tissue	-	-
H8[c]	48	M	Tongue	Normal tongue tissue	-	-
H9[c]	50	M	Salivary gland	Normal salivary gland tissue	-	-
H10[c]	22	M	Salivary gland	Normal salivary gland tissue	-	-

a: malignant tissues, b: metastatic tissues, c: Normal tissues, d: Normal adjacent tissue, e: benign tissues, f: hyperplastic tissues, g: cancer adjacent tissue, h: inflammation.

Table 2.1: Oral cavity tissue array details (OR802).

2.2.2. Immunohistochemistry (IHC)

The expression of Akt isoforms was determined by IHC. Briefly, the TMA slides were treated in xylene to remove the paraffin, rehydrated through serial dilutions of alcohol, followed by washings with PBS (pH 7.2). Subsequently, the slides were placed in a citrate buffer (pH 6.0) and heated on a plate for 15-min for antigen retrieval. The samples were then incubated with a monoclonal anti-rabbit Akt1, Akt2 and Akt3 antibody (Akt1; CST, Cat No. 2938, Akt2; Abcam, Cat No Ab66129, Akt3; CST, Cat No. 3788) for overnight at 25^{o}C. The following day it was incubated with the streptavidin conjugated secondary antibody for 30 mins in moist chamber (IHC Kit Histostain Plus, Invitrogen, Cat No. 859043). The 3,3'-diaminobenzidine tetrahydrochloride (DAB) compound was used for signal development and subsequently the cells were counter-stained with hematoxylin. The slides were mounted with DPX and the pictures were captured by the Nikon Eclipse T100 microscopic and Nikon Digital Camera System for study comparison (Cheng et al 2007).

2.2.3. Scoring of IHC Staining

The tissue microarray slides were examined using a Nikon Eclipse Ti-E microscope. The scoring of the specimens was based on the staining intensity and percentage of positively stained cells. Every tumor was assigned a score according to the intensity of the nuclear or cytoplasmic staining (no staining=0; weak staining=1; moderate staining=2; intense staining=3) and the frequency of stained cells (Less than 10%=0; 10–25%=1; 26–50%=2; 51–75%=3; 76–100%=4). The final expression score was determined as a product of the intensity and extent of positivity scores of stained cells, with the minimum score of 0 and

a maximum score of 12 (Han et al 2009, Remmele and Schicketanz 1993, Matos et al 2006, Han et al 2008, Kamoi et al 2002).

2.2.4. TCGA analysis

The genetic alterations were analyzed in head and neck carcinoma patient samples which were obtained from the open data portal of "The Cancer Genome Atlas dataset (TCGA)" and cbioportal platform (http://www.cbioportal.org) (Cerami et al 2012, Gao et al 2013). For the evaluation of genetic alterations of Akt isoforms on prognostic of head and neck cancer patient the Kaplan-Meier survival curve was generated. Specifically, to obtain the data selection "Query" on the home page of the website www.cbioportal.org was made, select "Head and Neck Carcinoma (TCGA, Provisional)" from Select Cancer Study. In the "Select Genomic Profiles", select "Mutations and Putative copy-number alterations" was clicked. In "Enter Gene set", input "AKT1: Exp < -2", then click "Submit". The "Oncoprint" tab will appear initially. For obtaining the survival data the "Survival" tab was clicked, overall survival Kaplan-Meier Estimate and disease free survival will appear.

2.2.5. Statistical Analysis

The statistical analysis for the expression score was achieved by performing one-way ANOVA. A p-value of < 0.05 was accepted as statistically significant.

2.3. RESULT AND DISCUSSION

In the present study, we have performed IHC to analyze the expression of different isoforms of Akt (Akt1, 2 and 3) on TMA slides of oral cancer patient samples. The oral cancer patient samples consist of specimens from different organs, types, stages and grades. This tissue microarray also contains normal salivary gland tissues.

2.3.1. Expression of Akt isoforms in normal and malignant oral tissues

The analysis of the expression of Akt isoforms in TMA slides have shown that Akt1 and 2 are overexpressed while no significant change in the expression of Akt3 was observed as compared to the normal tissues (Fig 2.1B and C). Similar to this result, earlier study led by Iamaroon and Krisanaprakornkit have shown that Akt2 was overexpressed while Akt1 was constitutively expressed in OSCC (Iamaroon and Krisanaprakornkit 2009). Moreover, overexpression of Akt1 and 2 isoform has been observed in many other cancers such as breast, liver, lung, glioma, neuroblastoma etc. These studies have indicated that differential expression of Akt isoforms exists in different cancers (Roy et al 2017).

2.3.2. Expression of Akt isoforms with respect to Genders

Since oral cancer incidence has the higher percentage in male than female whether expression of Akt isoforms varies among genders (Geurink 2014) was studied. The IHC of TMA slides has shown no significant variation in the expression of Akt isoforms with respect to gender (Fig 2.2). It can be inferred that the expression of Akt isoforms are not associated with different genders. Similar to the observation made, the previous report has also shown that the Akt expression in tumor epithelial cells and stroma did not have any correlation with genders along with other parameters such as age, smoking, clinical performance status, vascular infiltration, tumor differentiation or histological type (Al-Saad et al 2009). Also, the phosphorylated form of Akt (p-Akt) has been shown to have no correlation with the genders of cancer patients in NSCLC and nasopharyngeal carcinoma (Zhou et al 2007, Wang et al 2014). Moreover, the expression patterns of Akt1

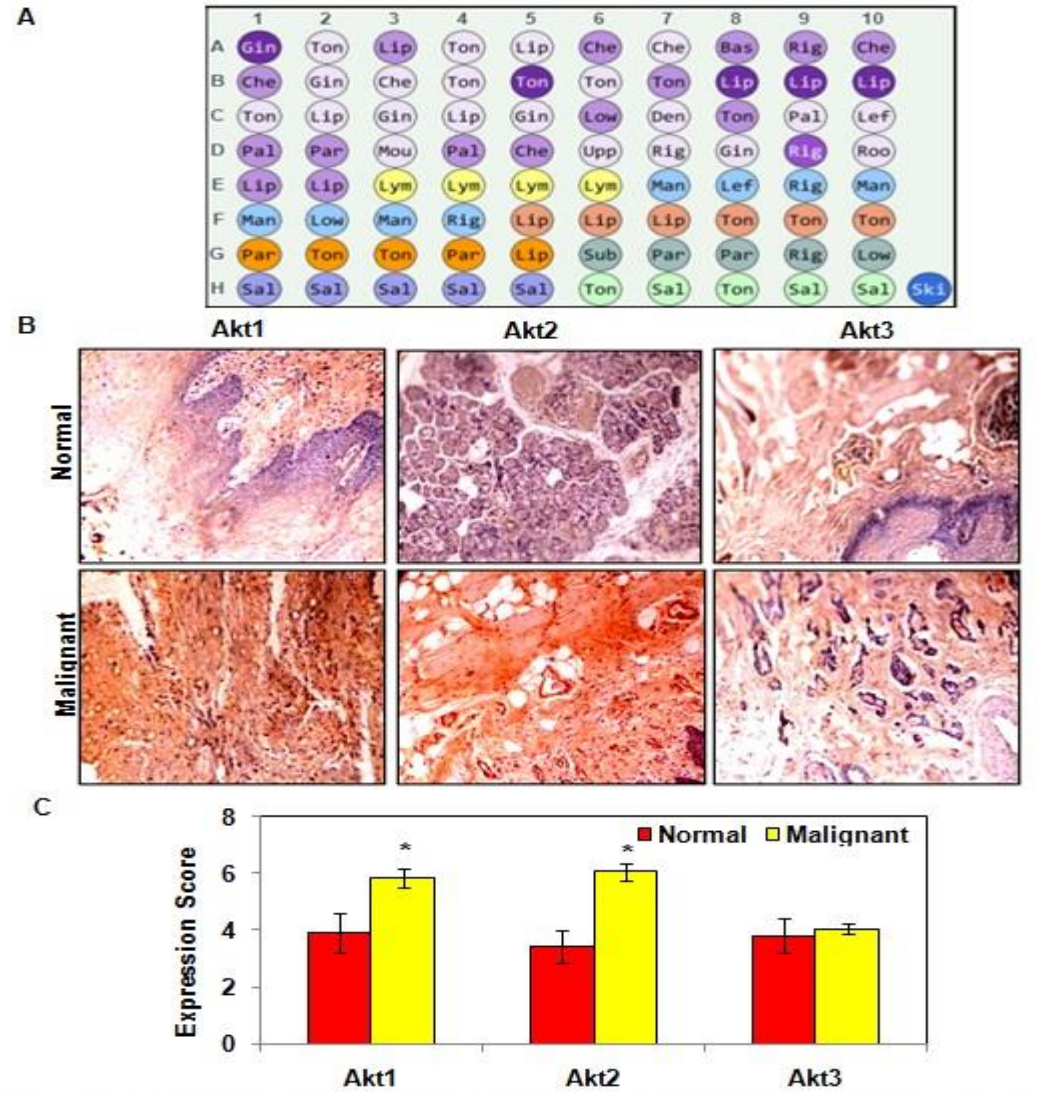


Figure 2.1: Expression of Akt isoforms in normal and malignant oral tissues: A; Schematic representation of TMA slides showing different tissue samples. B; Representative images showing the expression of Akt isoforms in tissues C; Bar graph of the expression score for the normal (n=10) and malignant (n=70) tissues. Bas: Base of tongue, Che: Cheek, Den: Dental alveoli, Gin: Gingiva, Lef: Left mandible, Lip: Lip, Low: Lower lip, Lym: Lymph node, Man: Mandible, Mou: Mouth floor, Pal: Palate, Par: Parotid gland, Rig: Right cheek, Roo: Root of tongue, Sal: Salivary gland, Sub: Submaxillary gland, Ton: Tongue, Upp: Upper lip. Data are expressed as the mean ± SE. *=p<0.05.

-AT, - Benign tumor, - Hyperplasia, - Inflammation, - Malignant tumor, - Malignant tumor (stage I), - Malignant tumor (stage II), - Malignant tumor (stage III), - Malignant tumor (stage IV), - Metastasis, - NAT, - Normal tissue.

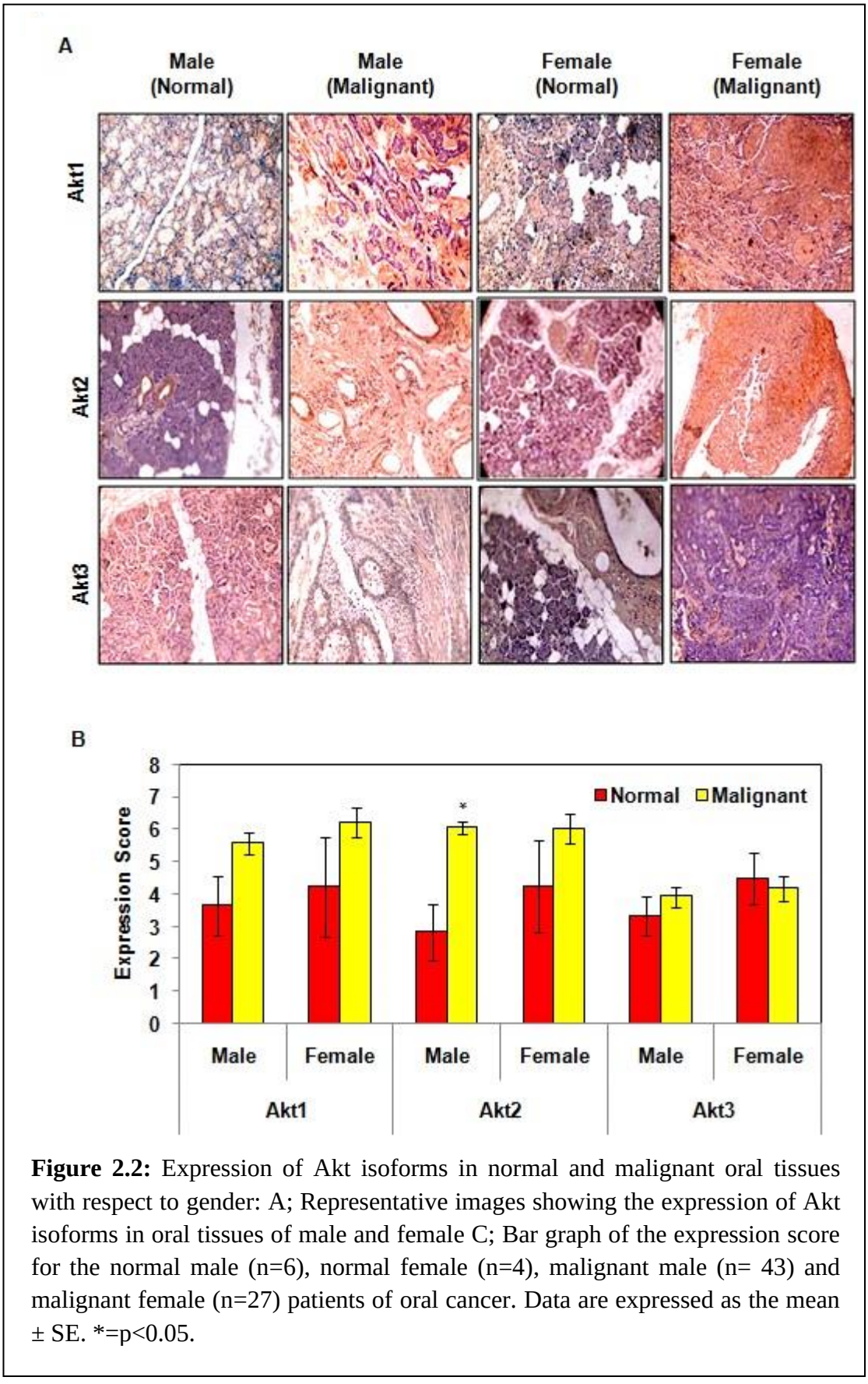


Figure 2.2: Expression of Akt isoforms in normal and malignant oral tissues with respect to gender: A; Representative images showing the expression of Akt isoforms in oral tissues of male and female C; Bar graph of the expression score for the normal male (n=6), normal female (n=4), malignant male (n= 43) and malignant female (n=27) patients of oral cancer. Data are expressed as the mean ± SE. *=$p<0.05$.

and 2 exhibited no statistically significant difference according to patient gender in case of osteosarcoma and NSCLC (Zhu et al 2017, Pu et al 2010).

2.3.3. Expression of Akt isoforms in different tissue types of oral cancer

The expression of Akt isoforms was also evaluated with respect to different tissue types such as normal, inflammation, hyperplasia, and cancer adjacent tissues (CAT) and malignant tissues. It was found that the maximum expression of Akt1 and 2 was in malignant tumor types in comparison to normal tissues (Fig 2.3). The role of Akt isoforms has been shown in inflammatory conditions especially in vascular diseases. (Di Lorenzo et al 2009, Yu et al 2015). The significance of Akt isoforms in hyperplasia has earlier been shown by the work of Bernal-Mizrachi and group who have reported the constitutive expression of Akt1 in the induction of hypertrophy, hyperplasia, and hyperinsulinemia in Islet β cell (Bernal-Mizrachi et al 2001). However, Akt isoform-specific involvement in other tissue types is yet to be established. It appears that the variation of expression of Akt isoforms in different stages is cancer-specific.

2.3.4. Expression of Akt isoforms in different stages of oral cancer

The expression of Akt isoforms has been examined in different stages (Stage I, II, III and IV) of oral cancer development. It was found that Akt1 and 2 were overexpressed while the expression of Akt3 isoform remains unchanged (Fig 2.4). Also, it was found that the expression of Akt1 and 2 gradually increased with respect to advanced stages of oral cancer. In contrast to the result obtained the study by Rychahou and group have shown the similar expression levels of Akt1 and 2 mRNA in all colorectal cancer samples irrespective of tumor stage (Rychahou et al 2008). However, Riggio et al have shown that Akt1 and 2 behave differently in early and late stages of breast cancer and they perform

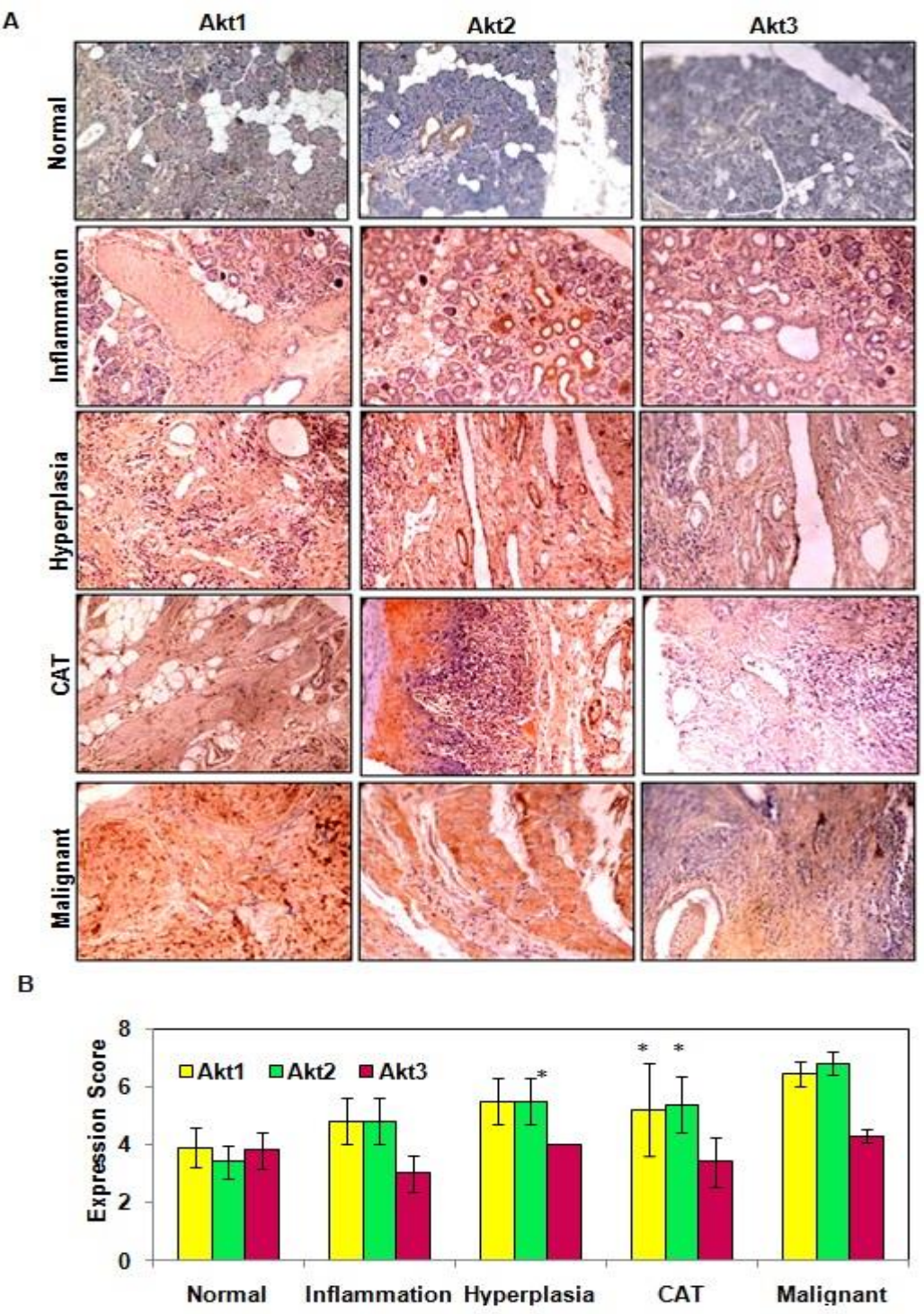


Figure 2.3: Expression of Akt isoforms in different tissue types of oral cavity. CAT: Cancer adjacent tissue. A; Representative images of expression of Akt isoforms in different tumor types of oral cancer. B; Bar graph of the expression score for the Normal tissues (n= 10), inflammation (n= 5), hyperplasia (n= 5), CAT (n=5), malignant tissues (n=42). Data are means ± SE. *= $p<0.05$.

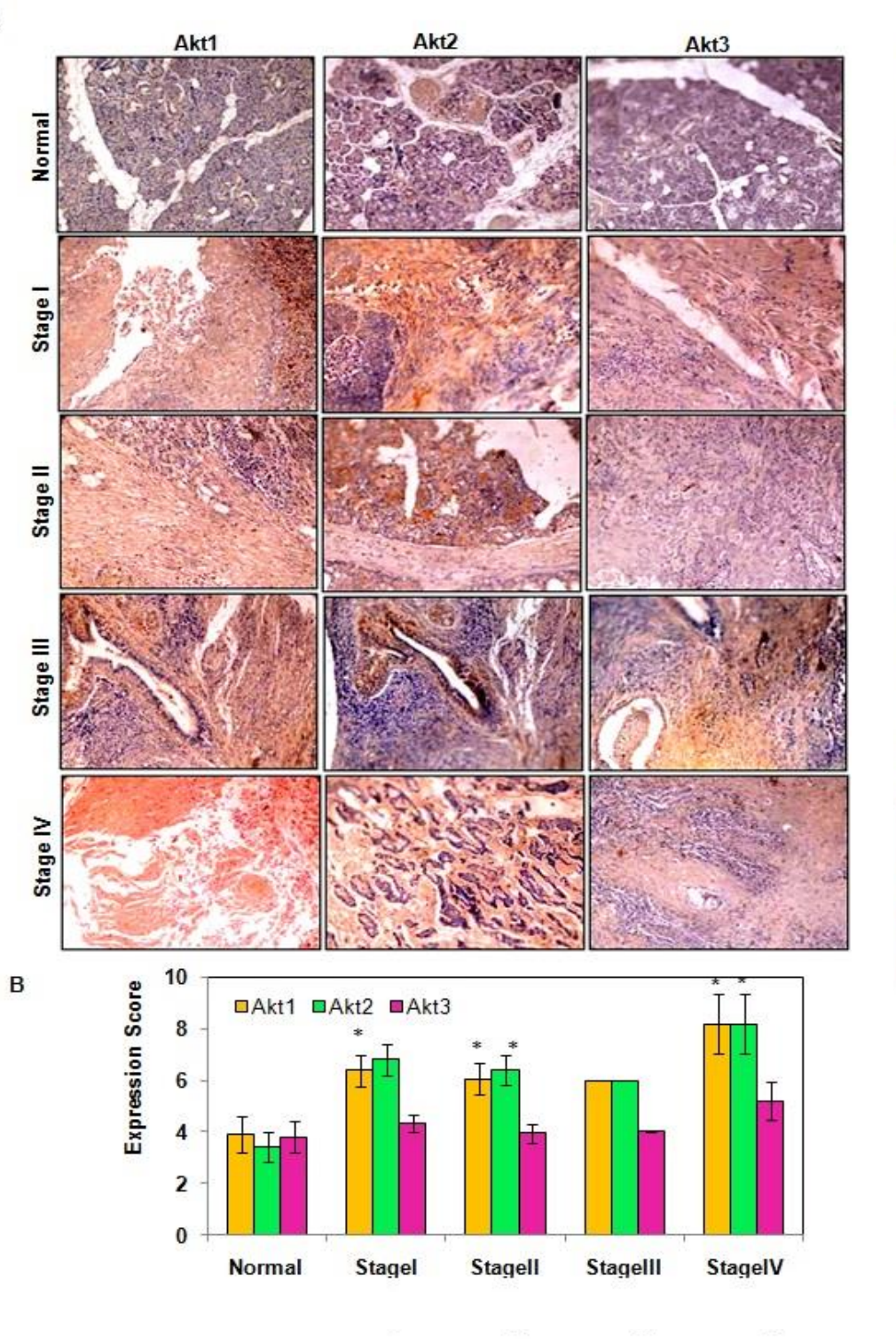


Figure 2.4: Expression of Akt isoforms in different stages of oral cancer: A; Representative images of expression of Akt isoforms in different stages of oral cancer. B; Bar graph of the expression score for the normal tissues (n=10) and malignant tissues of stage I (n=21), stage II (n=23), stage III (n=1) and stage IV (n=5). Data are expressed as the mean ± SE. *=$p<0.05$.

unique functions in breast cancer progression through the regulation of specific downstream proteins (Riggio et al 2017).

2.3.5. Expression of Akt isoforms in different regions of oral cavity

The expression of Akt isoforms in different regions of oral cavity such as lip, gingiva, palate, mandible, parotid gland, lymph node, cheek, and tongue have shown the increased expression of Akt1 and 2 as compared to normal tissues. Though, the palate region has not shown significant increase in expression of Akt1 and 2 isoforms. The maximum expression of Akt1 was observed in tongue tissues followed by gingiva and cheek while for Akt2, gingiva and tongue showed the maximum expression followed by cheek (Fig 2.5). Few other studies have reported the activation of Akt in tongue cancer and were found to be associated with adverse outcomes in patients (Watanabe et al 2009, Massarelli et al 2005). Thus, it appears that out of all the three isoforms, only Akt1 and 2 take active part in tongue cancer development but not Akt3. Nevertheless, more focused studies in this regard need to be undertaken to infer convincingly.

2.3.6. Mutational status of Akt isoforms from TCGA data portal

In view of the fact that through IHC, only expression of the Akt isoforms in the cancer tissues was analyzed but information on the mutational status of the sample could not be retrieved. So, an attempt has been made to analyze the mutational status of Akt isoforms in tissues of different cancer patients. From "The Cancer Genome Atlas (TCGA)" datasets, the mutational status including the data sets of DNA amplifications, mutations, and deletions of 504 patients of HNSCC was obtained and analyzed. The maximum genetic alteration was found in Akt1 with a percentage of 2.8% followed by Akt3 (2.5%) and Akt1 (2%). On careful examination of heatmap against the genetic alterations, it

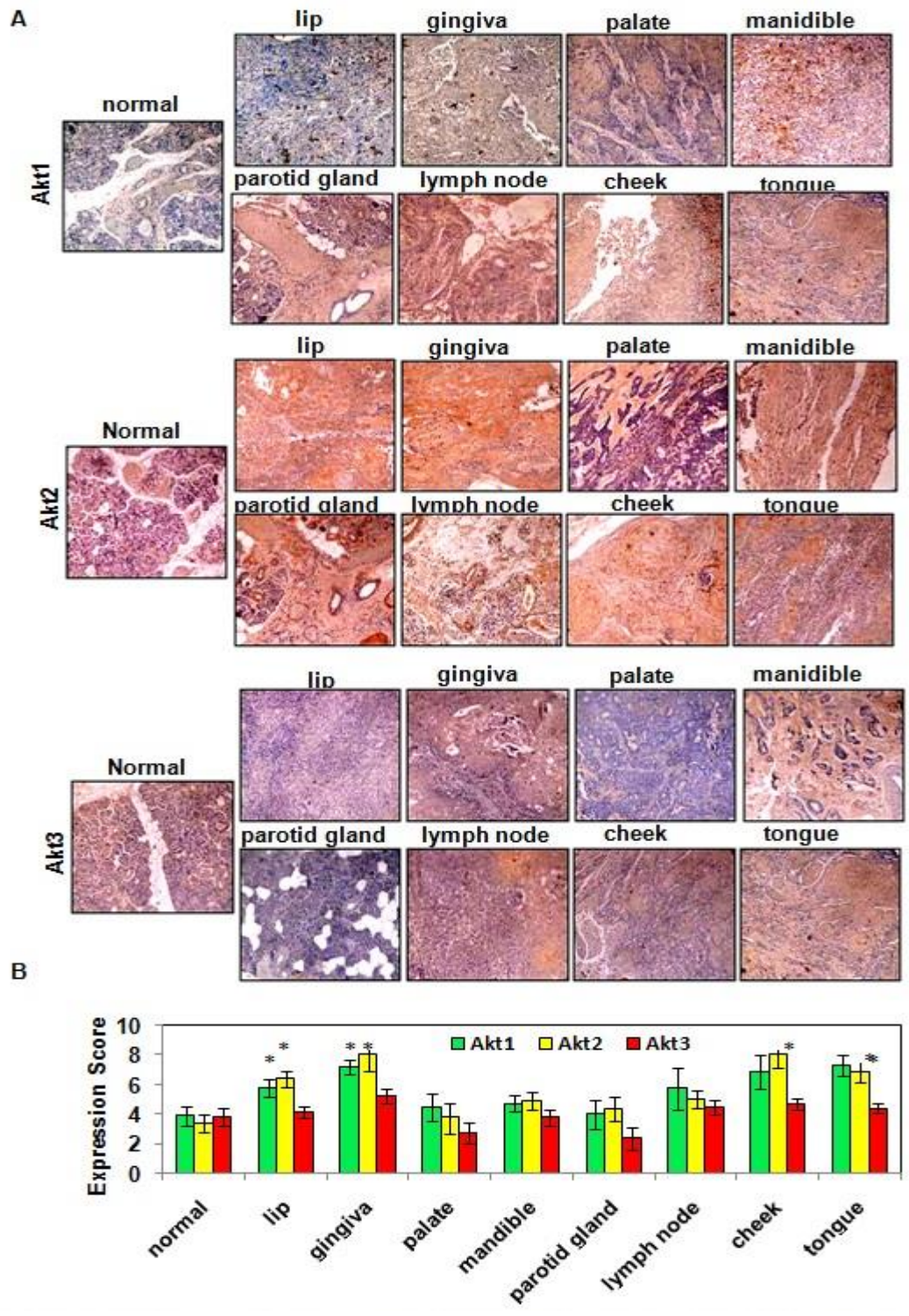


Figure 2.5: Expression of Akt isoforms in different organs of oral cavity: A; Representative images of expression of Akt isoforms in different organs of oral region, B; Bar graph of the expression score for the normal (n=10), lip (n=15), gingiva (n=5), palate (n=4), mandible (n=11), parotid gland (n=5), lymph node (n=4), cheek (n=7) and tongue (n=15) . Data are expressed as the mean ± SE. *=$p<0.05$.

becomes evident that the gene amplification led to increase in the mRNA expression for both Akt1 and 2 gene. However, in case of Akt3 gene, such observation was not made except in few cases. The previous report has suggested that the genes which are normally silent even when amplified don't play any significant role in tumor progression. The weak associations between mRNA level and protein accumulation and/or activity hint towards the importance of the translational or post-translational events in the final outcome of gene amplification (Vogt et al 2010) (Fig 2.6). In case of Akt1, in addition to amplification mutation, deep deletion and missense mutations were found. Deep deletion was found to decrease the expression of Akt1 gene at the mRNA level. Two missense mutations of E17K and E49K were observed in the PH domain of the Akt1protein. These mutations lead to hyperactivation of Akt1 and was reported in different cancers such as breast, colorectal, ovarian, endometrial cancers, bladder cancer and lung cancer (Jung et al 2016, Askham et al 2010, Yu et al 2015). In the present data, E17K mutation was showing the elevated expression of mRNA (1.8) while the E49K showed decreased mRNA expression (-1.7). In case of Akt2 isoform, five mutations Y351C, P471S, D388N, and G394Wfs*48 were observed. The previous report has been shown the involvement of Y351C in HNSCC. Y351 is considered as a putative phosphorylation site present in Akt2 and is found to be conserved among multiple species (Giudice and Squarize 2013). It was found that the sample carrying the Y351C mutation has elevated mRNA expression with an increase infold change of 1.9 (Table 2.2). The impact of all other mutations in protein function of Akt2 and on the cell as a whole is not well understood. In case of Akt3, mutations such as R343M, S403G, S136C, K152R, and

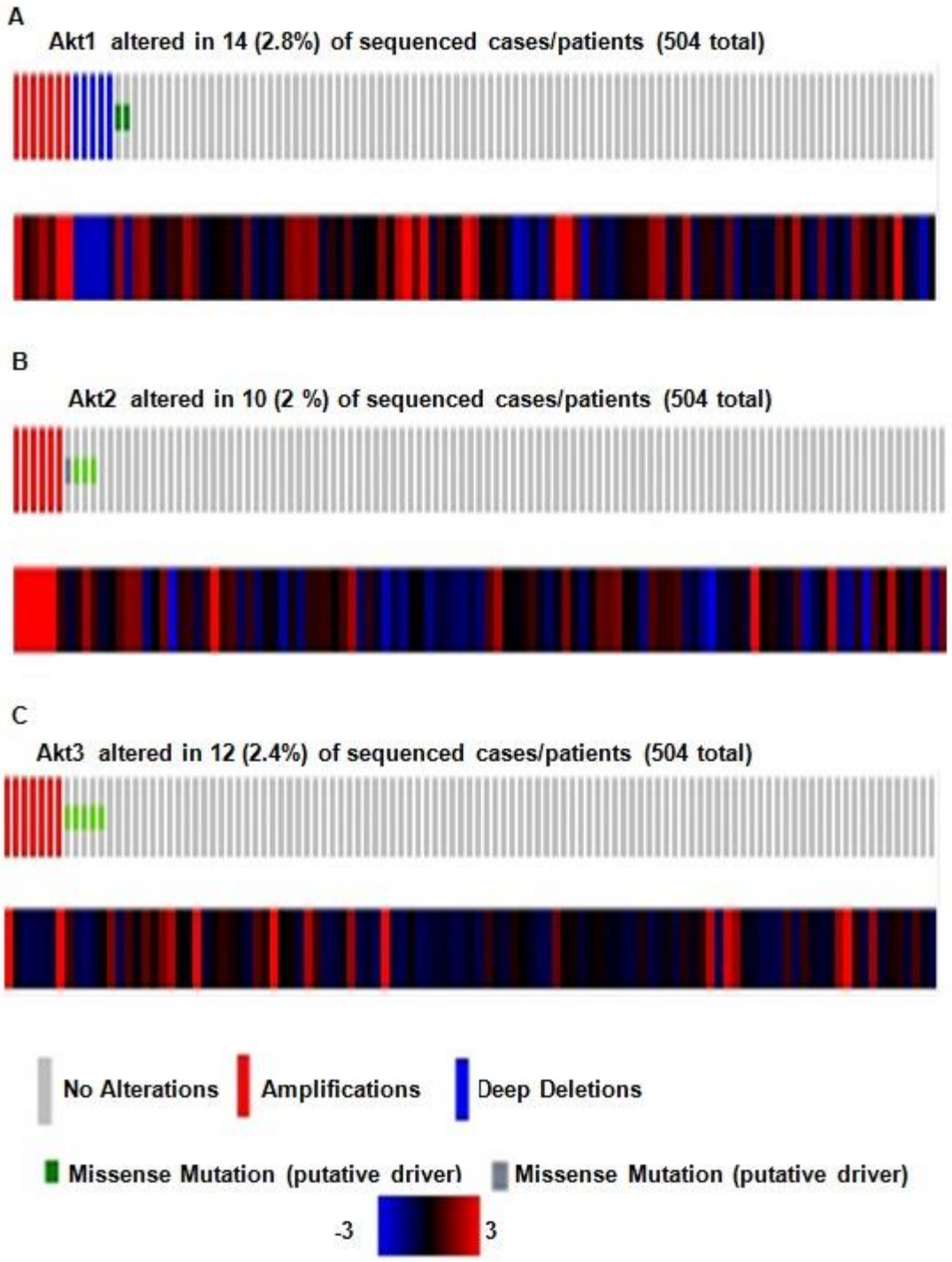


Figure 2.6: Genetic alterations of Akt isoforms present in HNSCC samples (n = 504) (www.cbioportal.org): A; Genetic alterations present in Akt1 (2.8%), B; Akt2 (2%), C; and Akt3 (2.4%) along with the heatmap showing their outcome on the level of mRNA transcript. The maximum percentage of alterations was present in Akt1 followed by Akt3 and Akt1, of which amplification of gene was most predominant.

Reference No.	Akt Isoform	Genetic Alteration	mRNA Z-Scores
TCGA-CV-5973	Akt1	Amplication	2.9317
TCGA-CN-6994	Akt1	Amplication	0.27
TCGA-CV-6934	Akt1	Amplication	0.9579
TCGA-CV-6951	Akt1	Amplication	1.9371
TCGA-HD-A4C1	Akt1	Amplication	0.7723
TCGA-HD-A4C1	Akt1	Amplication	10.587
TCGA-CN-A63W	Akt1	Amplication	8.5591
TCGA-CN-6020	Akt1	Deep deletion	-2.0056
TCGA-CV-6433	Akt1	Deep deletion	-2.2758
TCGA-CR-7399	Akt1	Deep deletion	-2.1521
TCGA-BB-4228	Akt1	Deep deletion	-2.3457
TCGA-CN-4728	Akt1	Deep deletion	-1.0963
TCGA-CV-6936	Akt1	Missense Mutation (E49K)	1.8099
TCGA-CV-5443	Akt1	Missense Mutation (E17K)	-1.7001
TCGA-CN-6012	Akt2	Amplication	23.2331
TCGA-CV-5434	Akt2	Amplication	7.2546
TCGA-CV-7415	Akt2	Amplication	8.164
TCGA-CV-7421	Akt2	Amplication	7.7094
TCGA-F7-A623	Akt2	Amplication	9.7156
TCGA-QK-A6IF	Akt2	Amplication	0.5676
TCGA-CN-4740	Akt2	Truncating Mutation (G394Wfs*48)	-0.6521
TCGA-DQ-5625	Akt2	Missense Mutation (D388N)	0.3533
TCGA-CN-6998	Akt2	Missense Mutation (Y351C)	1.9428
TCGA-CV-7252	Akt2	Missense Mutation (P471S)	0.5172
TCGA-BA-6871	Akt3	Amplication	5.0563
TCGA-CV-7235	Akt3	Amplication	-0.2159
TCGA-BB-8601	Akt3	Amplication	-0.974
TCGA-CN-A49B	Akt3	Amplication	-0.7115
TCGA-P3-A6SX	Akt3	Amplication	-0.7754
TCGA-UF-A71B	Akt3	Amplication	-0.5326
TCGA-CN-4735	Akt3	Amplication	12.7024
TCGA-CR-7367	Akt3	Missense Mutation (S403G)	0.7667
TCGA-CV-7407	Akt3	Missense Mutation (S136C)	-0.691
TCGA-CN-A63U	Akt3	Missense Mutation (M145K)	-1.0717
TCGA-P3-A6T0	Akt3	Missense Mutation (K152R)	-0.6309
TCGA-BA-4074	Akt3	Missense Mutation (R343M)	0.0302

Table 2.2: Outcome of different genetic alterations of Akt isoforms of HNSCC samples on the expression profile of transcripts (n = 504) (www.cbioportal.org).

M145K was found in cancer patients (Fig 2.7). It was observed that the present mutations of Akt3 could not elevate the expression of Akt3 at mRNA level. Furthermore, till date, the information regarding the role of these mutations in protein functions and its relevance to cancer has not been studied yet. As a whole, it was observed that the most of the mutations related to Akt1 and 2 led to elevated mRNA expression as observed from the heatmap while there was no such effect in Akt3 gene. Since the role of these mutations in oral cancer is unknown, therefore, more elaborate studies must be undertaken extensively to explore their proper function.

2.3.7. Correlation of genetic alterations of Akt isoforms with PFS and DFS

The univariate analysis for survival data of 504 patient samples of HNSCC from TCGA datasets indicate that the increasing abundance of genetic alterations of Akt2 isoform lead to worst overall survival as well as disease-free survival as compared to Akt1 and 3 (Fig 2.8 and 2.9). It was observed that the cases with genetic alterations of Akt2 gene have median survival months of 27.56 months as compared to the cases with no alteration of the Akt2 gene (56.44 months). Moreover, the DFS has shown disease free median months of 34.76 as compared to the 72.44 in case of the unaltered Akt2 isoform. In case of Akt1 gene alteration, median survival was found to be 45.93 months as compared to 56.44 months in cases of unaltered Akt1 gene while DFS months could not be obtained. The survival data for Akt3 could not be obtained for the concerned cancer patients from the TCGA datasets. Several earlier reports have also suggested the profound importance of Akt1 and 2 gene expression in the prognosis of different cancers. In one such study, it was indicated that the increased expression of p-Akt1 was associated with decreased patient survival (Zhu et al 2015). Recently, a similar observation was made for the studies

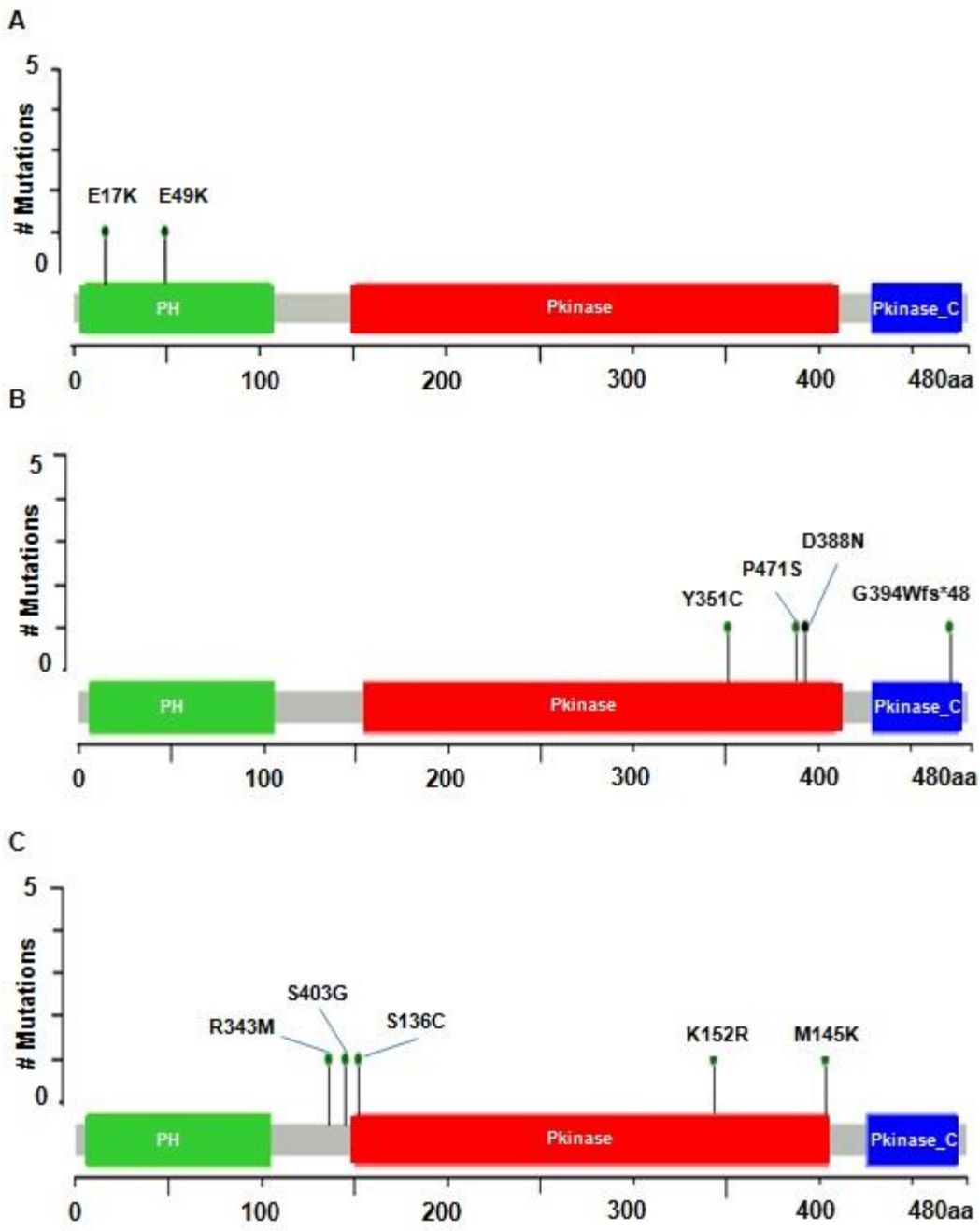
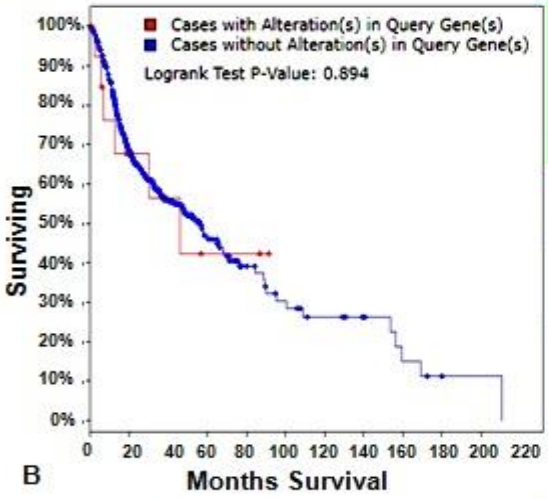


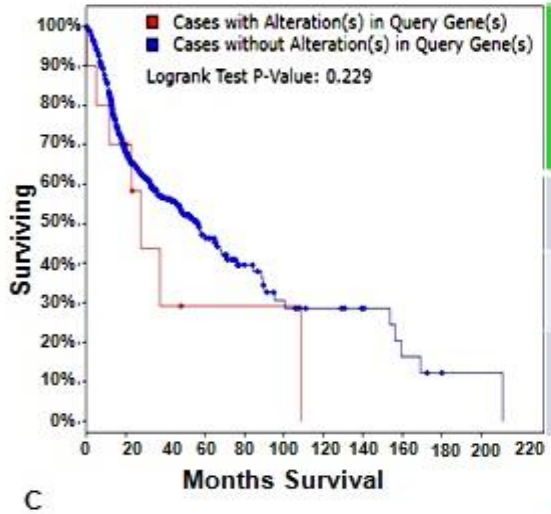
Figure 2.7: Graphical summary of mutations of Akt isoforms present in HNSCC samples (n = 504) (www.cbioportal.org): A; Akt1, B; Akt2 and C; Akt3 isoforms. Green dots represent sites for which the majority of alterations are missense mutations. Black dots represent sites for which the majority of alterations are in-frame deletions.

A



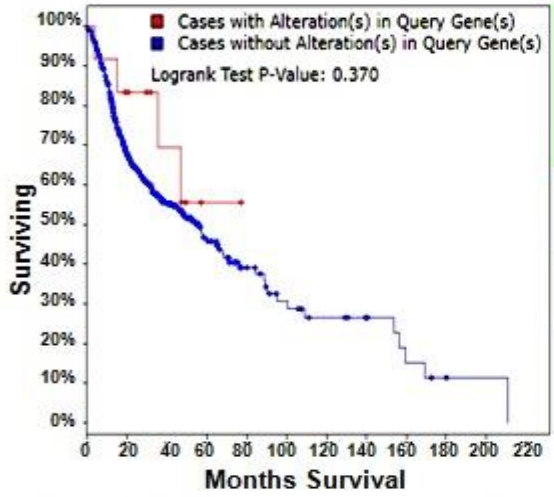
Akt1	Cases with Alterations in Query Gene	Cases without Alterations in Query Gene
#total cases	14	487
#cases deceased	6	209
median months survival	45.93	56.44

B



Akt2	Cases with Alterations in Query Gene	Cases without Alterations in Query Gene
#total cases	10	491
#cases deceased	7	208
median months survival	27.56	56.44

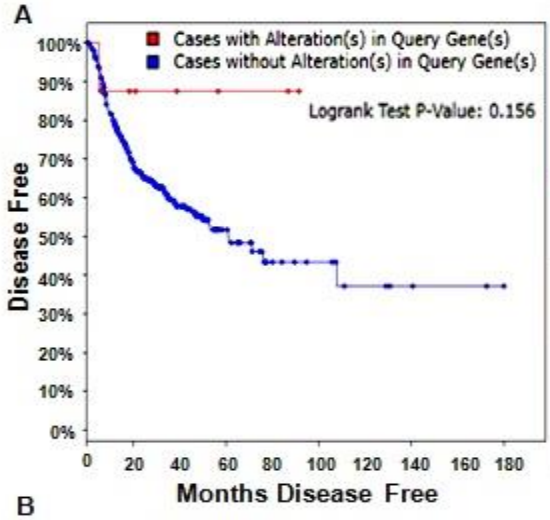
C



Akt3	Cases with Alterations in Query Gene	Cases without Alterations in Query Gene
#total cases	12	489
#cases deceased	4	211
median months survival	NA	56.44

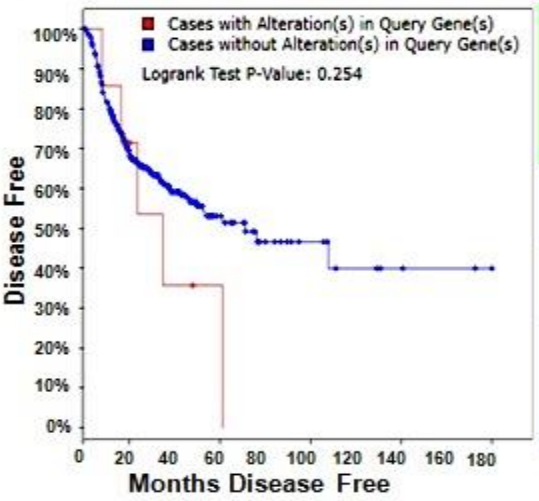
Figure 2.8: The survival curve of the reference population showing the genetic alterations of A; Akt1, B; Akt2 and C; Akt3 isoforms in correlation with overall survival. The worst overall survival was observed in alterations related to Akt2 followed by Akt1. For Akt3 the data could not be obtained.

A



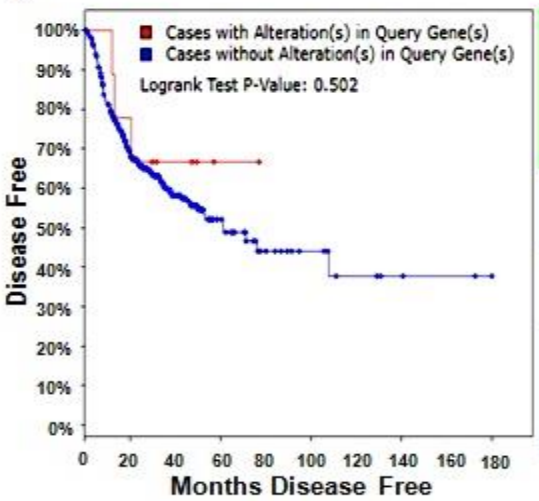
Akt1	Cases with Alterations in Query Gene	Cases without Alterations in Query Gene
#total cases	9	369
#cases relapsed	1	140
median months disease free	NA	56.44

B



Akt2	Cases with Alterations in Query Gene	Cases without Alterations in Query Gene
#total cases	7	371
#cases relapsed	5	136
median months disease free	34.76	71.22

C



Akt3	Cases with Alterations in Query Gene	Cases without Alterations in Query Gene
#total cases	9	369
#cases relapsed	3	138
median months disease free	NA	61.07

Figure 2.9: The survival curve of the reference population showing the mutation alterations of A; Akt1, B; Akt2 and C; Akt3 isoforms in correlation with disease free survival. The disease free survival was 34.76 months. For Akt1 and Akt3 the data could not be obtained.

related to phosphorylated Akt1. It was used for predicting the locoregional recurrence in patients with oesophageal squamous cell carcinoma (ESCC). It was observed that increased expression of p-Akt1 correlates with decreased locoregional-progression free survival (LPFS) in patients with ESCC (Yu et al 2017). The Akt2 gene expression has also been correlated with the overall survival of NSCLC patients. It was found that positive rate of p-Akt2 and Akt2 expression was associated with PFS as well as OS. Consequently, it appears that Akt1 and 2 both plays a significant role in clinical outcomes of cancer patients including oral cancer patients. Therefore, a through mechanistic approach must be undertaken in order to understand and decipher their distinct role in oral cancer development.

2.4. CONCLUSION

The IHC analysis of tissues of oral cancer samples have shown the overexpression of Akt1 and 2 isoforms while no significant change in Akt3 expression was found. Also, the increase in expression was correlated with aggressive tumor types and stages; though, gender-wise difference was absent. Furthermore, the mutational analysis of cancer samples obtained from TCGA portal showed that the maximum percentage of alteration was present in Akt1 followed by Akt3 and Akt2. Most of the mutations of Akt1 and 2 led to increase in the expression of Akt isoforms at mRNA level. However, the exact role of these mutations of Akt isoforms in oral cancer remains elusive. The survival analysis curve has shown that the patients carrying the genetic alteration in Akt1 and 2 isoforms have worst survival as compared to the Akt3 isoform. As a whole, it appears that both Akt1 and 2 isoforms may play significant role in oral cancer development and their precise mechanism of action need to be examined through in-depth analysis.

Chapter 3

Effect of Tobacco Components on the Expression of Akt Isoforms and Establishment of their Roles in Oral Cancer

3.1. INTRODUCTION

In the previous chapter, we have observed that Akt1 and 2 isoforms were overexpressed in oral cancer tissues as compared to the normal tissues. Therefore, we next analyzed what leads to the overexpression of Akt1and 2 in oral cancer. Since tobacco is known to be the major risk factor for oral cancer development and progression, we hypothesized that tobacco may induces the overexpression of Akt 1and 2 isoforms. Tobacco use is the leading preventable cause of premature death worldwide. In South East Asia especially in India and Pakistan, around 90% of global smokeless tobacco usage is prevalent (Khan et al 2012, Nisar and Iqbal 2011). Smokeless tobacco is used in many forms varying from chewing tobacco not mixed with any other ingredient to a mixture of tobacco with other ingredients such as in betel quid, areca nut with tobacco, Naswar, paan-masala with tobacco, Gutkha, Khaini, and Mishri. Smokeless tobacco contains around 28 known carcinogens. These include the non-volatile alkaloid-derived tobacco-specific N-nitrosamine and N-nitrosamino acids, volatile tobacco-specific nitrosamines, volatile aldehydes, and some polynuclear agents have been found in smokeless tobacco (Rodu and Jansson 2004, Madani et al 2012, Khan et al 2014). In lung cancer and head and neck cancer, many studies have shown that the PI3K/Akt pathway gets activated on treatment with the tobacco and its components (West et al 2003, Tsurutani et al 2005). Moreover, studies particularly focused on Akt kinase have also indicated the activation of Akt through phosphorylation. The activation of Akt in response to tobacco components can increase in the cell survival through direct phosphorylation and inactivation of the pro-apoptotic proteins like Bad and Bax. Also, Akt increases the cell survival by indirectly inducing the anti-apoptotic protein survivin and the transcription factor NF-κB. Tobacco

component-induced activation of Akt can also increase the cell growth and proliferation through the activation of the mTOR pathway (Memmott and Dennis 2010, Macha et al 2011, Islam et al 2014, Nooshinfar et al 2017). These studies have shown the direct associations of Akt with the promotion and progression of different cancers. However, no reports on the effect of tobacco and its components on the oral cancer cells in relation to Aktisoform-specific associations are available. In the present study aqueous tobacco extract (TE), BAP, and nicotine were chosen to analyze the expression of Akt isoforms in oral cancer cells. Furthermore, in the earlier chapter, we observed that the genetic alterations present in Akt1 and 2 isoforms were responsible for poor PFS and DFS. Therefore, we hypothesized that overexpression of Akt1 and 2 isoforms might play a key role in the development and progression of oral cancer. Similar to our result, several lines of evidence have suggested that the overexpression of Akt1 and 2 isoforms in different cancers (Roy et al 2017). However, the specific role of Akt isoforms in the pathogenesis of the oral cancer is not well understood. Therefore, it becomes essential to understand the specific roles of Akt isoforms and the associated molecular mechanisms that determine the functional specificity in the process of oral carcinogenesis. In the present chapter, an attempt has been made to elucidate the functional role of Akt isoforms in oral cancer.

3.2. MATERIALS AND METHODS

3.2.1. Cell culture

Human oral cancer cell line SAS was obtained from Rajiv Gandhi Centre for Biotechnology (RGCB), Trivandrum, India. The cells were maintained and seeded in six-

well plates and cultured in Eagle's DMEM high glucose media supplemented with 10% v/v FBS at 37°C in a humidified 5% CO_2 atmosphere.

3.2.2. Preparation of TE

The dried leaves of tobacco were purchased from the local market and it was powdered using mixer grinder. For the preparation of TE, 4g of dry leaf powder was dissolved in 100ml of distilled water in a conical flask. It was stirred on an orbital shaker for 24h and then filtered, followed by lyophilization (Fig 3.1). The stock concentration of 50mg/ml was prepared and stored at 4^{o}C for further experiments.

3.2.3. Cell proliferation assay (MTT assay)

To measure the proliferative effect of tobacco and its components, cell proliferation study was performed with the conventional MTT reduction assay on the SAS cancer cells. In short, the cells in the logarithmic phase of growth were seeded in 96-well plates at a density of 4,000 cells/100µl per well. The cells were allowed to grow for 24h for the acclimatization and treated with various concentrations of TE, BAP, and nicotine. For tobacco and BAP, the concentrations used between the ranges of 25-500ng/ml, and for nicotine the concentration chosen were between 0.05µM-1µM (a minimum of 6 wells were plated for each concentration). For the period of 0h and 24h 10µl of, MTT solution (5mg/ml) was added to each well and incubated for 2h. It was followed by the removal of the culture medium and the blue-violet formazan crystals were dissolved by adding 100µl of DMSO. Finally, the absorbance for each well was measured spectrophotometrically using a microplate reader (TECAN Infinite 200 PRO multimode reader) at 570 nm. The percentage of proliferation was calculated by normalizing with 0h absorbance and taking the untreated control as 100% and subsequently the IC_{50} was determined.

3.2.4. Reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was extracted using Trizol reagent (Sigma) and cDNA synthesis was done by taking 2µl of 10X RT buffer (Invitrogen), 1µg of RNA, 2µl of 10X oligo dT primer, 0.8µl of 25X dNTPs, and 1µl of SuperScript III Reverse Transcriptase enzyme and final volume was made to 20µl. The cDNA was synthesized according to manufac 61 protocol. Nucleic acids were mixed with loading buffer and run on a 1 % agarose/ethidium bromide/1XTAE gel. 0.7µg Ready load 100bp DNA ladder (Invitrogen) was run as a standard. The gel band was quantified by Image lab software.

Gene	Primer Pairs	No. of Bases	Annealing Temp (ºC)	Amplification length
Akt1	5'- CACCATGAGCGACGTGGCTAT -3'	21	61	1200 bp
	5'- CTGCATGATCTCCTTGGCGT -3'	20		
Akt2	5'- TTG CCAAGGATGAAG TCGCT -3'	20	61	934 bp
	5'- AACCACCCAGCGGTGATGG -3'	20		
Akt3	5'- GTGAAAGAAGGTTGGGTTCA -3'	22	53	1280 bp
	5'- CTAGTATCTGTCTCAGATGTT -3'	25		
Alpha-tubulin	5′- TAT CGA GCG CCC AAC CTA CAC T -3′	20	53	683 bp
	5′- CCT CAC CCT CTC CTT CAA CAG AAT C -3′	21		

Table 3.1: Primer sequences used for RT-PCR analysis.

3.2.5. Transfection of SAS cells

The silencing of Akt1 and 2 isoforms was accomplished by transfection with specific siRNAs. The sequence of siRNAs was obtained from the research of Hara et al 2008. Before initiating the transfection, the cells were allowed to reach 50%-60% confluency. For siRNA inhibition studies, siRNAs were transfected into the cells using the Lipofectamine RNAiMAX reagent (Invitrogen) as per the manufacturer's instructions

with 10nM of siRNA and 7µl of transfection reagent per well of the six-well plate. After transfection, the cells were harvested after 24 hours for the protein extraction and additional analysis.

3.2.6. Cell-cycle analysis

The different phase of the cell cycle was analyzed by DNA flow cytometry analysis. SAS cells were cultured in 6-well plates overnight and then transfected with siRNA for Akt1 and 2 isoforms. Scramble siRNA was used as a negative control. Initially, the cells were trypsinized and fixed in cold 70% ethanol for 1h and then stained with propidium iodide (PI) solution (10µg/µl PI and 0.125% RNaseA; Sigma Aldrich, St. Louis, MO) at room temperature for 15 minutes. An average of 10,000/sample cells was examined utilizing Cytomic FC500 flow cytometer (BD Biosciences FACSCalibur) with excitation at 488 nm and emission at 617 nm. The percentages of cells in each cell-cycle phase were analyzed using FCS express software.

3.2.7. Clonogenic assay

For the clonogenic assay, 2000 or 1000 cells of untreated and tobacco and its components-treated were seeded in 3ml fresh media in six-well plate and incubated for 10 days with media replacement in 3 days consecutively. After 10 days, formed colonies were fixed with chilled 6.0% glutaraldehyde, stained with crystal violet (4% w/v) for 2 min and then counted. The plating efficiency and survival fraction were further determined (Franken et al 2006).

Plating efficiency (%) = ((Number of colonies counted/Number of cells plated)) ×100

Survival fraction = (Plating efficiency of treated cells/Plating efficiency of control cells)

3.2.8. Migration assay

To perform migration assay, 7 X 10^5 cells were plated into 6-well cell culture plate. Cells were allowed to grow in 10% FBS containing DMEM to confluence, later it was washed with serum-free medium and serum starved for 8h. A wide scratch was made across the cell layer using a 10µl sterile pipette tip. Plates were photographed immediately after scratching. Cells were treated with tobacco and its components. Plates were photographed after 12h and 24h at the identical location of the initial image (Rodriguez et al 2005, Liang et al 2007).

3.2.9. Flow cytometric assessment of cell viability

The effect of knockdown of Akt1 and 2 on the cell viability was evaluated by propidium iodide (PI) exclusion assay through FACS. Transfected cells were collected together with dead floating cells in the medium, washed with 1X PBS twice before re-suspension in PI to achieve a final concentration of 10µg/ml PI and the % of dead cells up-taking PI was measured by flow cytometry (BD Biosciences FACSCalibur) with excitation at 488nm and emission at 617nm.

3.2.10. Protein extraction and western blot analysis

Protein extraction and western blot analysis were performed to examine the expression of Akt isoforms and other proteins. Briefly, the SAS cells were used for total cell lysate preparation. Homogenization of the sample was accomplished in 100µl of Lysis buffer containing fresh protease and phosphatase inhibitors (20mM HEPES, 2mM EDTA, 250mM NaCl, 0.1% Triton-X, 2µg/ml aprotinin, 1mM PMSF, 0.5µg/ml of DTT, 2µg/ml leupeptin). The concentration of the protein in lysates was determined by Bradford assay, with BSA as standard. Aliquots of samples were stored at -80°C until use. 40 µg of proteins were resolved by SDS-PAGE using the mini-PROTEIN 3-electrophoresis

module assembly (Bio-Rad, Hercules, CA). It was further transferred to a nitrocellulose blot membrane (Amersham Biosciences) through a transfer apparatus as per the manufacturer's protocols (Bio-Rad). The membrane was then incubated with 5% nonfat milk in TBST buffer (10mM Tris, pH 8.0, 150mM NaCl, 0.5% Tween 20) for 2h, the membrane was washed thrice with TBST buffer and incubated with antibodies against Akt1 (2938, 1:1000, Cell signaling tech), Akt2 (2964, 1:1000, Cell signaling tech), Akt3 (3788, 1:1000, Cell signaling tech 1:1000) and GAPDH (2118S, 1:1000, Cell signaling tech) at 4°C for overnight. Membranes were washed three times for 10 min each and incubated with a 1:6000 dilution of horseradish peroxidase-conjugated anti-rabbit (ab97080, Abcam) and anti-mouse secondary antibody (ab97040, Abcam) for 3 hours. Blots were washed with TBST thrice and developed with Optiblot ECL Detection Kit (ab133406, Abcam). Densitometry analysis on the developed blots was performed by the NIH Imager software (NIH, Bethesda, MD).

3.2.11. Statistical analysis

One-Way ANOVA was used to test the statistical significance of the differences between the various group parameters. p values of less than 0.05 were considered statistically significant.

3.3. RESULT AND DISCUSSION

In the present chapter, we have examined the effect of TE and other tobacco components such as BAP and nicotine on SAS oral cancer cells in relation to Akt isoforms. Initially, we determine the effect TE, BAP and nicotine on the proliferation of SAS cells. Then, we analyzed their effect on the expression of Akt isoforms by semiquantitative PCR. Moreover, we also determined their effect on clonogenicity and migration of SAS cells.

To establish the role of Akt1 and 2 in oral cancer we silenced these genes using siRNA technology and further examined their outcome.

3.3.1. Effect of TE, BAP, and nicotine on the proliferation of SAS cells

Initially, the effect of TE, BAP, and nicotine was assayed to check their effect on the proliferation of SAS cells. After 24h of incubation with TE and nicotine, it was found that it induces the proliferation of SAS cells. Though, for BAP the increase in the percentage of proliferation was not significant (Fig 3.1 and 3.2). In line with our result, the earlier reports have also suggested that exposure of tobacco on cancer cells to increase the proliferation of cells (Tsurutani et al 2005, Sobus and Warren, 2014). Moreover, many reports have suggested that BAP exposure can also lead to increase in the proliferation of normal and several cancer cells such as ovarian, breast, lung, gastric etc (Stopper et al 2003, Burdick et al 2003, Kometani et al 2009, Wei et al 2016). Nicotine also has been associated with various cancers such as lung cancer, head and neck, gastric, pancreatic, gallbladder, liver, colon, breast, cervical, urinary bladder and kidney cancers (Grando 2014). Nicotine and its derivatives can initiate tumorigenesis via nicotinic acetylcholine receptors (nAChRs) in target cells (Arrendondo et al 2006). It is also known to increase the proliferation of immortalized oral keratinocytes and other cancer cells (Lee et al 2005, Schaal and Chellappan 2014). Furthermore, nicotine was also shown to promote tongue squamous cell carcinoma progression (Wang et al 2017).

3.3.2. Effect of TE, BAP, and nicotine on the expression of Akt isoforms in SAS cells

The effect of tobacco and its components were checked on the expression of Akt isoforms (Fig 3.1 and 3.2). It was found that after 24h treatment of TE, BAP, and nicotine increased the transcript level of Akt1 and 2 whereas no significant change in Akt3

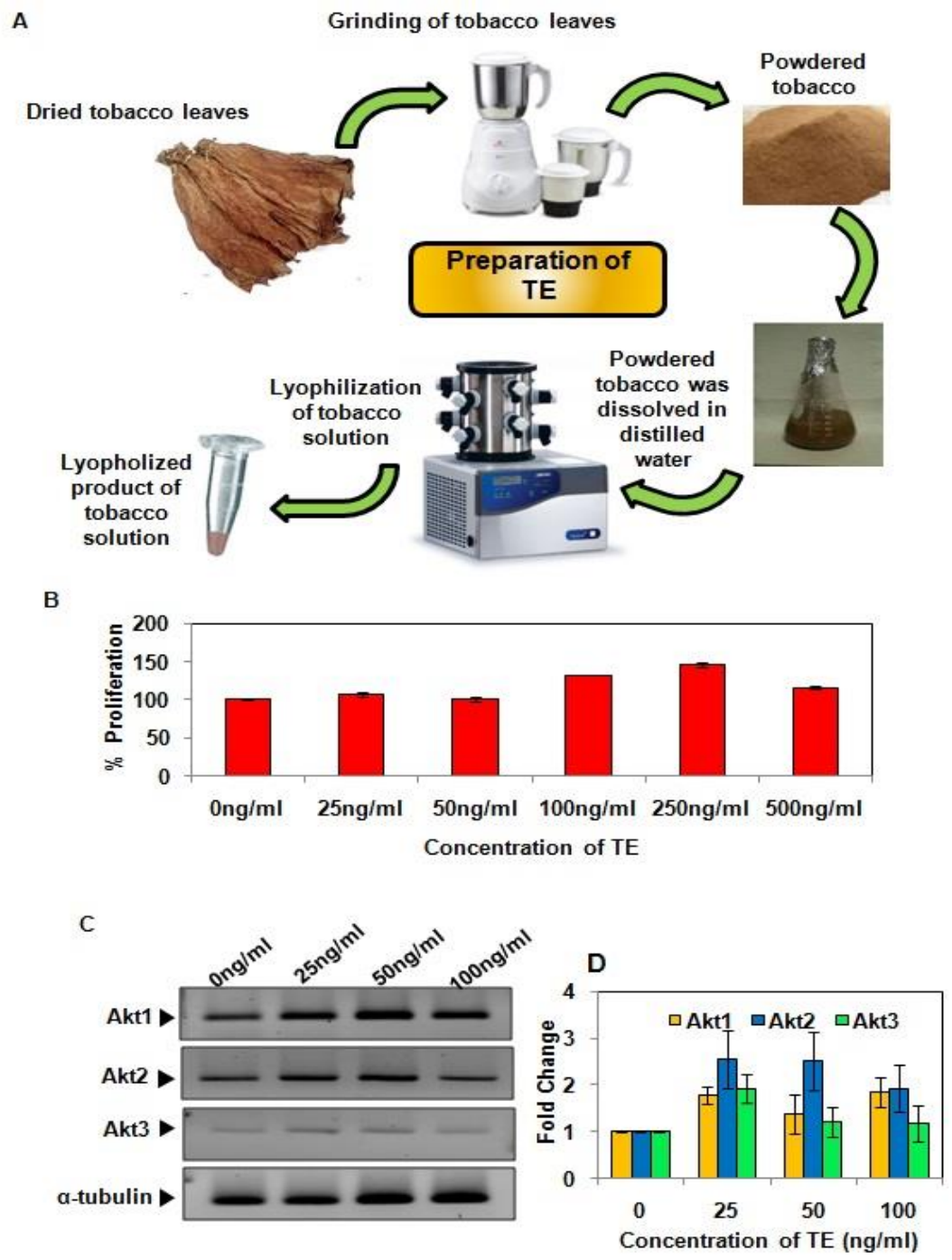


Figure 3.1: Effect of TE on the expression of Akt isoforms in oral cancer. A; Schematic representation of method used for preparation of TE, B; Effect on the proliferation as analyzed by MTT assay. (% Proliferation values shown are the average of two independent experiments), C; RT-PCR analysis of the expression of Akt isoforms on treatment of TE, D; Bar graph of mRNA expression of Akt isoforms in TE treated SAS cells over untreated control. Fold change in the expression was analyzed using Image Lab software. Data are means ± SE*= $p<0.05$.

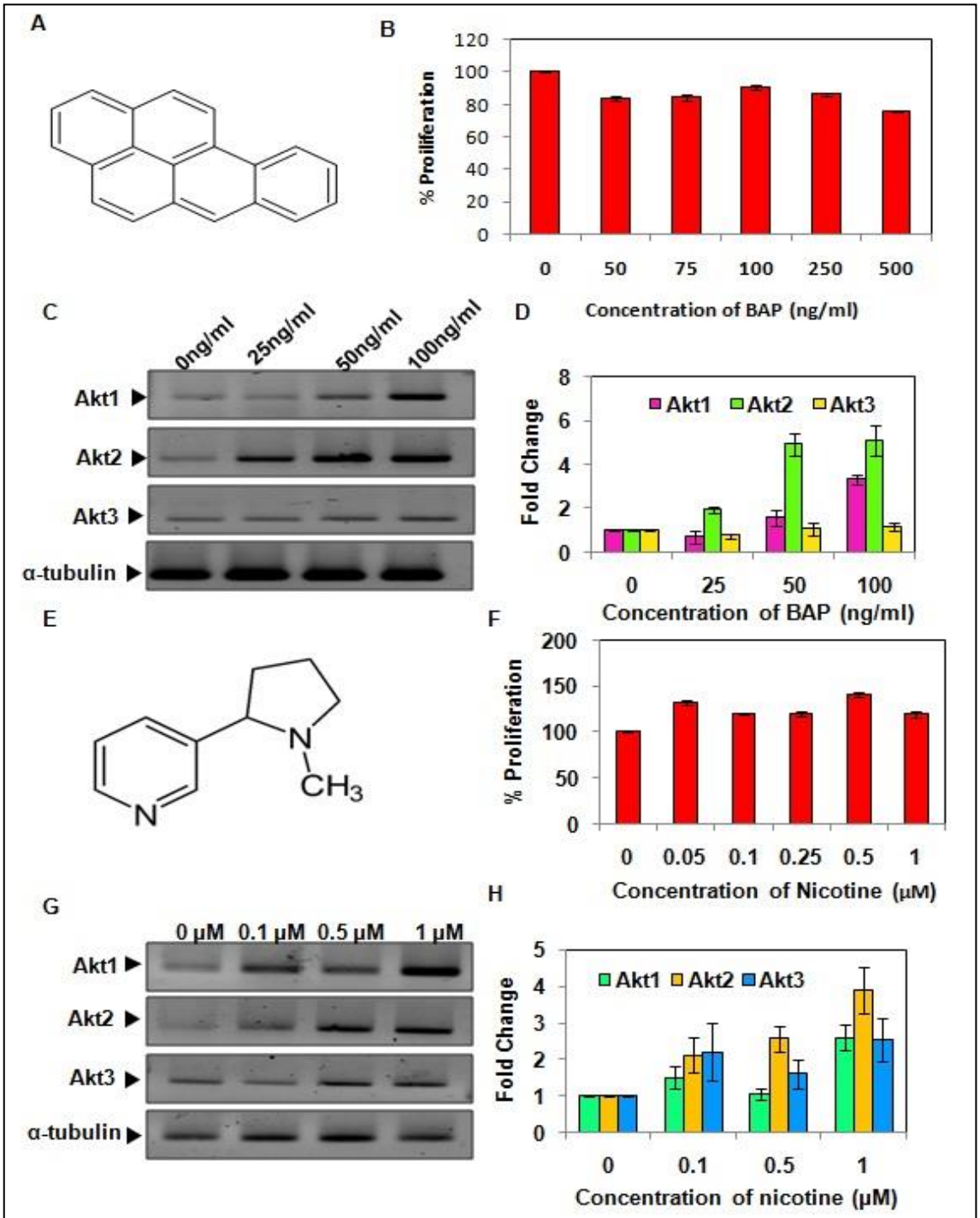


Figure 3.2: Effect of BAP and nicotine on the expression of Akt isoforms in oral cancer. A and E; Structure of BAP and nicotine, B and F; Effect of BAP and nicotine on the proliferation as analyzed MTT assay. (% Proliferation values shown are the average of two independent experiments), C and G; RT-PCR analysis of the expression of Akt isoforms on treatment of BAP and nicotine, D and H; Bar graph of mRNA expression of Akt isoforms in BAP and nicotine-treated SAS cells over untreated control. Fold change in the expression was analyzed using Image Lab software. Data are means ± SE *= $p<0.05$.

isoform was found. Increasing lines of evidence suggested the Akt isoform-specific involvement in tobacco-induced cancer. In one such study, it was shown that Akt1 plays an important role in lung cancer development and its deletion can prevent lung tumorigenesis by mutant K-ras. It was also observed that the deletion of Akt1 or Akt2 reduced the NNK-induced lung tumor formation by 90% but deletion of Akt2 alone failed to decrease the lung tumorigenesis in two other mouse models driven by mutant K-ras (Hollander et al 2011a, Hollander et al 2011b). In case of urothelial cell carcinoma, it was found that both Akt1 and 2 was upregulated along with HRAS and RAC1 in tobacco-treated samples (Brait et al 2013).

3.3.3. Silencing of Akt1 and 2 in SAS cells

To understand the role of Akt1 and 2 in oral cancer, we have silenced these isoforms in SAS cells using siRNA technology. Synthetic siAkt1 and siAkt2 was transfected into SAS cells at a concentration of 10nM, and the effect was evaluated by Western blot analysis. It was observed that the specific siRNAs reduced the expression of the respective gene by 80–90% and the effects were found to be isoform-specific (Fig 3.3).

3.3.4. Effect of silencing Akt1 and 2 isoforms in cell cycle arrest of SAS cells

The effect of silencing of Akt1 and 2 isoforms was analyzed on the cell cycle arrest of SAS cells. It was found that the ablation of Akt1 and 2 induces the cell cycle arrest in G2/M phase (Fig 3.3). Similar to our result, an earlier report has also indicated the involvement of Akt in the G2/M cell cycle arrest. It was shown that activation of Akt can overcome a G2/M cell cycle checkpoint (Kandel et al 2002). Moreover, in another study of NSCLC, the treatment of Akt2 siRNA was found to arrest cell at G2/M phase (Lee et

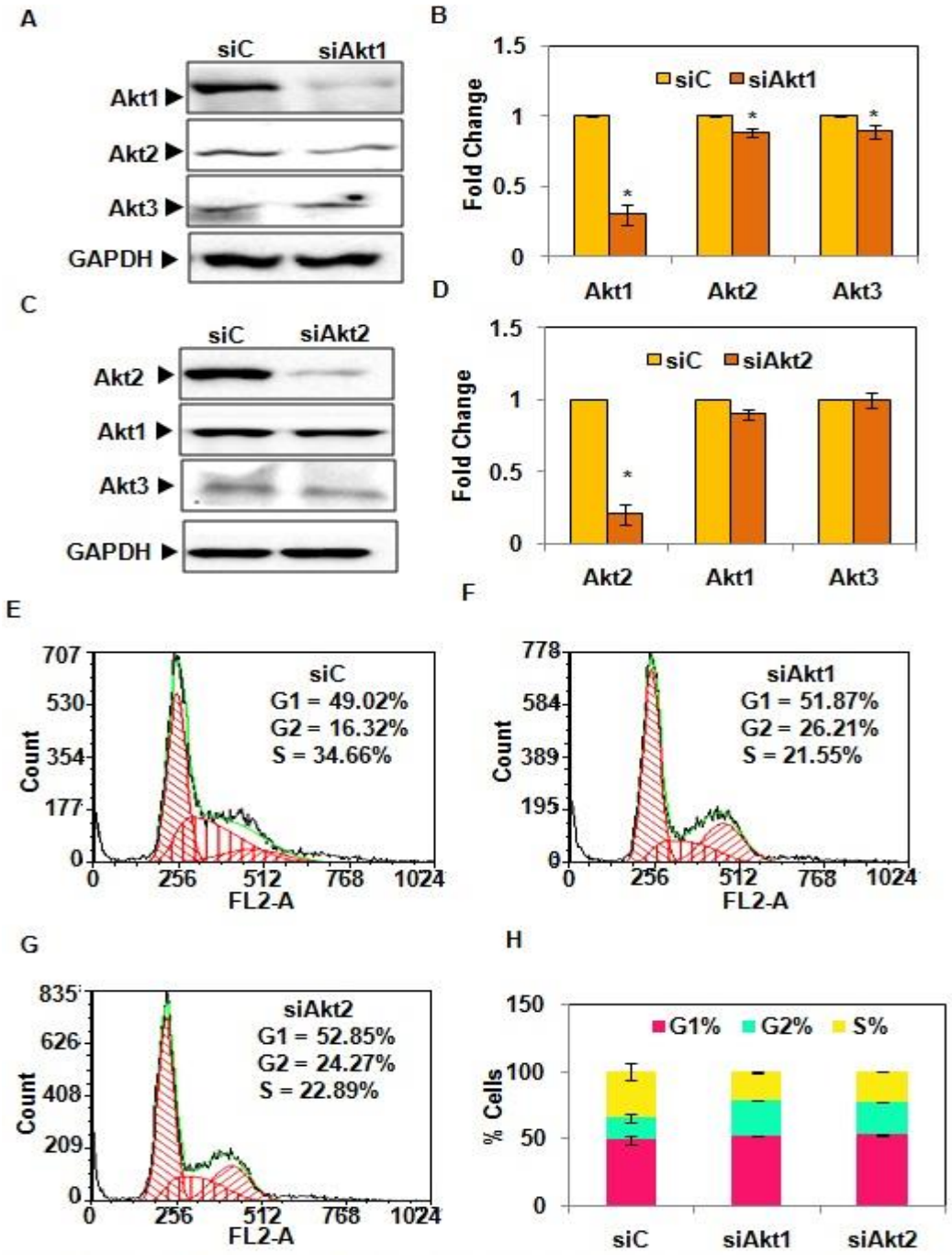


Figure 3.3: Knockdown of Akt1 and Akt2 in SAS cells through siRNAs and the effect on cell cycle arrest, A and C; Specific gene silencing of Akt1 and Akt2 by siRNA (siAkt1 and siAkt2) as analyzed by western blot assay, B and D; Fold change in expression of proteins on Akt1 and Akt2 knockdown, Representative image of the cell cycle distribution as determined by DNA flowcytometric analysis in E; siC-treated cells, F; siAkt1-treated cells, G; siAkt2-treated cells, H; Bar graph showing the % of cells in G1, G2 and S phase. Fold change in the expression was analyzed using Image Lab software. Data are means ± SE *= $p<0.05$.

al 2011). It is believed that the arrest in the G2/M phase leads to apoptosis of cancer cells (DiPaola 2002).

3.3.5. Effect of tobacco components and silencing of Akt1 and 2 isoforms on the clonogenicity of SAS cells

The effect of tobacco and its components were examined on the clonogenic potential of cancer cells. Subsequently, the effect of silencing of Akt1 and 2 isoforms was analyzed in the same experimental conditions. The tobacco-treated samples were found to have increased potential for colony formation up to certain concentration. The treatment of TE up to 250ng/ml increased the colony forming efficiency up to 1.6 fold. In case of BAP treatment the survival capacity was increased up to 250ng/ml it was increased the survival fraction was found to be 1.7 times. A similar report was also presented by Barkley and Santocanale where they proposed that it is benzo(a)pyrene dihydrodiol epoxide (BPDE) regulates cell survival through MicroRNA-29a (Barkley and Santocanale 2013). However, contrasting results were also observed in breast cancer where survival efficiency was reported to be reduced on the treatment with BAP (Davis et al 2002, Hewitt et al 2007). The treatment of nicotine was found to increase the clonogenic potential up to 0.5μM concentration with a survival fraction reaching to a maximum of 1.3 (Fig 3.4-3.6). In line with our observation, few reports have shown that both nicotine and BAP can increase the clonogenic potential of cancer cells. In HNSCC also nicotine was shown to enhance the ability of 10B and HN-1 cells to form colonies under low serum conditions, suggesting increased survival capacity (Yu et al 2012). In addition, Puliyappadamba et al have shown that the nicotine-induced increase in the number and size of the clones in H1299 and A549cells (Puliyappadamba et al 2010). The

effect of knockdown of Akt1 and 2 isoforms by siRNAs on colony forming potential was also analyzed. It was observed that silencing of Akt1 and 2 lead to the reduction in the potential of colony formation of SAS cells. The level of reduction of colony forming efficiency was similar in both Akt1 and 2 knockdown cells. Moreover, it was found that the silencing caused the reduction in the TE, BAP, and nicotine-induced clonogenic potential (Fig 3.4- 3.6). Several lines of evidence have suggested the association of Akt pathway in assisting the colony forming potential of different cancer such as glioblastoma, breast cancer, pancreatic cancer, esophageal cancer etc (Qi et al 2013, Cairns et al 2018, Taga et al 2009, Li et al 2014). The involvement of specific Akt isoforms has also been implicated in different cancers through regulation of various molecular mediators. The silencing of each Akt isoforms caused a decrease in the colony forming efficiency of glioma cells. However, the effect was more pronounced in Akt2 and 3-knockdown cells than Akt1 isoform (Joy et al 2016, Mure et al 2009). Although, in another study of glioblastoma cell lines, only Akt3 isoform was found to be important for colony formation while no such significant change was observed for Akt1 and 2 isoforms (Endersby et al 2011). In case of lung cancer, the treatment of Akt1 siRNA markedly decreased the colony but Akt2 siRNA had no such effect on the cancer cells (Lee et al 2011). The neuroendocrine tumor cells have shown the reduction in the colony forming ability of Akt1 and Akt3-knockdown cells while Akt2-knockdown was showing the opposite effect (Zitzmann et al 2012). Furthermore, in case of chronic myeloid leukemia, pharmacological inhibition of either Akt1 or Akt2 separately or Akt1 and 2 in combination was ineffective in reducing the colony formation of BCR-ABL transformed cells. However, the inhibitions of all the isoforms by an inhibitor successfully caused the

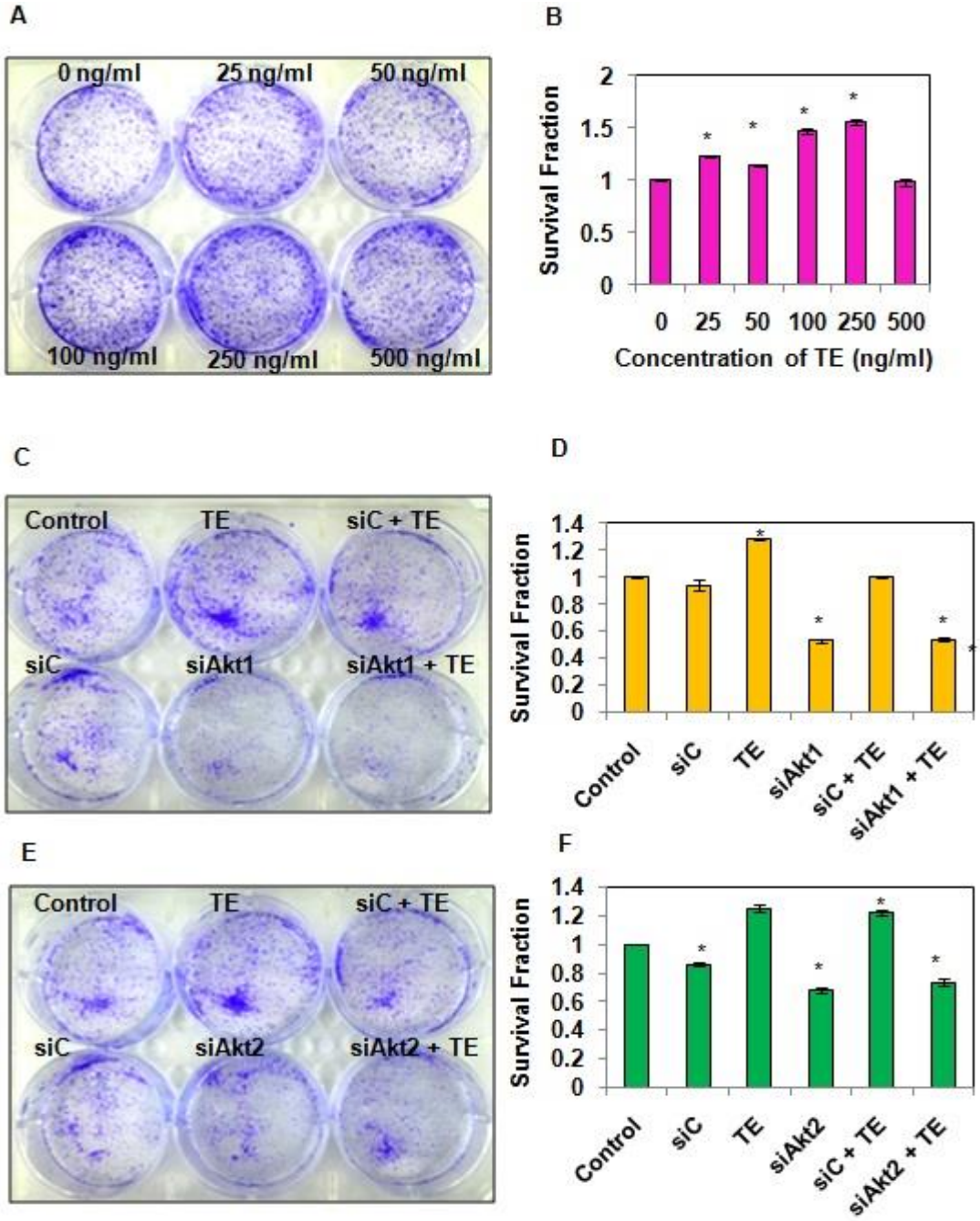


Figure 3.4: Effect of TE and silencing of Akt1 and 2 on the survival of SAS cells. A, C, and E; Representative image showing the clonogenic potential of SAS cells on treatment with TE and silencing of Akt1 and Akt2 isoforms, B, D, and F Bar graph of clonogenic assay revealing the survival efficiencies of TE-treated SAS cells and silencing of Akt1 and Akt2 isoforms (siC; Scramble control, siAkt1; siRNA for Akt1, siAkt2; siRNA for Akt2, TE; 50ng/ml TE). Data are means ± SE *= $p<0.05$.

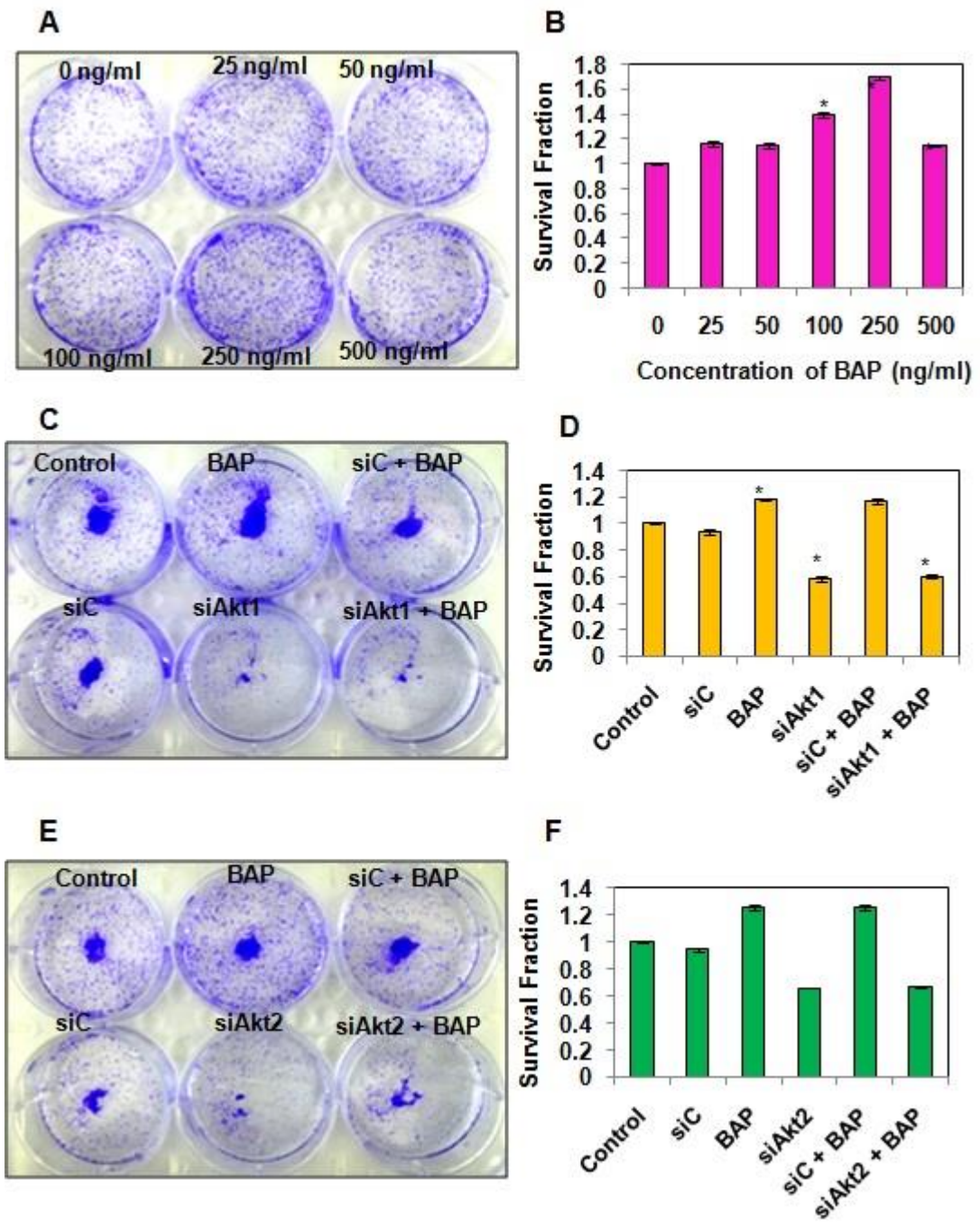


Figure 3.5: Effect of BAP and silencing of Akt1 and 2 on the survival of SAS cells. A, C, and E; Representative image showing the clonogenic potential of SAS cells on treatment with BAP and silencing of Akt1 and Akt2 isoforms, B, D; and F; Bar graph of clonogenic assay revealing the survival efficiencies of BAP-treated SAS cells and silencing of Akt1 and Akt2 isoforms (siC; Scramble control, siAkt1; siRNA for Akt1, siAkt2; siRNA for Akt2, BAP; 50ng/ml of BAP). Data are means ± SE *= $p<0.05$.

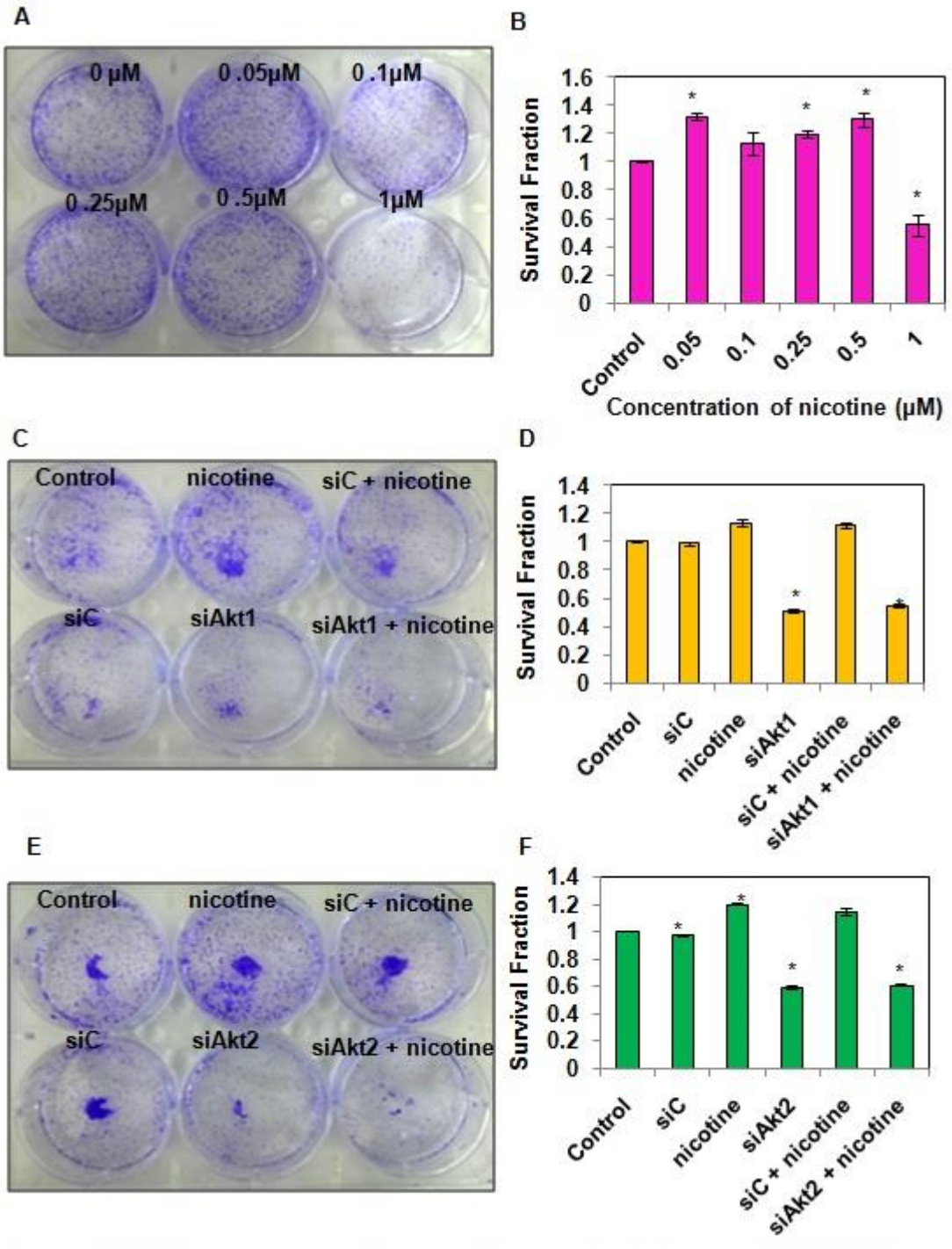


Figure 3.6: Effect of nicotine and silencing of Akt1 and 2 on the survival of SAS cells. A, C, and E; Representative image showing the higher clonogenic potential of nicotine-treated SAS cells which was further reduced by silencing of Akt1 and Akt2 gene, B, D, and F; Bar graph showing the survival fraction of SAS cells (siC; Scramble control, siAkt1; siRNA for Akt1, siAkt2; siRNA for Akt2, nicotine; 0.5µM of nicotine). Data are means ± SE *= $p<0.05$.

elimination of colony forming potential of cancer cells (Watkins 2012). From these studies, it becomes evident that these isoforms play a discrete role in colony formation in different cancers.

3.3.6. Effect of tobacco components and silencing of Akt1 and 2 isoforms on the migration of SAS cells

For analyzing the migration of cancer cells on induction with TE, BAP, and nicotine and silencing of Akt1 and 2 isoforms, the wound healing assay was performed. On increasing the concentration of TE it was found that percentage of wound area significantly decreased and it healed in most of the treated concentration in 24h interval time. For BAP and nicotine also with an increase in the concentration of the compound the percentage of wound area reduced, though the healing capacity was less as compared to TE as most of the treated wounds were not healed in 24h (Fig 3.7-3.9). There are several reports available which have suggested that tobacco and its components can increase the migration potential of different cancer such as breast, colon and lung cancers (Hung et al 2011, Wei et al 2011, Di Cello et al 2013, Warren and Singh 2013, Sobus and Warren 2014). Recently, a report from Zhang and group have suggested that BAP promotes lung cancer A549 cell migration and invasion through up-regulating the cytokine IL8 and chemokines CCL2 and CCL3 expression (Zhang et al 2016). The silencing of Akt2 gene led to the reduction in the migration potential of the cancer cells as compared to the untreated cells. However, silencing of Akt1 failed to decrease the migration potential of SAS cells (Fig 3.10-3.12). In consistent with the observation made, a similar result was shown in breast cancer where cell migration was reduced on knockdown of Akt2 by shRNA (Riggio et al 2017). In mesencymal stem cells also, it was observed that

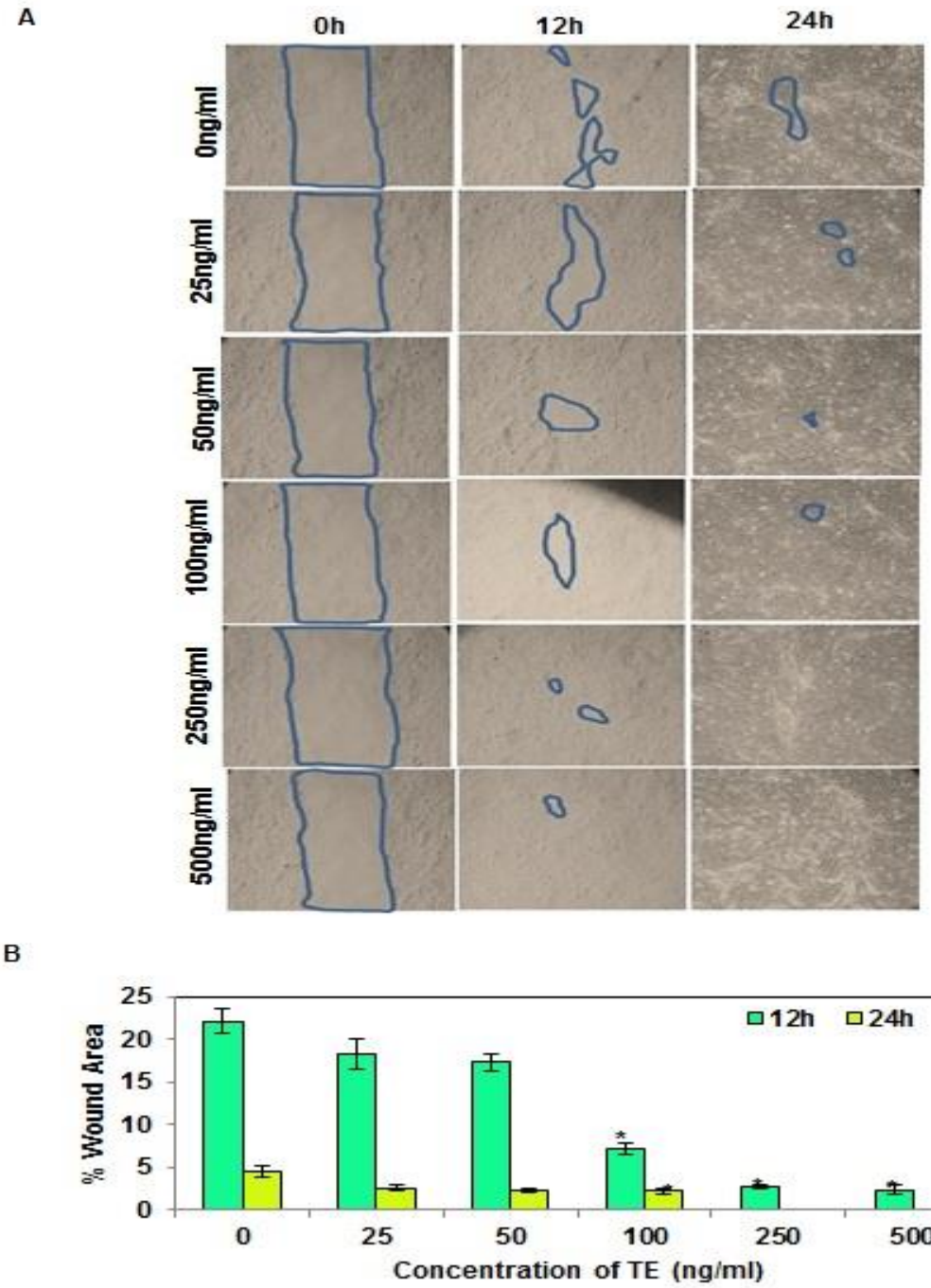


Figure 3.7: Effect of TE on the migration of SAS cells. A; Representative image of the cell migration in control and TE-treated SAS cells after 12 hours (12 hr) and 24 hours (24 hr), B; The % of cell-covered area (wound area) are shown in the bar diagram as mean ±SE from two independent experiments Percentage of wound area was estimated using Image J software.

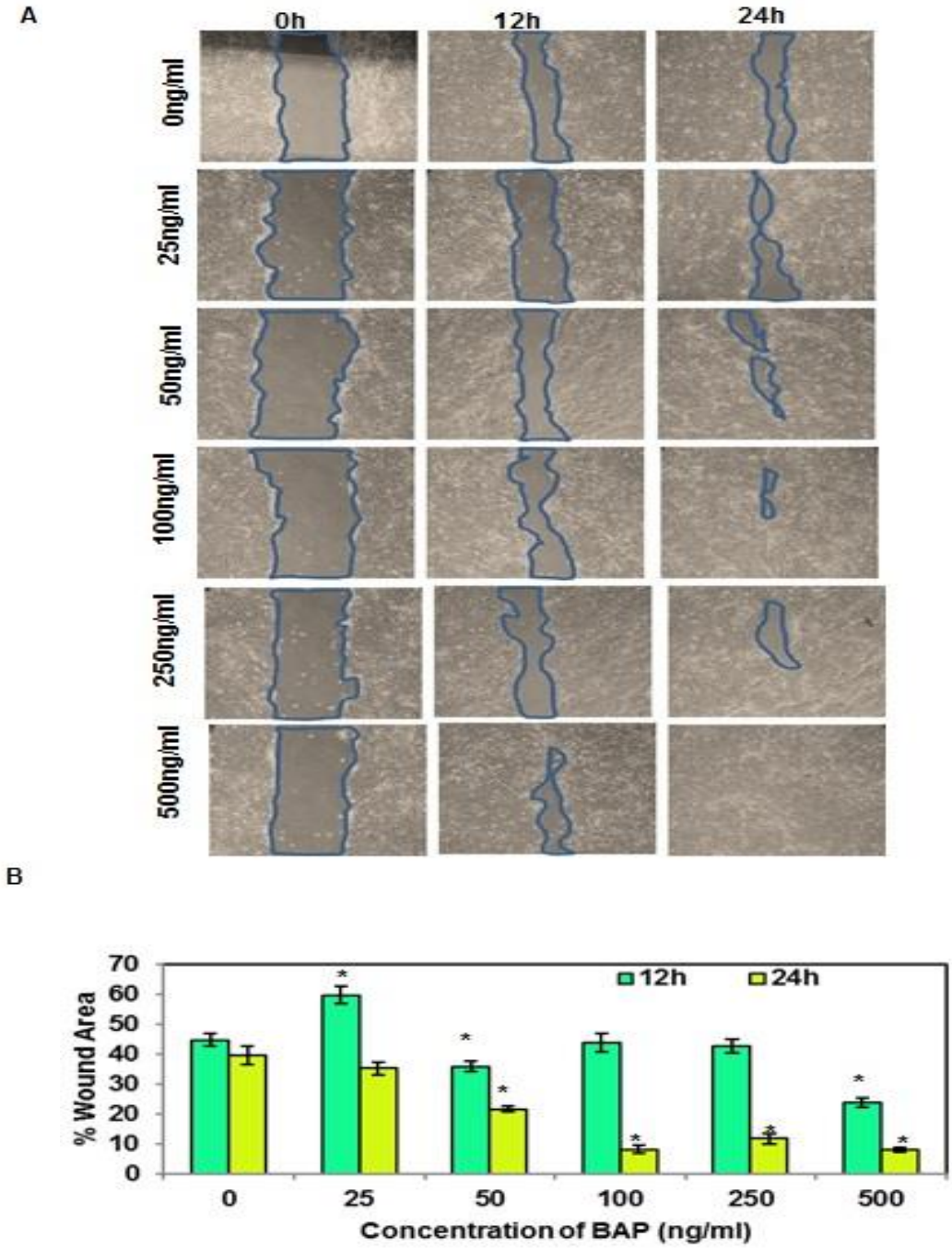


Figure 3.8: Effect of BAP on the migration of SAS cells. A; Representative image of the cell migration in control and BAP-treated SAS cells after 12 hours (12 hr) and 24 hours (24 hr), B; The % of cell-covered area (wound area) are shown in the bar diagram as mean ±SE from two independent experiments Percentage of wound area was estimated using Image J software. Data are means ± SE *= $p<0.05$.

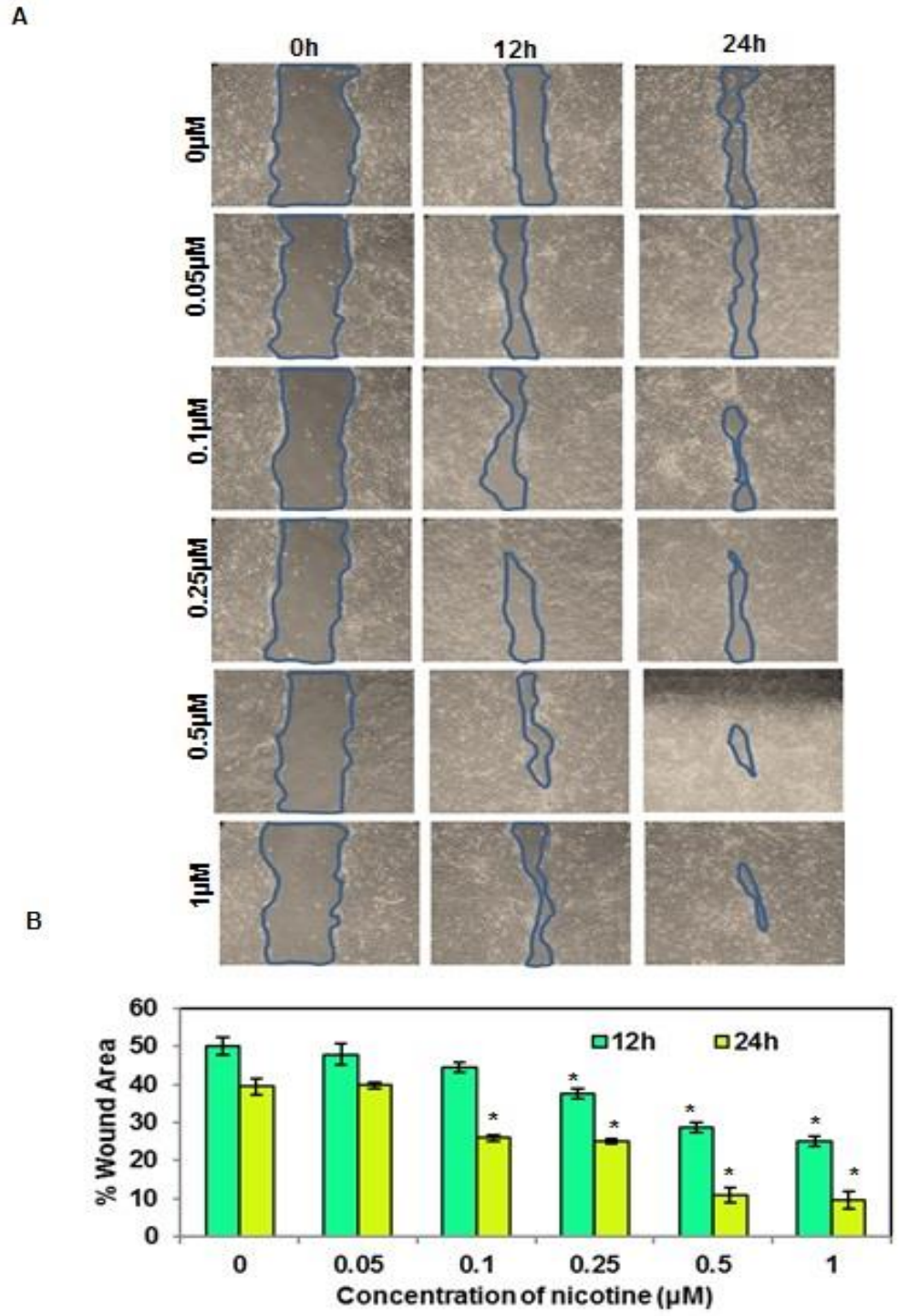


Figure 3.9: Effect of nicotine on the migration of SAS cells. A; Representative image of the cell migration in control and nicotine-treated SAS cells after 12 hours (12 hr) and 24 hours (24 hr), B; The % of cell-covered area (wound area) are shown in the bar diagram as mean ±SE from two independent experiments Percentage of wound area was estimated using Image J software. Data are means ± SE *= $p<0.05$.

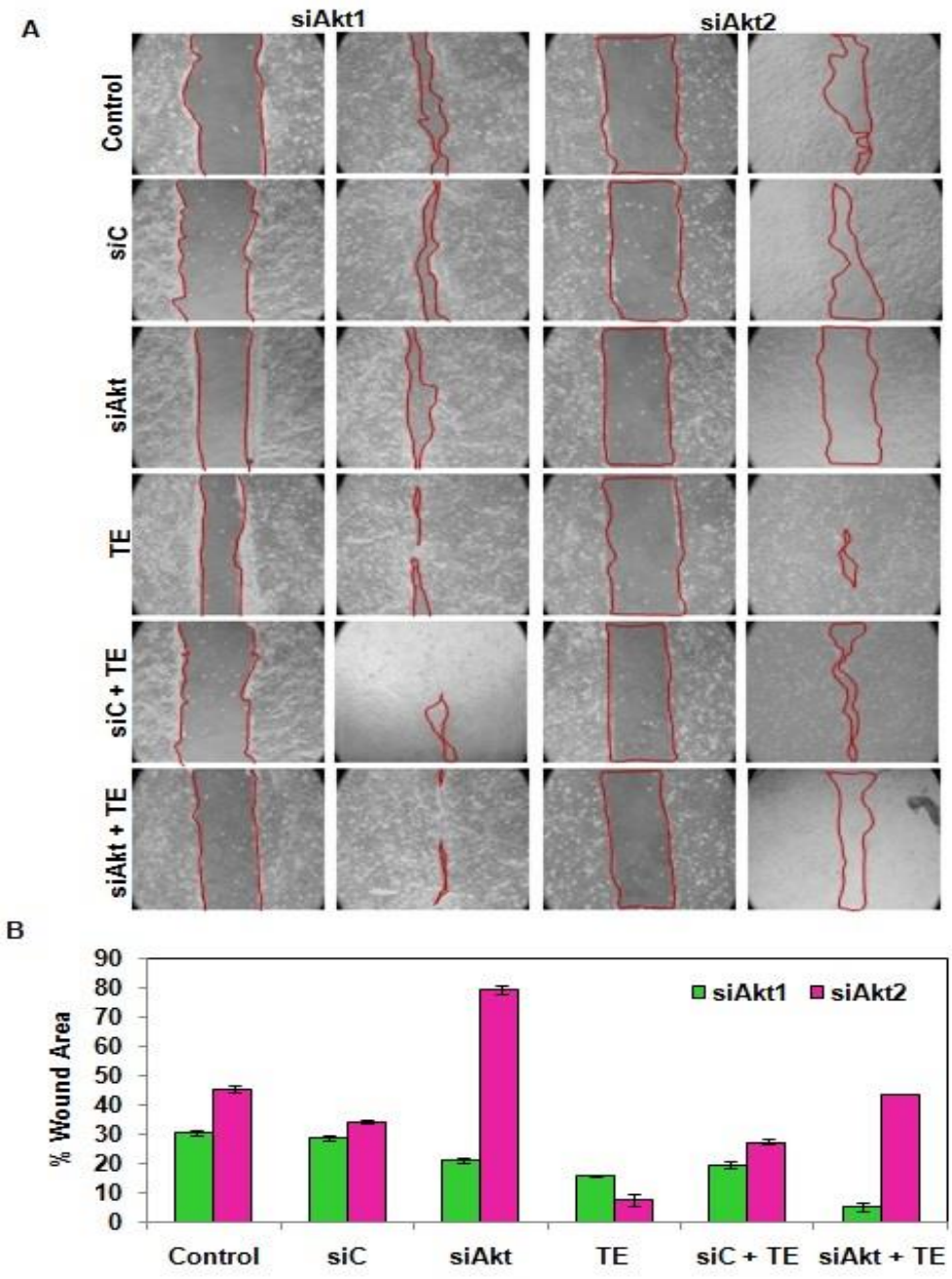


Figure 3.10: Effect of TE and silencing of Akt1 and 2 on the migration of SAS cells. A; Representative image of the cell migration in control, TE-treated and transfected SAS cells after 12 hours (12 hr), B; The % of cell-covered area (wound area) are shown in the bar diagram as mean ±SE from two independent experiments Percentage of wound area was estimated using Image J software (siC; Scramble control, siAkt1; siRNA for Akt1, siAkt2; siRNA for Akt2, TE; 50ng/ml of TE). Data are means ± SE *= $p<0.05$.

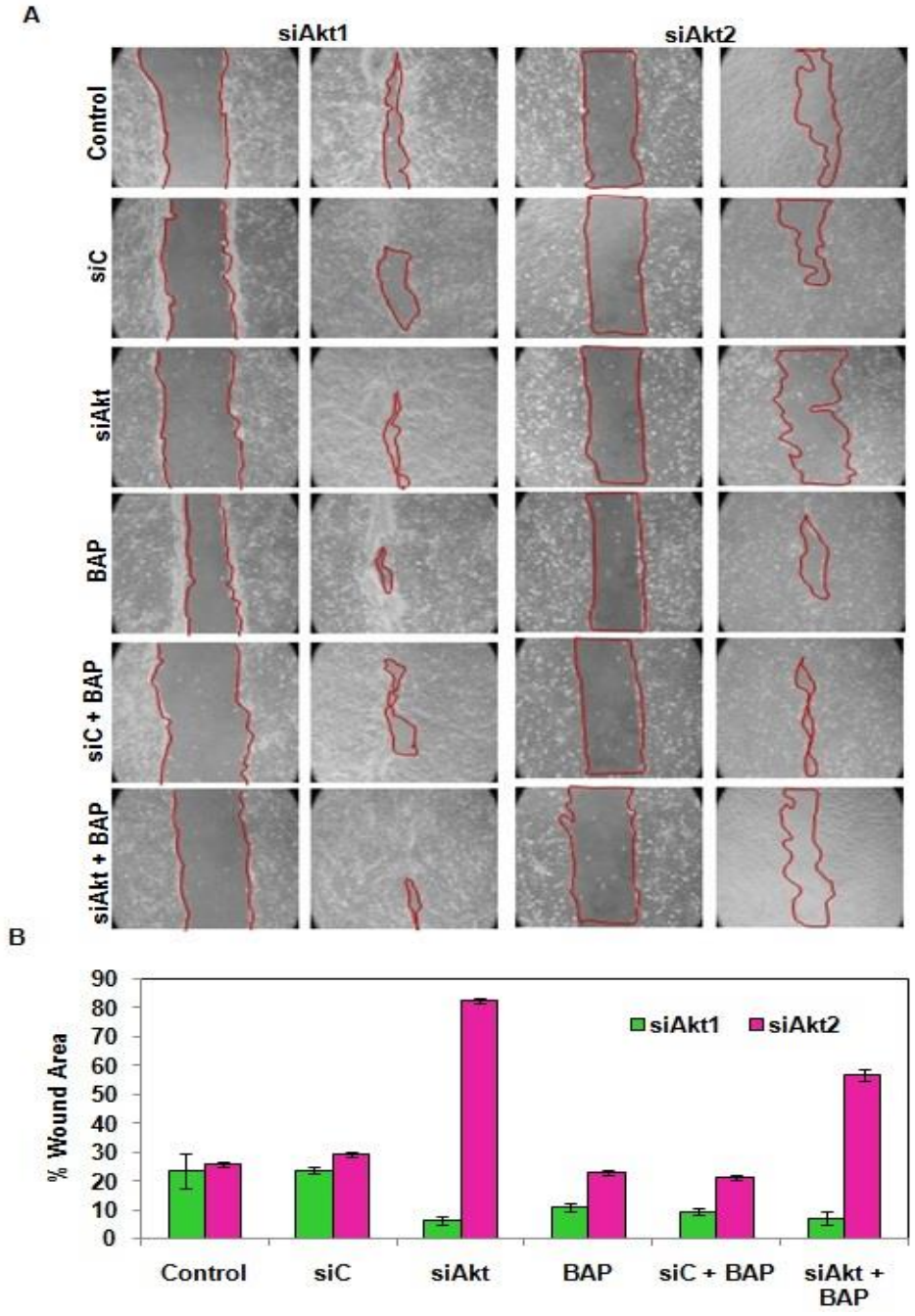


Figure 3.11 Effect of Effect of BAP and silencing of Akt1 and 2 on the migration of SAS cells. A; Representative image of the cell migration in control, BAP-treated and transfected SAS cells after 12 hours (12 hr), B; The % of cell-covered area (wound area) are shown in the bar diagram as mean ±SE from two independent experiments Percentage of wound area was estimated using Image J software (siC; Scramble control, siAkt1; siRNA for Akt1, siAkt2; siRNA for Akt2, BAP; 50ng/ml of BAP). Data are means ± SE *= $p<0.05$.

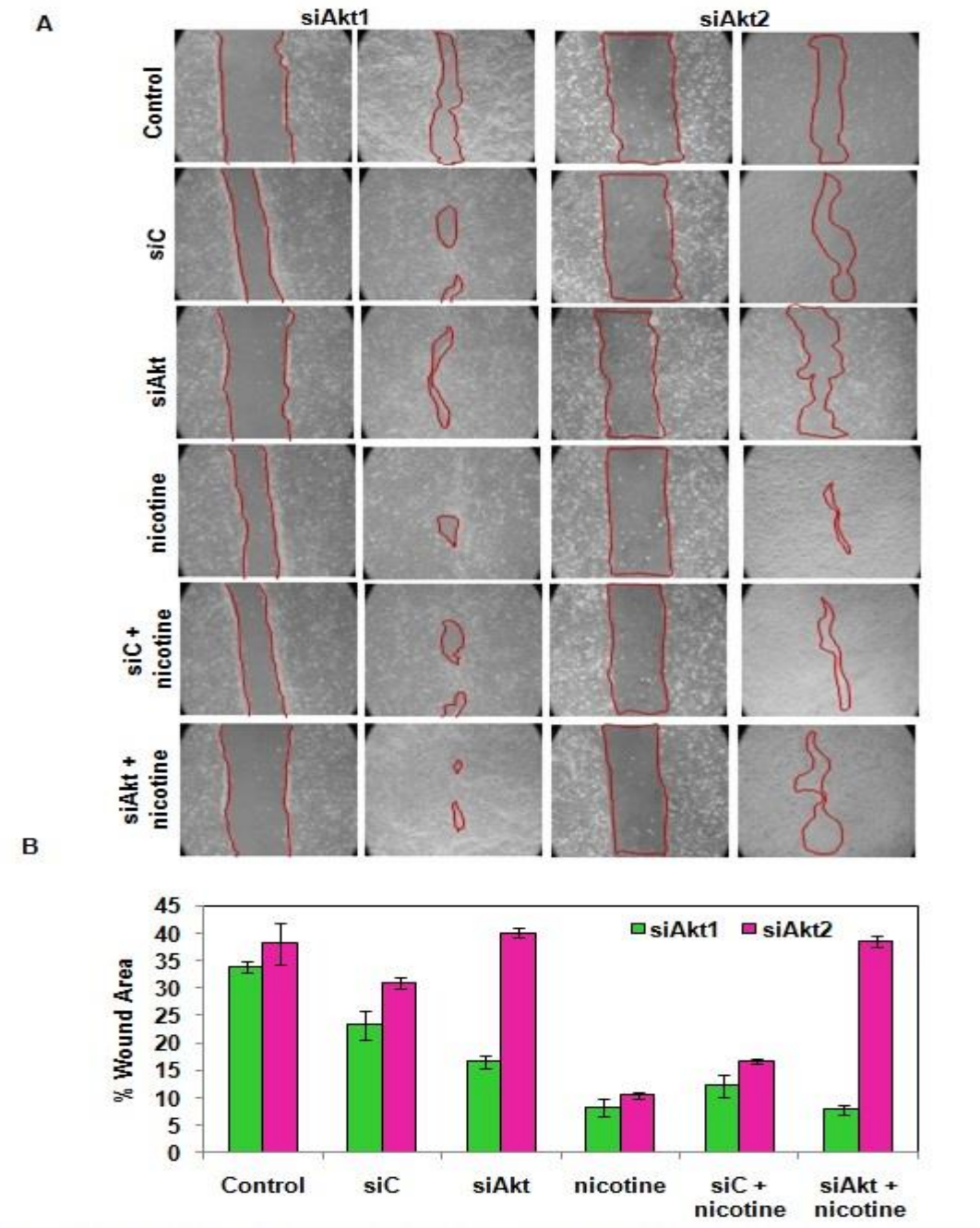


Figure 3.12: Effect of Effect of nicotine and silencing of Akt1 and 2 on the migration of SAS cells. A; Representative image of the cell migration in control, nicotine-treated and transfected SAS cells after 12 hours (12 hr), B; The % of cell-covered area (wound area) are shown in the bar diagram as mean ±SE from two independent experiments Percentage of wound area was estimated using Image J software (siC; Scramble control, siAkt1; siRNA for Akt1, siAkt2; siRNA for Akt2, nicotine; 0.5μM of nicotine). Data are means ± SE *= $p<0.05$.

pharmacological inactivation of Akt2 but not Akt1 significantly decreased cell migration and invasion (Bulj et al 2013). However, in lung cancer, partial reduction in wound healing migration was observed in Akt2 knockdown cells while the prominent effect was observed in Akt1 knockdown cells (Lee et al 2011). Interestingly, in prostate cancer cells both Akt1 and 2 was reported to work as negative regulators of cell migration and invasion (Virtakoivu et al 2012). Subsequently, analysis of the effect of tobacco components on directional cell migration and effect of Akt2 knockdown on it was studied. The TE (50ng/ml), BAP (50ng/ml) and nicotine (0.05μM) increased the aggressiveness of the cancer cells by increasing their migration potential as compared to the non-treated cells. Furthermore, this assisted migration was reduced by knockdown of Akt2 gene on SAS cells.

3.3.7. Effect of silencing Akt1 and 2 isoforms on the survival of SAS cells

The effect of Akt1 and 2 gene silencing was examined on the viability of SAS cells by PI exclusion method using FACS. It was found that the knockdown of Akt1 and 2 was cytotoxic to cancer cells as near to 26% of cell death was induced (Fig 3.13). The previous studies have also suggested the importance of Akt1 and 2 in cell survival and it might be achieved through different routes (Lee et al 2011, Sugatani et al 2005, Calamito et al 2010). Kim et al 2009 have shown that Akt2, but not Akt1, is required for cell survival upon UV irradiation and it prevents UV-induced cell death by inhibiting activation of JNK and p38. A similar observation was also made for cardiomyocyte where Akt2 was found to be critical for their survival and metabolism (Muslin 2011). However, it has been shown that PTEN-deficient tumors are dependent on Akt2 for maintenance and survival but not Akt1 (Chin et al 2014[b]).

3.3.8. Effect of silencing of Akt1 and 2 isoforms on the proteins associated with cell survival and proliferation.

The western blot analysis has shown that the expression of cell survival and proliferation-related proteins was decreased in Akt1 and 2 knockdown cells (Fig 3.13). The effect was found to be more prominent in Akt2-knockdown cells than Akt1. The expression of Cox-2 was found to be decreased in Akt1 and 2 knockdown cells. Various reports have suggested that the Cox-2 is an important molecular mediator of apoptosis in different cancer cells such as colorectal, breast, prostate, and blood cancers. Also, in endometrial and lung cancer, it is reported it to be a downstream target of Akt and takes an active part in the process of apoptosis of cancer cells (Sobolewski et al 2010, St-Germain et al 2004, Chen et al 2010, Yu et al 2012). The knockdown of Akt2 caused a marked decrease in the expression of Cyclin D1, Bcl-2 and Survivin while no significant change was observed in case of Akt1-knockdown cells. Cyclin D1 is known to encode the regulatory subunit of the holoenzyme which is involved in phosphorylation and inactivation of retinoblastoma protein thus promoting the progression of cells through G1/S phase of the cell cycle (Fu et al 2004). Several lines of evidence have indicated that the PI3K/Akt pathway is a strong activator of Cyclin D1 that is involved in apoptosis. It is well known that GSK-3 is involved in Cyclin D1 degradation which is inactivated by Akt through phosphorylation (Chang et al 2003). Though in our study, we found that silencing of Akt2 led to the reduction in the expression of Cyclin D1 but cell cycle arrest was not observed in G1 phase but in G2 suggesting some other novel mechanisms by which cells are undergoing apoptosis. In lung and breast cancer, contrary to our result, it was shown

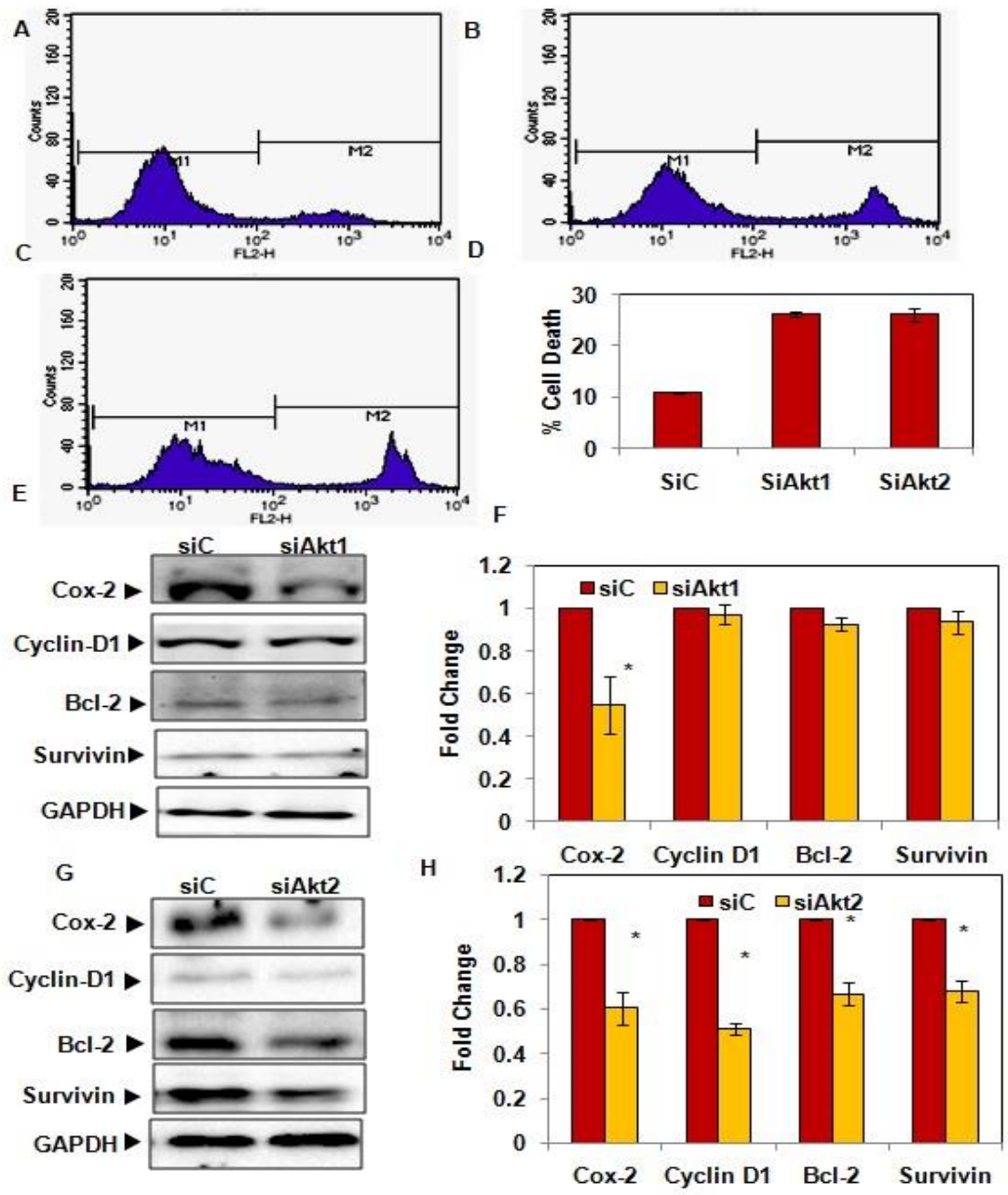


Figure 3.13: Effect of silencing of Akt1 and 2 isoforms on cell death and proteins associated with cell survival and proliferation. A; siC, B; siAkt1 and C; siAkt2 as analyzed by PI uptake method, D; Bar graph showing the percentage of cell death, E and G; Effect of gene silencing of Akt1 and Akt2 by siRNA (siAkt2) in SAS cells as analyzed by western blot assay, F and H; Relative bar graph showing the fold change of proteins of Cox-2, Cyclin D1, Bcl-2 and Survivin in Akt1 and Akt2-knockdown SAS cells over scramble control. Fold change in the expression was analyzed using Image Lab software. Data are means ± SE *= $p<0.05$.

that Akt1 modulates the expression of Cyclin D1 but not Akt2 (Grabinski et al 2011, Riggio et al 2017). Akt activation is believed to be an important signaling pathway that suppresses apoptosis pathways, in part by regulation of members of the Bcl-2 family of proteins which include Bcl-2, an anti-apoptotic protein (Song et al 2005, Green et al 2013). The Pugazhenthi and group have reported that the Akt upregulates Bcl-2 expression through cAMP-response element-binding protein (Pugazhenthi et al 2000).The expression of survivin is known to be regulated by a number of cell survival signaling cascades, which includes PI3K/Akt in different cancers (Chen et al 2016).

3.4. CONCLUSION

The treatment of TE and other tobacco components such as BAP and nicotine was found to increase the aggressiveness of cancer cells in terms of proliferation, clonogenic and migration potential. Furthermore, their treatment caused the increase in the transcript level of Akt1 and 2 gene isoforms. In addition, the silencing of Akt1 and 2 isoforms caused cell cycle arrest in G2/M phase and it induces cell death in oral cancer cells through apoptosis. Moreover, the silencing of Akt1 and 2 was able to reduce the normal and tobacco-induced clonogenic potential of oral cancer cells. However, silencing of Akt2 isoform only decreased the migration potential of cancer cells but no such effect was observed in case of Akt1-knockdown cells. Further, the expression of different molecular mediators involved in apoptosis was found to get decreased in Akt1 and 2-knockdown cells. Though, this effect was more prominent in case of Akt2-knockdown cells. Overall, it appears that Akt1 and 2 both are involved in the pathogenesis of oral cancer cells albeit through different mechanism affecting the different process of carcinogenesis.

Chapter 4

Demarcation of Natural Agents and General Akt Kinase Inhibitors into Akt Isoform-specific Inhibitors

4.1. INTRODUCTION

In the previous chapters we have observed that, out of the three Akt isoforms, only Akt1 and 2 isoforms play a major role in oral neoplasia. Moreover, it was found that both Akt1 and 2 regulate the distinct process of oral cancer pathogenesis. Furthermore, on examining the effect of pure TE and other tobacco components such as BAP and nicotine on the expression of Akt isoforms, it was ascertained that only the expression of Akt1 and 2 varies while the Akt3 remains unchanged. Despite the fact that, the structures of Akt isoforms are similar but their non-conserved residues are different, which might contribute in differential interactions with various inhibitors. There are many reports available which suggest that the non-conserved residues present in the catalytic pockets as well as outside lead to differential activity in the interactions with the inhibitors due to the involvement of different types of interactive bonds such as hydrophobic bond, lipophilic forces, van der wall forces etc (Frazzetto et al 2008). Moreover, it is noteworthy that the mutations in the non-conserved residues are very critical and have a significant clinical outcome towards applied treatment indicating that these residues might play an important role in the interactions (Vogiatzi and Giordano 2007). Therefore, in the present chapter we hypothesize that the general Akt kinase inhibitors can further be demarcated into Akt isoform-specific inhibitors based on the difference in the interaction with the available inhibitors. Moreover, it is well known fact that, nature has the vast reserve of countless drugs with enormous potential against many human diseases including cancer. The present day has observed a considerable shift in research related to drug development from natural products. Until now, approximately 50% of the drugs are derivative of botanicals, and this enormous resource is yet to be explored to the fullest for

developing the efficacious novel chemotherapeutic agents against cancer. Hence, we also consider the natural compounds for our study and attempted to group the available natural compounds into Akt isoform-specific inhibitors.

4.2. MATERIALS AND METHODS

4.2.1. Data retrieval

The structure of different natural agents and general Akt inhibitors were obtained from the Pubchem database to demarcate them more specifically into isoform-specific inhibitors (Fig 4.1 and 4.2). The amino acid sequences of human Akt1, Akt2 and Akt3 were derived from UniProtKB/Swiss-Prot (http://www.uniprot.org/; IDs: P31749, P31751, Q9Y243, respectively). The 3D structure of human Akt1 and 2 were obtained from Protein Data Bank (PDB, http://www.rcsb.org) having PDB ID of 3MVH and 3E9D respectively. These are crystal structures of the kinase domain of Akt1 and 2 in complex with ATP-competitive inhibitors. These structures were selected for the study of the docking targeting the ATP-competitive inhibitors and it needed a clue for the ATP-competitive site from the bound ATP-competitive inhibitor. Different natural agents were selected for the docking studies.

4.2.3. Protein sequence alignment of Akt isoforms and homology modeling of human Akt3

The protein sequences of Akt isoforms was aligned using PRofile ALIgNEment (PRALINE) which is a fully customizable multiple sequence alignment application. In addition to a number of available alignment strategies, this application can integrate information from database homology searches to generate a homology-extended multiple alignments (http://ibivu.cs.vu.nl/programs/pralinewww/) (Simossis and Heringa 2005).

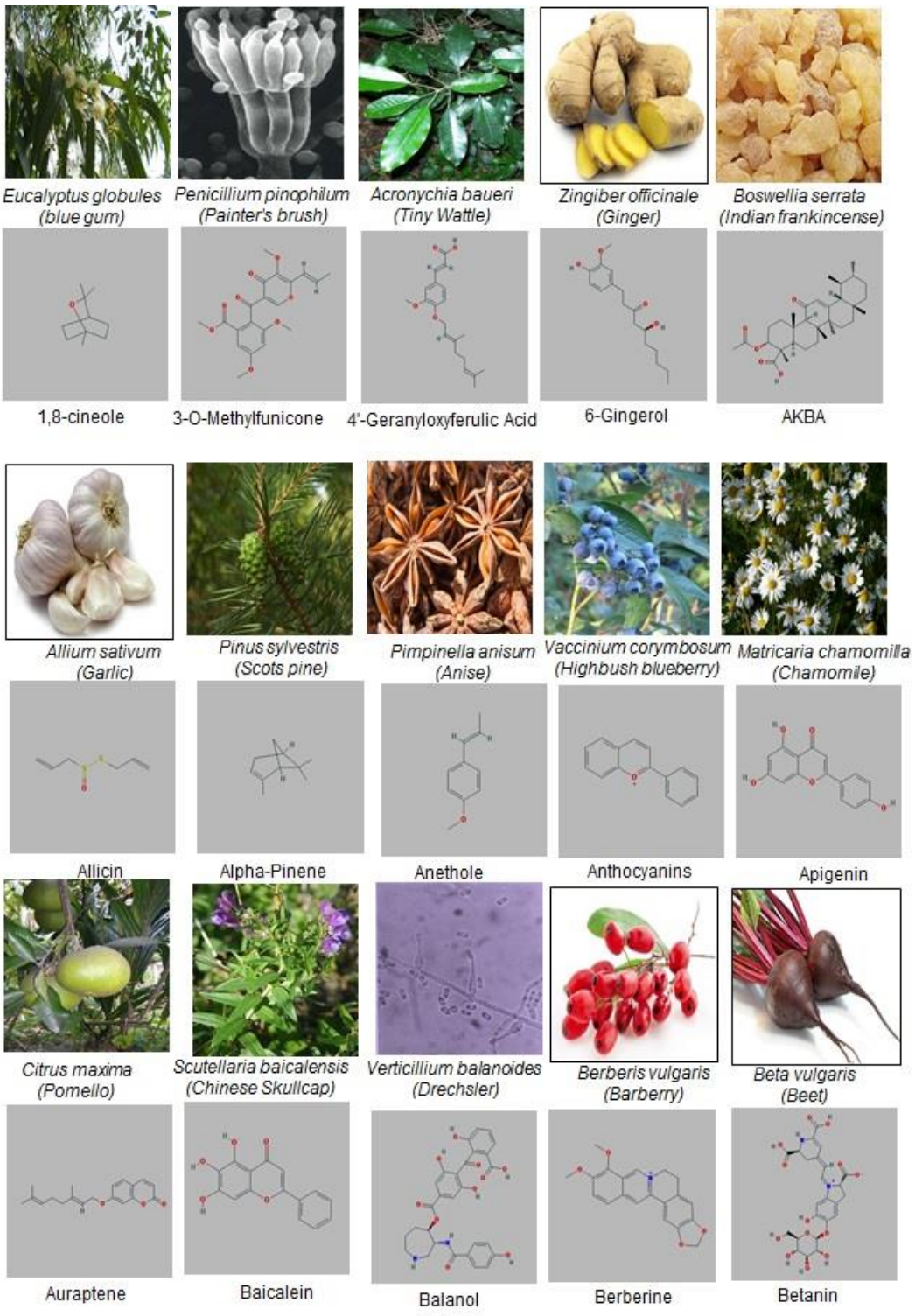


Figure 4.1: Structures of natural inhibitors used for the docking studies and their sources. The structures of the inhibitors were obtained from the Pubchem database (Image courtesy: Annexure I).

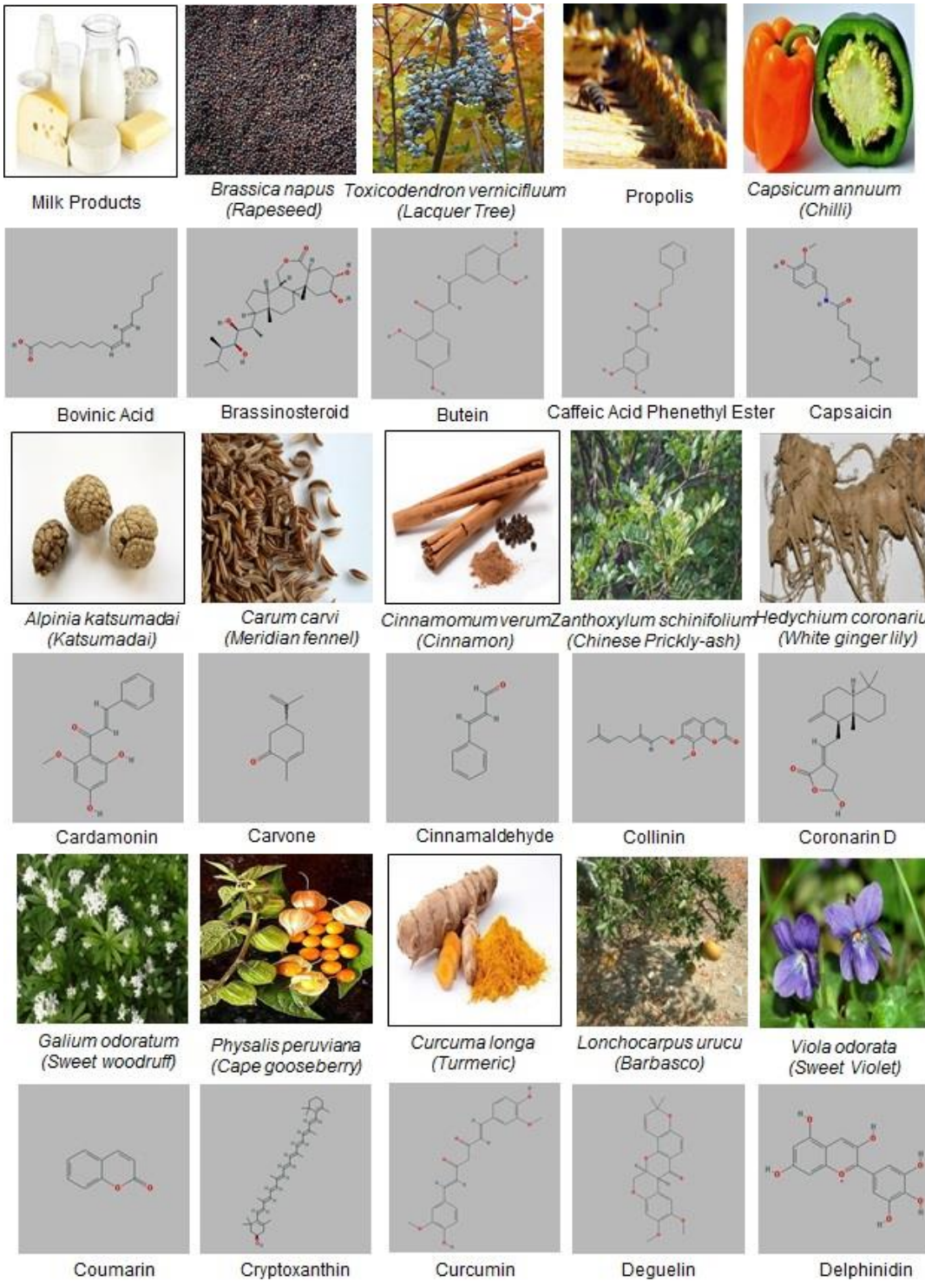


Figure 4.1contd: Structures of natural inhibitors used for the docking studies and their sources. The structures of the inhibitors were obtained from the Pubchem database (Image courtesy: Annexure I).

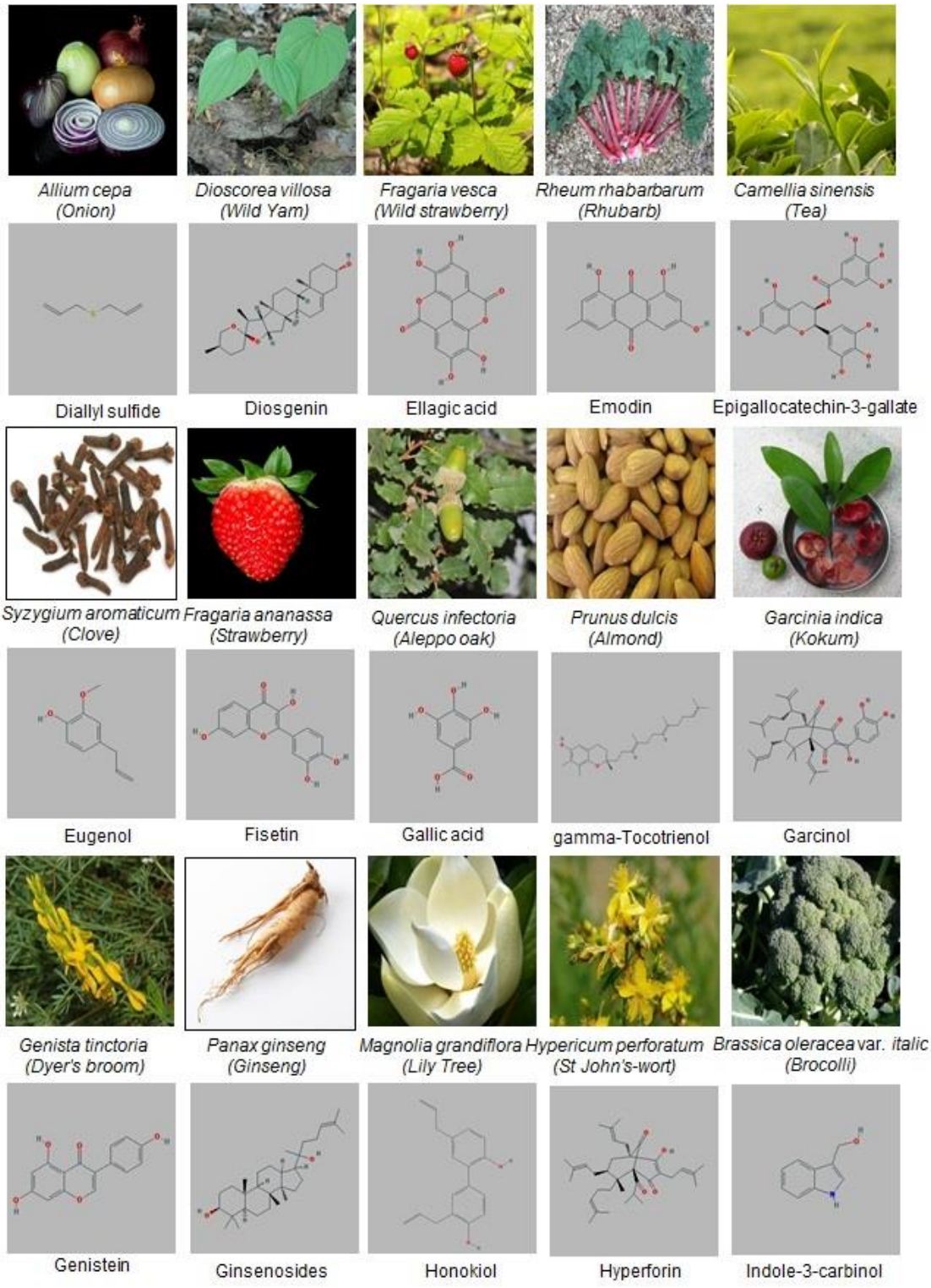


Figure 4.1contd: Structures of natural inhibitors used for the docking studies and their sources. The structures of the inhibitors were obtained from the Pubchem database (Image courtesy: Annexure I).

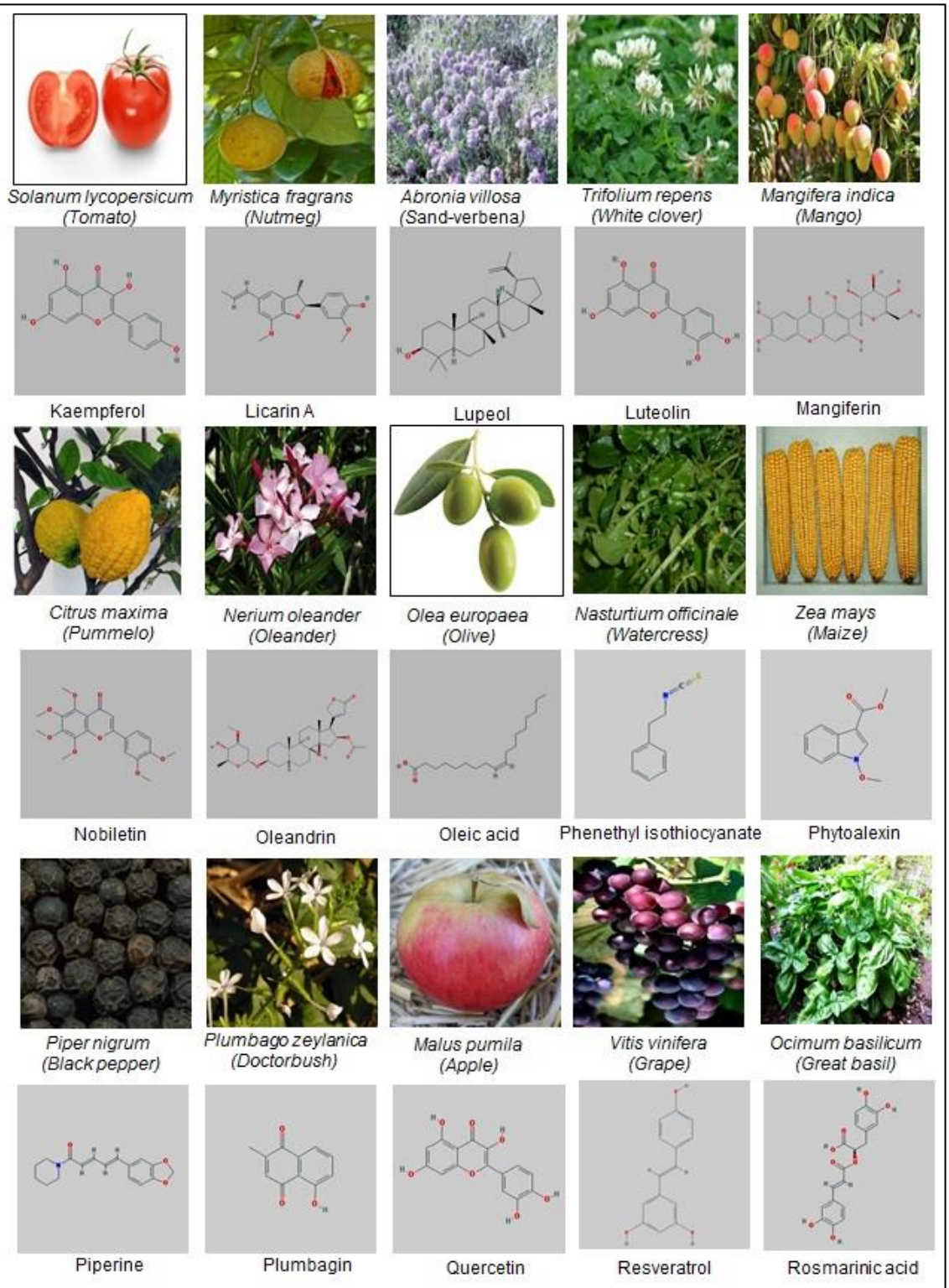


Figure 4.1contd: Structures of natural inhibitors used for the docking studies and their sources. The structures of the inhibitors were obtained from the Pubchem database (Image courtesy: Annexure I).

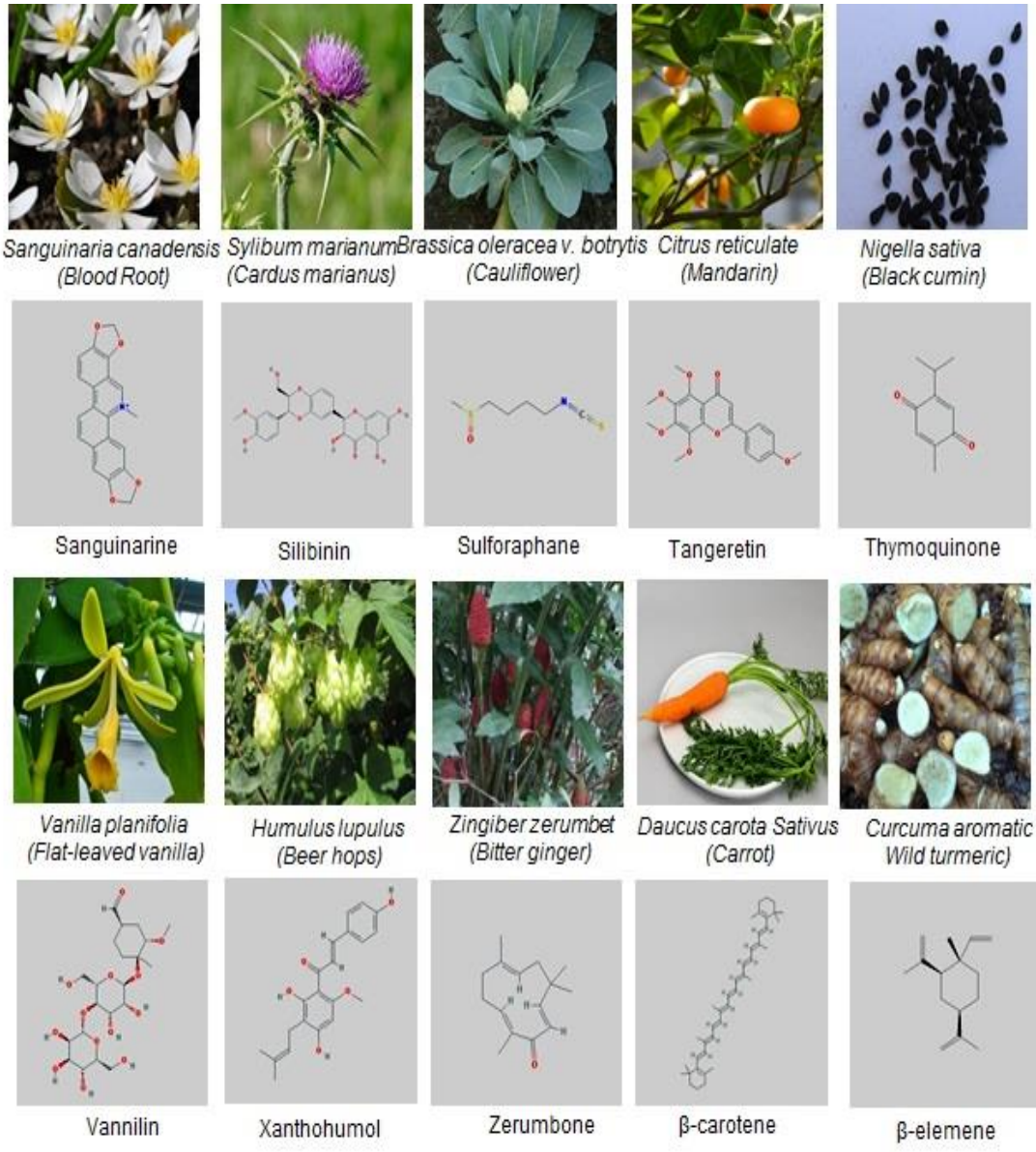


Figure 4.1contd: Structures of natural inhibitors used for the docking studies and their sources. The structures of the inhibitors were obtained from the Pubchem database (Image courtesy: Annexure I).

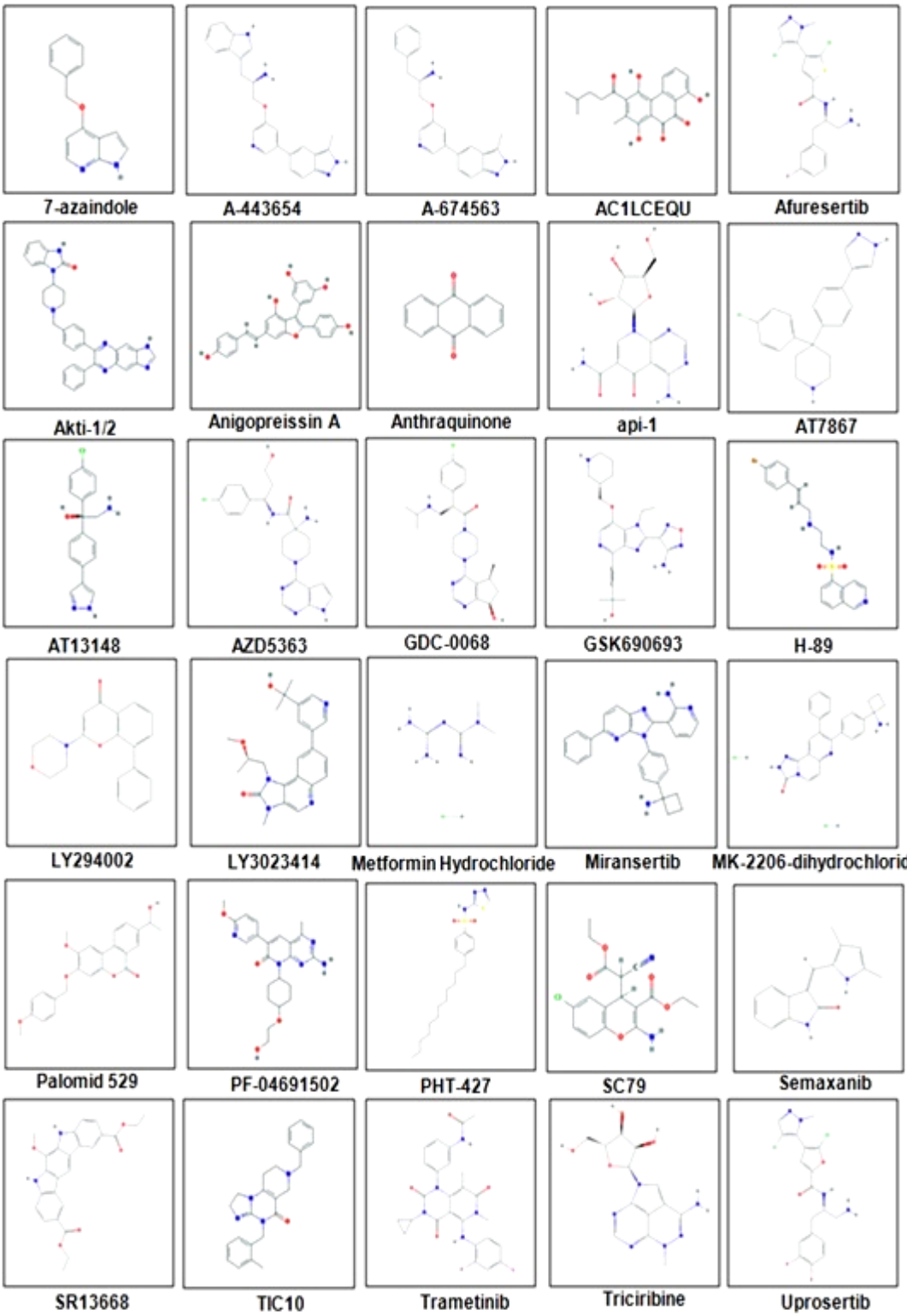


Figure 4.2: Structures of general Akt kinase inhibitors used for the docking studies. The structures of the inhibitors were obtained from Pubchem database.

The homology model of human Akt3 was obtained by submitting the sequence in ModWeb Server. ModWeb is a web server for automated comparative modeling that relies on PSI-BLAST, IMPALA and MODELLER used for protein structure modeling (http://salilab.org/modweb) (Sanchez and Sali 1998). It accepts one or many seqı 94 in the FASTA format (Pearson 2000) and calculates models for them depending on the best available template structures from the Protein Data Bank (PDB) (http://www.rcsb.org) (Berman et al 2002). The model was generated by *ModWeb version r175* based on the template of Akt1 (PDB id: 4EJN) with which it showed 87% sequence identity. The model was generated by considering different modes of selection criteria such as MPQS, TSVMOD, Longest DOPE, and DOPE (Sali and Blundell 1993). The evaluation of generated model was assessed for stereochemical properties by using PROCHECK (Laskowski et al 1993).

4.2.4. Molecular docking

Molecular docking of the Akt isoforms, Akt1, Akt2 and Akt3 was performed by using Schrödinger *Glide* software within *Maestro* 9.5. The used software undertakes a correction of the raw PDB structure by incorporating some modifications such as the addition of hydrogen atoms, assigning bond orders, creating disulfide bonds, fixing of the charges, and orientation of groups. To start with the process the protein was first prepared with the help of Protein Preparation wizard. The selected inhibitors were prepared by using LigPrep for the corrections on the ligands including the addition of hydrogens, bond lengths and bond angles, low energy structure, and ring conformation etc succeeded by minimization and optimization in the optimized potential for liquid simulations (OPLS 2005) force field. For docking analysis, *Glide;* a ligand docking program was used for

predicting the protein-ligand binding modes and ranking of ligands was performed through high-throughput virtual screening. *Glide* molecular docking needs an X-ray crystal structure of protein complexed with ligands for determining the active site receptor grid. Receptor grid-based molecular docking helps the ligands to bind in more than one possible conformation. *Glide* examines for favorable interactions between one or more ligand molecules and a protein. The van der Waals radii of the receptor atoms with partial atomic charges should be less than the specified cutoff. The scaling factor and partial charge cutoff were 1 and 0.25A°, respectively. The G score was calculated in kcal/mol and it includes ligand–protein interaction energies, hydrophobic interactions, hydrogen bonds, internal energy, p–p stacking interactions, and root mean square deviation (RMSD) and desolvation. For the present study, XP Glide Score was used to group the inhibitors. Grids were generated using *Glide* version 9.5 as per the standard procedure recommended by Schrodinger (Schrödinger *et al.*, 2013).

4.3. RESULT AND DISCUSSION

4.3.1. Protein sequence alignment of Akt isoforms and homology modeling of human Akt3

The protein sequence alignment of Akt isoforms was performed to explore the dissimilar residues and to analyze their role in the interactions with the inhibitors. A PRALINE amino acid alignment of the Akt isoforms highlights the conserved residues and the divergent residues as well (Fig 4.3). The percent sequence identity was found to be 0.81. The consistency score of 5 or less than that are considered to be unreserved (Simossis and Heringa 2005). The 3D structure of human Akt3 having around 484 amino acids was generated using Akt1 as a template by using *ModWeb version r175.* MODWEB relies on MODPIPE, a completely automated software pipeline for comparative protein structure

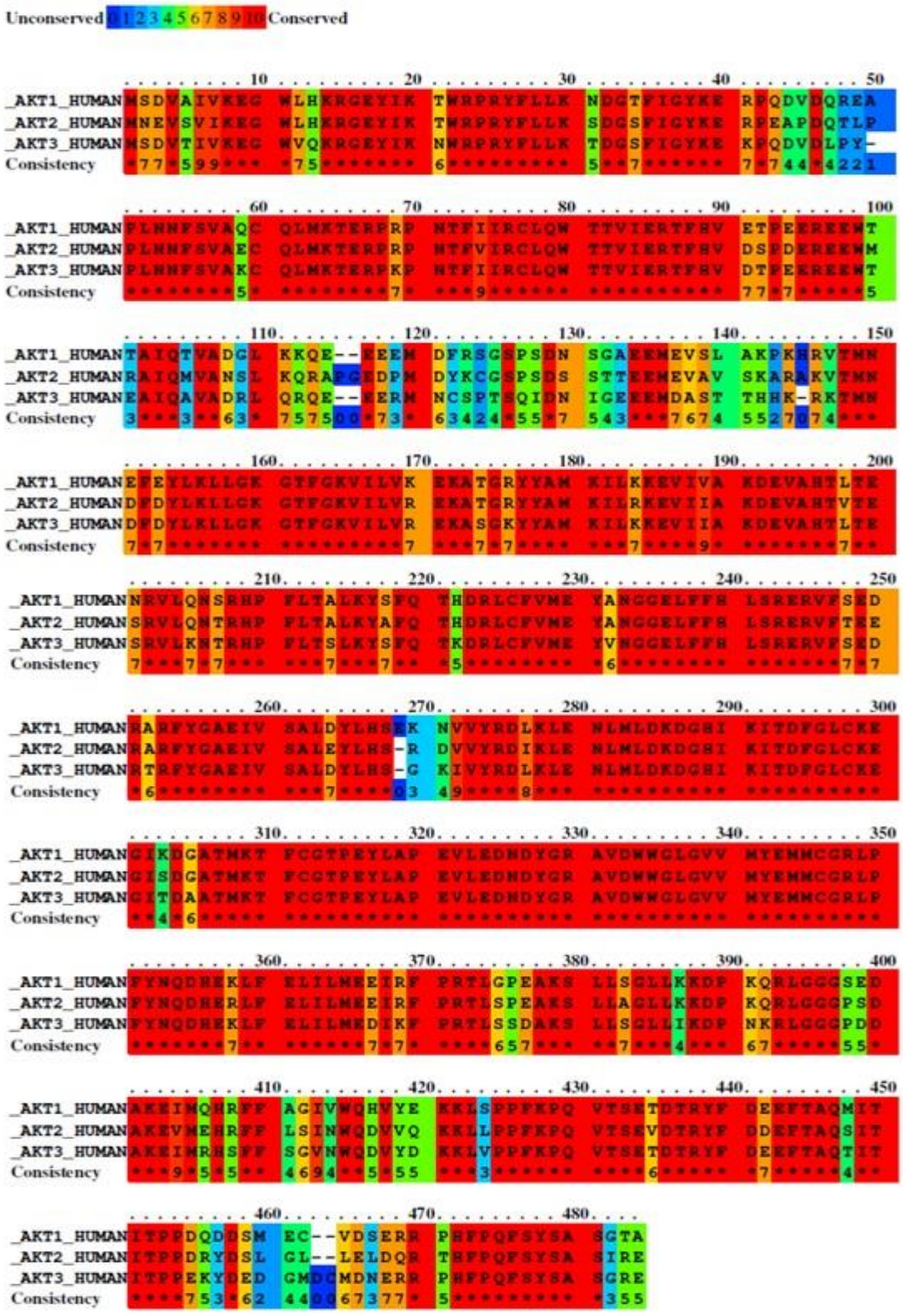


Figure 4.3: Multiple sequence of Akt isoforms (Akt1, Akt2 and Akt3) using PRALINE alignment program along with conservedness scale to show the conserved and non-conserved region Akt isoforms.

modeling, which can calculate comparative models for a large number of protein sequences, using many different template structures and sequence-structure alignments. Sequence-structure matches are established by aligning the PSI-BLAST sequence profile of the target sequence against each of the template sequences extracted from PDB, as well as by scanning the target sequence against a database of the template profiles using IMPALA. Significant alignments covering distinct regions of the target sequence were chosen for modeling. Models were calculated for each of the sequence-structure matches using MODELLER (Pieper et al 2006, Bairoch et al 2005, Benson et al 2013, Eswar et al 2003, Shen et al 2006). The modeled structure of the Akt3 protein was found to be similar to other Akt isoforms having a bilobed structure with a deep cleft between the lobes. The smaller N-terminal lobe has a twisted five-stranded antiparallel β sheet and a partially ordered α helix (αC). The larger C-terminal domain contains mostly of α helices (Sanchez and Sali 1998, Huang et al 2003). These structures of Akt isoforms were chosen for the docking study (Fig 4.4). Further, the Akt3 modeled protein structure was analyzed by generating Ramachandran Plot through *PROCHECK* (Fig 4.4). The *PROCHECK* suite of programs provides a detailed check on the stereochemistry of a protein structure. Its outputs comprises of a number of plots in PostScript format and a comprehensive residue-by-residue listing. It provides an evaluation of the overall quality of the structure in comparison to well-refined structures of the same resolution and also highlights the regions that may need further investigation. The *PROCHECK* programs are useful not only for assessing the quality of protein structures in the process of being solved but also for existing structures and of those being modeled on known structures. It is based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater

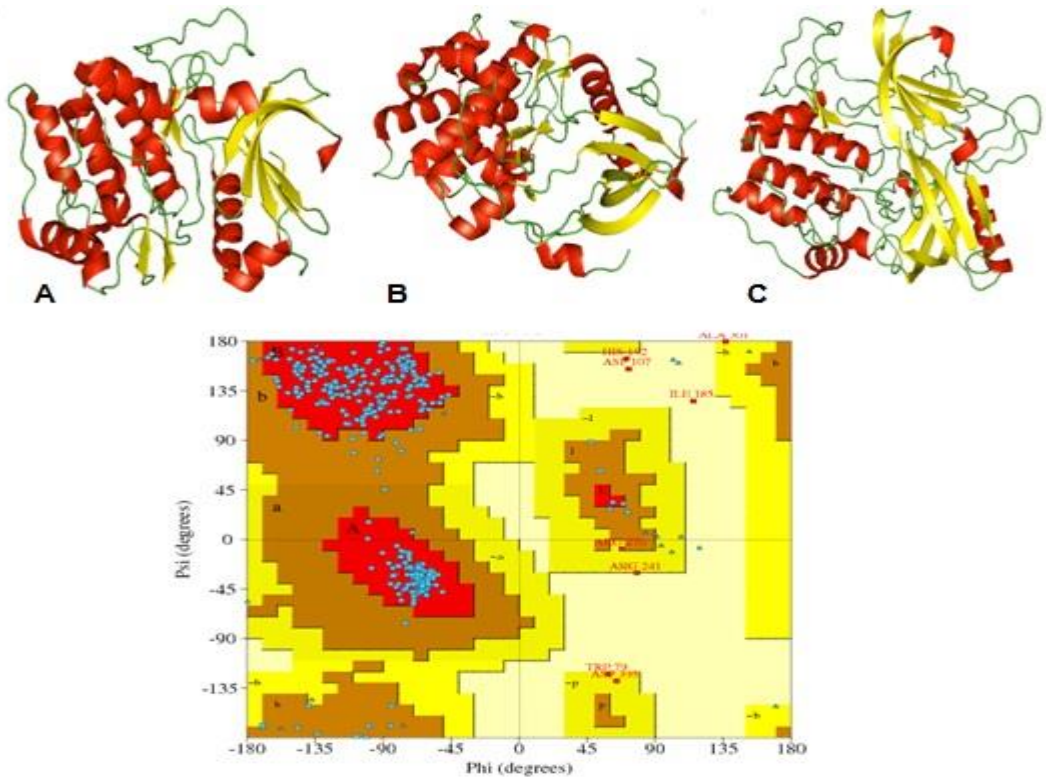


Figure 4.4: Structures of different isoforms of Akt obtained from the PDB database; A) Akt1, B) Akt2 and C) Akt3 (generated model ofAkt3 from modeller program). Ramachandran plot of modeled structure of Akt3 protein validated by PROCHECK program showing the percentage of amino acid residues present in allowed and disallowed region based on the psi and phi angles forming between amino acid residues.

	No. of residues	%-tage
Most favoured regions [A,B,L]	347	89.9%*
Additional allowed regions [a,b,l,p]	31	8.0%
Generously allowed regions [~a,~b,~l,~p]	4	1.0%
Disallowed regions [XX]	4	1.0%*
Non-glycine and non-proline residues	386	100.0%
End-residues (excl. Gly and Pro)	2	
Glycine residues	26	
Proline residues	18	
Total number of residues	**432**	

Table 4.1: Stereochemical evaluation of obtained Akt3 model in Ramachandran Plot analysis using Procheck. The stereochemical data showed the allowed and disallowed region for the present amino acid residues and dihedral angles present between the amino acid residues which show the favorability of the generated model for Akt3 protein.

than 20.0, a good quality model would be expected to have over 90% in the most favored regions (A, B, L) (Laskowski et al 1993). The obtained Ramachandran plot presented good quality of model having 89.9% of residues in the most favored region, 8% in additional allowed region, and 1% in the generously allowed region (Table 4.1). The previous studies have also considered a good model if 89.9% of residues reside in most favored region and proceeded for further studies (Krebs et al 2016, Ohno et al 2005, Huang et al 2009).

4.3.2. Molecular docking

Docking was achieved with the Glide (Grid-based Ligand Docking with Energetics) program, version 10.3. For docking, the scoring grids were centered on the crystal structure of ATP competitive inhibitor using the default bounding box sizes; with an inner box of 10A^{o} on each side and an outer box of 24A^{o} on each side. Flexible docking with default parameters was used. Glide XP (extra precision) was used for all docking calculations. It has been used in many earlier studies of docking (Friesner et al 2006, Tripathi et al 2013, Hao et al 2008, Kuck et al 2010). The best docked poses were selected as the ones with the lowest Glide Score; the more negative the Glide Score, the more favorable the binding is. To understand the binding mode of our selected inhibitors, they were docked with the crystal structure of the human Akt1 and 2 protein and modeled Akt3 protein. The docking was targeted against the ATP-competitive site of protein. The protein was first prepared using the Protein Preparation wizard and the docking studies were performed using the Schrödinger *Glide* software (*Maestro* 9.5) with extra-precision (XP). The obtained G-Score have shown that, Betanin have the maximum affinity for Akt1 protein with a maximum GScore of -10.45. For Akt2 protein, AT13148 with a

GScore of -12.43 showed the highest affinity. In case of Akt3 protein, Balanol exhibited the best docked pose (GScore: -9.89) (Table 4.2). The 2D interaction diagram of the Akt isoforms shows involvement of different residues such as acidic, hydrophobic, basic, polar, and other residues at the active site in the interactions with the inhibitors differently (Fig 4.5). Interestingly some of the active residues involved in the interactions were present in the non-conserved region which might be responsible for differential interaction of Akt isoforms with the inhibitors. The non-conserved residues have been exploited in some studies for specific binding to the inhibitors. In one such study, inhibitors were designed to specifically interact with the non-conserved residues of thymidylate synthase of *Lactobacillus casei* in order to differentiate it from the human enzyme (Stout et al 1999). Moreover, the role of non-conserved residues has been well established in dissecting the isoform-selectivity of PI3kinase inhibitors (Frazzetto et al 2008). Furthermore, a systematic mutational analysis of non-conserved residues within the extracellular entry pathway and the high affinity binding site in norepinephrine transporter have shown that that the non-conserved binding site residues are critical determinants for inhibitor selectivity (Andersen et al 2015).

Protein	Compounds	GScore	LipophilicEvdW	PhobEnHB	HBond	Electro
Akt1	Betanin	-10.45	-3.42	0	-5.38	-1.4
	Vannilin	-10.22	-2.35	-1	-5.19	-1.15
	AT13148	-10.07	-3.89	-0.47	-1.95	-0.98
	(-)-Epigallocatechin gallate	-9.58	-3.16	0	-5.06	-1.55
	AZD5363	-9.39	-4.43	-1.5	-2.01	-0.85
Akt2	AT13148	-12.43	-4.01	-0.68	-1.95	-2.48
	A-443654	-11.45	-4.9	-1.5	-2.7	-2.44
	A-674563	-11.29	-4.81	-1.5	-2.58	-2.41
	Vannilin	-11.04	-2.64	-0.19	0	-6.02
	Quercetin	-10.13	-3.18	-0.65	-1	-1.11
Akt3	Balanol	-9.89	-3.87	-1	-3.04	-1.69
	Quercetin	-8.24	-2.7	-1	-2.85	-1.11
	AT13148	-8.1	-3.22	0	-1.44	-0.88
	CCT128930	-7.8	-1.74	-3	-1.37	-0.85
	CURCUMIN	-7.76	-3.66	-0.97	-2.04	-0.53

Table 4.2: Lowest binding energy for the ligand-protein interaction as detected by GLIDE molecular docking.

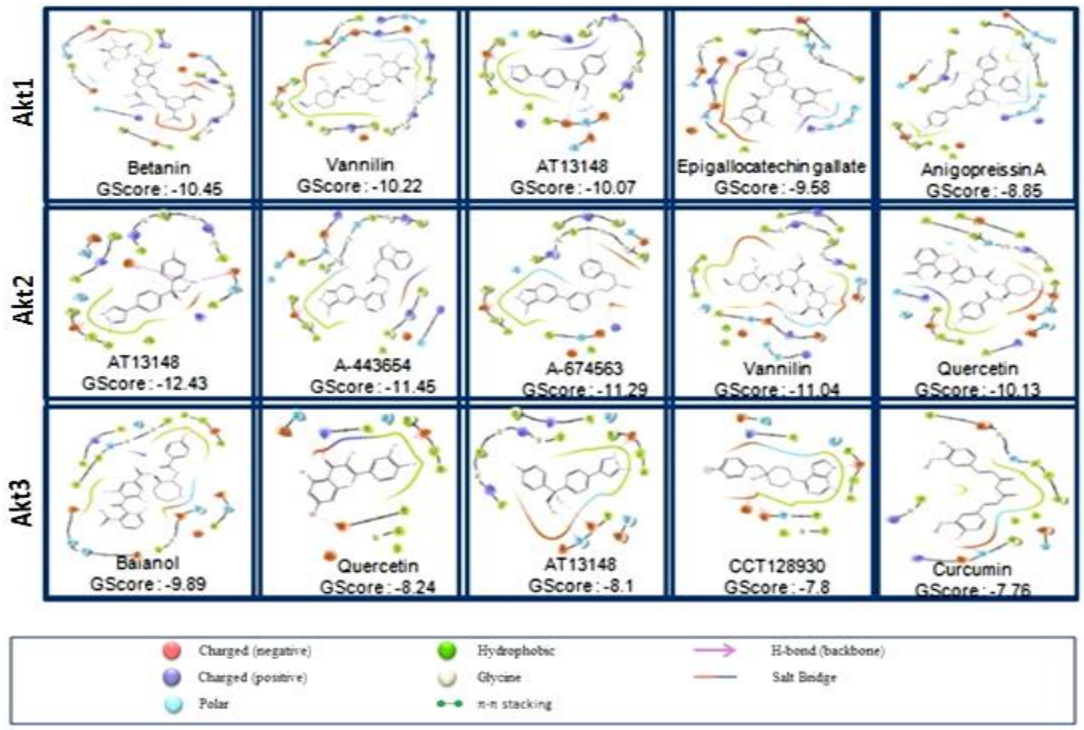


Figure 4.5: 2D diagram showing the interactions of Akt isoforms with selected inhibitors and their GScore. The Betanin was showing the maximum affinity for Akt1 protein with the maximum affinity with an Gscore of -10.45. AT13148 was having maximum affinity for Akt2 protein. For Akt3 protein, Balonol with a Gscore of -9.89 was showing highest affinity.

4.4. CONCLUSION

In the present study, we segregated the general Akt kinase inhibitor and other natural agents into Akt isoform-specific inhibitors. It was accomplished using the *in silico* docking analysis by Schrodinger Maestro program and is particularly focused on ATP competitive site. However, the interaction of the selected compounds with the allosteric site remains unclear and it can be performed in order to achieve in-depth understanding. Nevertheless, the present investigation is a preliminary attempt to categorize the natural and general Akt inhibitor into Akt-isoform specific inhibitors and the research must be extended to in vitro and preclinical models before proceeding for clinical studies.

Chapter 5

Conclusion and Future Prospects

5.1. CONCLUSION

Despite the remarkable progress made in the oral cancer diagnostics and therapeutics, the overall survival of cancer patients has not been increased in the last two decades. The main reasons for the failure of these advancements are late diagnosis and poor prognosis. Moreover, the emergence of chemoresistance, radioresistance and tumor recurrence in oral cancer has augmented the limitations further. The aggravated situation can only be overcome by exploring the new molecular signatures that can pave the way for early diagnosis as well as can provide novel targets against which effective chemotherapeutics can be developed. Akt kinase is one such molecular mediator which acts as a central signalling node for all the important cellular processes involved in human physiology and diseases. Though it has three isoforms whose functions are mutually exclusive, they perform distinct functions in various cancers. To analyze their role in oral cancer we initially determined the expression and mutation status of Akt isoforms in different stages of OSCC of patient samples. The expression of Akt isoforms in patient samples was studied by tissue microarray analysis. Our results showed that Akt1 and 2 were overexpressed while no significant change in the expression of Akt3 isoform was observed. There was no significant variation in the expression of different isoforms of Akt in different genders. With respect to different tumor tissue types, we found the maximum expression of Akt1 and 2 isoforms in malignant tissue types. Also, we observed that the increased expression of Akt1 and 2 isoforms were found to be correlated with different stages of oral cancer development. We observed the maximum expression of Akt1 in cheek tissues and Akt2 in tongue tissues. To evaluate the mutational status of Akt isoforms in HNSCC patients, the data of 504 patient samples from TCGA data portal was obtained. We found that Akt1 has the maximum genetic

alteration of 2.8% followed by Akt3 (2.4%) and Akt2 (2%). Moreover, on analyzing the mutation status from the portal we have observed different mutations in various regions of the Akt isoforms protein. In case of Akt1, only two missense mutations ie., E17K and E49K were observed in the PH domain of the protein which is known to hyperactivate the Akt1 isoform. For Akt2 isoform, five mutations; Y351C, P471S, D388N, and G394Wfs*48 were observed. Different mutations such as R343M, S403G, S136C, K152R, and M145K were found in Akt3 isoform. Moreover, the cases of Akt1 and 2mutations were found to have higher mRNA transcript level. Also, the univariate analysis of 504 samples of HNSCC has shown that the increase in the abundance of genetic alterations of Akt1 and 2 was correlated worst OS and DFS as compared to Akt3 isoform. Consequently, it can be inferred that Akt1 and 2 play a significant role in clinical outcomes of HNSCC patients.

Subsequently, we examined the effect of pure TE and other commercially available tobacco components on the expression Akt in OSCC cells. It is a well-known fact that tobacco is the one of the main risk factor responsible for oral cancer. It was found that after 24h treatment of TE, BAP, and nicotine, the transcript level of both Akt1 and 2 isoforms were increased but no change in Akt3 isoform was found. Subsequently, we studied the role of Akt isoforms in the development of OSCC. For achieving this, we silenced the Akt1 and 2 using specific siRNAs. The effect of silencing of Akt1 and 2 on the different hallmarks of cancer was evaluated. The cell cycle arrest was observed by FACS assay where we observed the arrest in G2/M phase which might induces the cell to undergo apoptosis. Subsequently, the effect of tobacco components and silencing of Akt1 and 2 isoforms was analyzed on the clonogenic potential of cancer cells. It was found that

the treatment of tobacco components increased the potential for colony formation up to a certain concentration. The Akt1 and 2-silenced cells eliminated the effect of tobacco and its component on colony forming ability of cancer cells; however, silencing of Akt 1 shows more significant effect. Next, we analyzed the effect of different isoforms of Akt on the migration of oral cancer cells by wound healing assay. On increasing the concentration of TE, it was found that percentage of wound area significantly decreased and the wound was healed in most of the treated concentration in 24h interval time. A similar effect was also observed with BAP and nicotine. We found that the assertiveness of tobacco components on the migration of cancer cells was nullified by the knockdown of Akt2 in oral cancer cells. However, the silencing of Akt1 could not inhibit the migration of the SAS cells. The wound healing assay suggested the reduction in the migration of cancer cells on the knockdown of Akt2 gene signifying their role in the migration of cancer cells. Though, in case of the Akt1 knockdown, no such effect was observed. The clonogenicity of the SAS was found to be declined in Akt1 and 2 knockdown cells as compared to the untreated cells. The silencing of Akt1 and 2 isoforms in SAS cells increased the percentage of cell death as observed through PI-FACS method. Furthermore, silencing of Akt2 isoforms decreased the expression of proteins associated with cancer cell survival and apoptosis such as Survivin, Cox-2, Bcl-2, and Cyclin-D1. Though, the silencing of Akt1 doesn't have a significant effect on these proteins except Cox-2. It appears that Akt1 and 2 are regulating the distinct processes the oral cancer development through different molecular mediators. Finally, we made an effort to demarcate the general Akt kinase inhibitors into Akt isoform-specific inhibitors based on the docking studies. It was commenced with obtaining the crystal

structure of Akt1 and 2 from PDB. The homology model of human Akt3 was obtained by submitting the sequence in ModWeb Server. ModWeb is a web server for automated comparative modeling that relies on PSI-BLAST, IMPALA and MODELLER used for protein structure modeling (http://salilab.org/modweb). Further, the obtained modeled structure was evaluated by generating Ramachandran Plot through PROCHECK. It gives information about the overall quality of the structure as compared with well-refined structures of the same resolution and also highlights regions that may need further investigation. The protein sequences of Akt isoforms was aligned using PRofile ALIgNEment (PRALINE) which is a fully customizable multiple sequence alignment application (http://ibivu.cs.vu.nl/programs/pralinewww/). The molecular structures of selected general Akt inhibitors and natural agents were retrieved from PubChem compound database. To understand the binding mode of our selected 103 compounds, they were docked with the crystal structure of the human Akt1 and 2 protein and modeled Akt3 protein. The docking was targeted at the ATP-competitive site of the protein. The protein was first prepared using the Protein Preparation wizard and the docking studies were performed using the Schrödinger Glide software (Maestro 9.5) with extra-precision (XP). Based on the GScore obtained, the ranking of Akt inhibitors was done with the maximum score of -9.39 (AZD5363), -11.45 (A-443654) and -7.8 (CCT128930) for Akt1, Akt2, and Akt3 respectively. The 2D interaction diagram of the Akt isoforms shows the involvement of different residues such as acidic, hydrophobic, basic, polar, and other residues at the active site in the interactions with the inhibitors differently.

Overall, the present study was aimed at evaluating the expression and delineating the role of different Akt isoforms in the development of oral cancer. The tissue microarray

analysis showed the overexpression of Akt1 and 2 in oral cancer patients as compared to the Akt3 isoform. The mutational data obtained from the TCGA have shown the worst survival of cancer patients having alterations in Akt1 and 2 isoforms as compared to Akt3 isoform. Tobacco and its components increased the expression of Akt1 and 2 in oral cancer cells and it also increased the aggressiveness of cancer cells by increasing the proliferation, clonogenicity and migration potential of cancer cells. The knockdown of Akt1 and 2 in the cancer cells led to the reduction in the aggressiveness by reducing the clonogenicity and migration potential of cancer cells. The knockdown of Akt1 and 2 led to cell cycle arrest which might drive the oral cancer cells to undergo apoptosis and we observed increased percentage of cell death. It also further reduced the expression of proteins such as Survivin, Bcl-2, Cox-2, and Cyclin-D1 important for promotion and progression of cancer cells. Though, the effect was more prominent in Akt2-silenced cells. Through docking studies we demarcated the general Akt inhibitors and natural agents into Akt isoform-specific inhibitors based on the docking score. It can be helpful in precise targeting of involved-isoform instead of in general targeting.

4.2. FUTURE PROSPECTS

There were certain limitations in the present study. We analyzed the expression of Akt isoforms in TMA slides which were procured from the US and lacks the cancer tissues originating from the Indian population. Therefore, it becomes imperative to examine the same in the Indian population in near future. Also, in the present study genetic alterations data of Akt isoforms were obtained from TCGA portal and their correlation with OS and DFS was made. However, these data could not be retrieved from the local population of cancer patients and warrants further investigation. Next, we tried to establish the role of

Akt isoforms in SAS cells by the application of siRNAs. The effect of knockdown of Akt1 and 2 was analyzed on the different process of oral carcinogenesis such as cell proliferation and survival, migration and apoptosis. The effect of silencing of these genes was studied on different molecular mediators involved in the pathogenesis of oral cancer. Though our study was based on SAS cells only and other cell lines must be included for more elaborate understanding. Also, the *in vitro* study must be extended further to *in vivo* stage to validate the obtained results. Later, we tried to examine the effect of pure TE and other commercially available tobacco components on OSCC cells in relation to Akt isoforms. We observed that the tobacco and its components such as nicotine and BAP increased the aggressiveness in terms of clonogenicity and migration. Also, it was found that these tobacco components induced the expression of Akt1 and 2 isoforms and by silencing these genes the increased aggressiveness can be reduced. This indicates the association of Akt1 and 2 in tobacco-induced oral carcinogenesis. However, the detailed mechanism was missing in the present study and future experiments need to be designed and accomplished to understand the associations between Akt isoforms and tobacco-induced oral neoplasia. Several lines of evidence have suggested that the Akt kinase is involved in chemoresistance of cancer cells including oral cancer. Although, in our study, such aspects of research was absent and need to be analyzed in near future. Additionally, if gene microarray analysis of Akt-isoform-knockdown cells can be performed then the thorough understanding of the complex network of genes involved in tumorigenesis can be identified. Further, an attempt was made to demarcate the general Akt kinase inhibitors into Akt isoform-specific inhibitors using Schrodinger program. These preliminary results must be validated by further preclinical and clinical studies.

BIBLIOGRAPHY

Abdullah LN, Chow EK. Mechanisms of chemoresistance in cancer stem cells. Clinical and translational medicine. 2013 Dec 1;2(1):3.

Agarwal E, Robb CM, Smith LM, Brattain MG, Wang J, Black JD, Chowdhury S. Role of Akt2 in regulation of metastasis suppressor 1 expression and colorectal cancer metastasis. Oncogene. 2017 Jun 1;36(22):3104-18.

Al-Saad S, Donnem T, Al-Shibli K, Persson M, Bremnes RM, Busund LT. Diverse prognostic roles of Akt isoforms, PTEN and PI3K in tumor epithelial cells and stromal compartment in non-small cell lung cancer. Anticancer research. 2009 Oct 1;29(10):4175-83.

Altomare DA, Guo K, Cheng JQ, Sonoda G, Walsh K, Testa JR. Cloning, chromosomal localization and expression analysis of the mouse Akt2 oncogene. Oncogene. 1995 Sep;11(6):1055-60.

Altomare DA, Testa JR. Perturbations of the AKT signaling pathway in human cancer. Oncogene. 2005 Nov;24(50):7455.

Alvi A. Cancer of the oral cavity. Cancer of the head and neck. 1996:321-60.

Amagasa T, Yamashiro M, Ishikawa H. Oral leukoplakia related to malignant transformation. Oral Science International. 2006 Nov 1;3(2):45-55.

Andersen J, Ringsted KB, Bang-Andersen B, Strømgaard K, Kristensen AS. Binding site residues control inhibitor selectivity in the human norepinephrine transporter but not in the human dopamine transporter. Scientific Reports. 2015 Oct 27;5:15650.

Arboleda MJ, Lyons JF, Kabbinavar FF, Bray MR, Snow BE, Ayala R, Danino M, Karlan BY, Slamon DJ. Overexpression of AKT2/protein kinase Bβ leads to up-regulation of β1 integrins, increased invasion, and metastasis of human breast and ovarian cancer cells. Cancer research. 2003 Jan 1;63(1):196-206.

Arnedos M, Scott V, Job B, De La Cruz J, Commo F, Mathieu MC, Wolp-Diniz R, Richon C, Campone M, Bachelot T, Dalenc F. Array CGH and PIK3CA/AKT1 mutations to drive patients to specific targeted agents: a clinical experience in 108 patients with metastatic breast cancer. European Journal of Cancer. 2012 Oct 1;48(15):2293-9.

Arredondo J, Chernyavsky AI, Grando SA. Nicotinic receptors mediate tumorigenic action of tobacco-derived nitrosamines on immortalized oral epithelial cells. Cancer biology & therapy. 2006 May 1;5(5):511-7

Askham JM, Platt F, Chambers PA, Snowden H, Taylor CF, Knowles MA. AKT1 mutations in bladder cancer: identification of a novel oncogenic mutation that can co-operate with E17K. Oncogene. 2010 Jan 7;29(1):150.

Awwad HK, Lotayef M, Shouman T, Begg AC, Wilson G, Bentzen SM, El-Moneim HA, Eissa S. Accelerated hyperfractionation (AHF) compared to conventional fractionation (CF) in the postoperative radiotherapy of locally advanced head and neck cancer: influence of proliferation. British journal of cancer. 2002 Feb 12;86(4):517.

Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, Bouvard V, Altieri A, Cogliano V, 2007, WHO International Agency for Research on Cancer Monograph Working Group. Carcinogenicity of alcoholic beverages.

Bacus SS, Altomare DA, Lyass L, Chin DM, Farrell MP, Gurova K, Gudkov A, Testa JR. AKT2 is frequently upregulated in HER-2/neu-positive breast cancers and may contribute to tumor aggressiveness by enhancing cell survival. Oncogene. 2002 May;21(22):3532.

Bagan JV, Scully C. Recent advances in Oral Oncology 2007: epidemiology, aetiopathogenesis, diagnosis and prognostication. Oral oncology. 2008 Feb 1;44(2):103-8.

Bairoch A, Apweiler R, Wu CH, Barker WC, Boeckmann B, Ferro S, Gasteiger E, Huang H, Lopez R, Magrane M, Martin MJ. The universal protein resource (UniProt). Nucleic acids research. 2005 Jan 1;33(suppl_1):D154-9.

Banno E, Togashi Y, de Velasco MA, Mizukami T, Nakamura Y, Terashima M, Sakai K, Fujita Y, Kamata K, Kitano M, Kudo M. Clinical significance of Akt2 in advanced pancreatic cancer treated with erlotinib. International Journal of Oncology. 2017 Jun 1;50(6):2049-58.

Barkley LR, Santocanale C. MicroRNA-29a regulates the benzo [a] pyrene dihydrodiol epoxide-induced DNA damage response through Cdc7 kinase in lung cancer cells. Oncogenesis. 2013 Jul 1;2(7):e57.

Bell RB, Kademani D, Homer L, Dierks EJ, Potter BE. Tongue cancer: is there a difference in survival compared with other subsites in the oral cavity?. Journal of oral and maxillofacial surgery. 2007 Feb 1;65(2):229-36.

Bellacosa A, Kumar CC, Di Cristofano A, Testa JR. Activation of AKT kinases in cancer: implications for therapeutic targeting. Advances in cancer research. 2005 Dec 31;94:29-86.

Bellacosa A, Staal SP, Tsichlis PN. A retroviral oncogene, akt, encoding a serine-threonine kinase containing an SH2-like region. Science. 1991 Oct 11;254(5029):274-7.

Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL. Nucleic Acids Res. GenBank. 2005;33:D34-8.

Berman HM, Battistuz T, Bhat TN, Bluhm WF, Bourne PE, Burkhardt K, Feng Z, Gilliland GL, Iype L, Jain S, Fagan P. The protein data bank. Acta Crystallographica Section D: Biological Crystallography. 2002 Jun 1;58(6):899-907.

Bernal-Mizrachi E, Wen W, Stahlhut S, Welling CM, Permutt MA. Islet β cell expression of constitutively active Akt1/PKBα induces striking hypertrophy, hyperplasia, and hyperinsulinemia. Journal of Clinical Investigation. 2001 Dec 1;108(11):1631.

Bhutani J, Sheikh A, Niazi AK. Akt inhibitors: mechanism of action and implications for anticancer therapeutics. Infectious agents and cancer. 2013 Dec;8(1):49.

Bishop JM. Molecular themes in oncogenesis. Cell. 1991 Jan 25;64(2):235-48.

Bitran JD, Samuels B, Klein L, Hanauer S, Johnson L, Martinec J, Harris E, Kempler J, White W. Tandem high-dose chemotherapy supported by hematopoietic progenitor cells yields prolonged survival in stage IV breast cancer. Bone marrow transplantation. 1996 Feb;17(2):157-62.

Bleeker FE, Felicioni L, Buttitta F, Lamba S, Cardone L, Rodolfo M, Scarpa A, Leenstra S, Frattini M, Barbareschi M, Del Grammastro M. AKT1 E17K in human solid tumours. Oncogene. 2008 Sep;27(42):5648.

Blot WJ, McLaughlin JK, Winn DM, Austin DF, Greenberg RS, Preston-Martin S, Bernstein L, Schoenberg JB, Stemhagen A, Fraumeni JF. Smoking and drinking in relation to oral and pharyngeal cancer. Cancer research. 1988 Jun 1;48(11):3282-7.

Boffetta P, Hashibe M. Alcohol and cancer. The lancet oncology. 2006 Feb 1;7(2):149-56.

Boormans JL, ermans KG, van Leenders GJ, Trapman J, Verhagen PC. An activating mutation in AKT1 in human prostate cancer. International journal of cancer. 2008 Dec 1;123(11):2725-6.

Boormans JL, Korsten H, Ziel-Van Der Made AC, Van Leenders GJ, Verhagen PC, Trapman J. E17K substitution in AKT1 in prostate cancer. British journal of cancer. 2010 May;102(10):1491.

Boufraqech M, Zhang L, Jain M, Patel D, Ellis R, Xiong Y, He M, Nilubol N, Merino MJ, Kebebew E. miR-145 suppresses thyroid cancer growth and metastasis and targets AKT3. Endocrine-related cancer. 2014 Aug 1;21(4):517-31.

Bouquot JE, Speight PM, Farthing PM. Epithelial dysplasia of the oral mucosa—Diagnostic problems and prognostic features. Current Diagnostic Pathology. 2006 Feb 1;12(1):11-21.

Bouzakri K, Zachrisson A, Al-Khalili L, Zhang BB, Koistinen HA, Krook A, Zierath JR. siRNA-based gene silencing reveals specialized roles of IRS-1/Akt2 and IRS-2/Akt1 in glucose and lipid metabolism in human skeletal muscle. Cell metabolism. 2006 Jul 1;4(1):89-96.

Braakhuis BJ, Bloemena E, Leemans CR, Brakenhoff RH. Molecular analysis of surgical margins in head and neck cancer: more than a marginal issue. Oral oncology. 2010 Jul 1;46(7):485-91.

Braakhuis BJ, Tabor MP, René Leemans C, van der Waal I, Snow GB, Brakenhoff RH. Second primary tumors and field cancerization in oral and oropharyngeal cancer: molecular techniques provide new insights and definitions. Head & neck. 2002 Feb 1;24(2):198-206.

Brait M, Munari E, LeBron C, Noordhuis MG, Begum S, Michailidi C, Gonzalez-Roibon N, Maldonado L, Sen T, Guerrero-Preston R, Cope L. Genome-wide methylation profiling and the PI3K-AKT pathway analysis associated with smoking in urothelial cell carcinoma. Cell Cycle. 2013 Apr 1;12(7):1058-70.

Brennan JA, Boyle JO, Koch WM, Goodman SN, Hruban RH, Eby YJ, Couch MJ, Forastiere AA, Sidransky D. Association between cigarette smoking and mutation of the p53 gene in squamous-cell carcinoma of the head and neck. New England Journal of Medicine. 1995 Mar 16;332(11):712-7.

Brodbeck D, Cron P, Hemmings BA. A human protein kinase Bγ with regulatory phosphorylation sites in the activation loop and in the C-terminal hydrophobic domain. Journal of Biological Chemistry. 1999 Apr 2;274(14):9133-6.

Budach W, Hehr T, Budach V, Belka C, Dietz K. A meta-analysis of hyperfractionated and accelerated radiotherapy and combined chemotherapy and radiotherapy regimens in unresected locally advanced squamous cell carcinoma of the head and neck. BMC cancer. 2006 Dec;6(1):28.

Bulj Z, Duchi S, Bevilacqua A, Gherardi A, Dozza B, Piccinini F, Adalgisa Mariani G, Lucarelli E, Giannini S, Donati D, Marmiroli S. Protein kinase B/AKT isoform 2 drives migration of human mesenchymal stem cells. International journal of oncology. 2013 Jan 1;42(1):118-26

Burdick AD, Davis JW 2nd, Liu KJ, Hudson LG, Shi H, Monske ML, Burchiel SW. Benzo(a)pyrene quinones increase cell proliferation, generate reactive oxygen species, and transactivate the epidermal growth factor receptor in breast epithelial cells. Cancer Res. 2003 Nov 15;63(22):7825-33.

Cai N, Wang YD, Zheng PS. The microRNA-302-367 cluster suppresses the proliferation of cervical carcinoma cells through the novel target AKT1. Rna. 2013 Jan 1;19(1):85-95.

Cairns J, Fridley BL, Jenkins GD, Zhuang Y, Yu J, Wang L. Differential roles of ERRFI1 in EGFR and AKT pathway regulation affect cancer proliferation. EMBO reports. 2018 Jan 15:e44767.

Calamito M, Juntilla MM, Thomas M, Northrup DL, Rathmell J, Birnbaum MJ, Koretzky G, Allman D. Akt1 and Akt2 promote peripheral B-cell maturation and survival. Blood. 2010 May 20;115(20):4043-50.

Califano J, Van Der Riet P, Westra W, Nawroz H, Clayman G, Piantadosi S, Corio R, Lee D, Greenberg B, Koch W, Sidransky D. Genetic progression model for head and neck cancer: implications for field cancerization. Cancer research. 1996 Jun 1;56(11):2488-92.

Carpten JD, Faber AL, Horn C, Donoho GP, Briggs SL, Robbins CM, Hostetter G, Boguslawski S, Moses TY, Savage S, Uhlik M. A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. Nature. 2007 Jul;448(7152):439.

Castellano E, Santos E. Functional specificity of ras isoforms: so similar but so different. Genes & cancer. 2011 Mar;2(3):216-31.

Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, Antipin Y. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data 2012.

Chang F, Lee JT, Navolanic PM, Steelman LS, Shelton JG, Blalock WL, Franklin RA, McCubrey JA. Involvement of PI3K/Akt pathway in cell cycle progression, apoptosis, and neoplastic transformation: a target for cancer chemotherapy. Leukemia. 2003 Mar;17(3):590.

Chen W, Bai L, Wang X, Xu S, Belinsky SA, Lin Y. Acquired activation of the Akt/cyclooxygenase-2/Mcl-1 pathway renders lung cancer cells resistant to apoptosis. Molecular pharmacology. 2010 Mar 1;77(3):416-23.

Chen X, Duan N, Zhang C, Zhang W. Survivin and tumorigenesis: molecular mechanisms and therapeutic strategies. Journal of Cancer. 2016;7(3):314.

Chen XN, Wang KF, Xu ZQ, Li SJ, Liu Q, Fu DH, Wang X, Wu B. MiR-133b regulates bladder cancer cell proliferation and apoptosis by targeting Bcl-w and Akt1. Cancer cell international. 2014 Dec;14(1):70.

Chen Y, He Y, Zhang S, Li L, Zhu X, Liu Y. The expression of oncogene AKT2 in laryngeal squamous cell carcinoma and its clinical significance. Lin chuang er bi yan hou tou jing wai ke za zhi= Journal of clinical otorhinolaryngology, head, and neck surgery. 2009 Jun;23(12):539-41.

Cheng JQ, Godwin AK, Bellacosa A, Taguchi T, Franke TF, Hamilton TC, Tsichlis PN, Testa JR. AKT2, a putative oncogene encoding a member of a subfamily of protein-serine/threonine kinases, is amplified in human ovarian carcinomas. Proceedings of the National Academy of Sciences. 1992 Oct 1;89(19):9267-71.

Cheng JQ, Ruggeri B, Klein WM, Sonoda G, Altomare DA, Watson DK, Testa JR. Amplification of AKT2 in human pancreatic cells and inhibition of AKT2 expression and tumorigenicity by antisense RNA. Proceedings of the National Academy of Sciences. 1996 Apr 16;93(8):3636-41.

Cheng YW, Wu MF, Wang J, Yeh KT, Goan YG, Chiou HL, Chen CY, Lee H. Human papillomavirus 16/18 E6 oncoprotein is expressed in lung cancer and related with p53 inactivation. Cancer Research. 2007 Nov 15;67(22):10686-93.

Cheung M, Testa JR. AKT3 (v-akt murine thymoma viral oncogene homolog 3, Protein Kinase B gamma) 2008.

Chiba I. Prevention of betel quid chewers' oral cancer in the Asian-Pacific area. Asian Pac J Cancer Prev. 2001;2(4):263-9.

Chin YR, Toker A. Akt2 regulates expression of the actin-bundling protein palladin. FEBS letters. 2010 Dec 1;584(23):4769-74.

Chin[a] YR, Yoshida T, Marusyk A, Beck AH, Polyak K, Toker A. Targeting Akt3 signaling in triple-negative breast cancer. Cancer research. 2014 Feb 1;74(3):964-73.

Chin[b] YR, Yuan X, Balk SP, Toker A. PTEN-deficient tumors depend on AKT2 for maintenance and survival. Cancer discovery. 2014 Aug 1;4(8):942-55.

Choi S, Myers JN. Molecular pathogenesis of oral squamous cell carcinoma: implications for therapy. Journal of dental research. 2008 Jan;87(1):14-32.

Chuthapisith S. Cancer stem cells and chemoresistance. InCancer Stem Cells Theories and Practice 2011. InTech.

COFFER PJ, WOODGETT JR. Molecular cloning and characterisation of a novel putative protein-serine kinase related to the cAMP-dependent and protein kinase C families. The FEBS Journal. 1991 Oct 1;201(2):475-81.

Cristiano BE, Chan JC, Hannan KM, Lundie NA, Marmy-Conus NJ, Campbell IG, Phillips WA, Robbie M, Hannan RD, Pearson RB. A specific role for AKT3 in the genesis of ovarian cancer through modulation of G2-M phase transition. Cancer Research. 2006 Dec 15;66(24):11718-25.

Cui Y, Wang Q, Wang J, Dong Y, Luo C, Hu G, Lu Y. Knockdown of AKT2 expression by RNA interference inhibits proliferation, enhances apoptosis, and increases

chemosensitivity to the anticancer drug VM-26 in U87 glioma cells. Brain research. 2012 Aug 21;1469:1-9.

Das BR, Nagpal JK. Understanding the biology of oral cancer. Medical Science Monitor. 2002 Nov 21;8(11):RA258-67.

Davies MA, Stemke-Hale K, Tellez C, Calderone TL, Deng W, Prieto VG, Lazar AJ, Gershenwald JE, Mills GB. A novel AKT3 mutation in melanoma tumours and cell lines. British journal of cancer. 2008 Oct;99(8):1265.

Davis C, Bhana S, Shorrocks AJ, Martin FL. Oestrogens induce G(1) arrest in benzo[a]pyrene-treated MCF-7 breast cells whilst enhancing genotoxicity and clonogenic survival. Mutagenesis. 2002 Sep;17(5):431-8.

De Vries N, Van der Waal I, Snow GB. Multiple primary tumours in oral cancer. International journal of oral and maxillofacial surgery. 1986 Feb 1;15(1):85-7.

DeBosch B, Treskov I, Lupu TS, Weinheimer C, Kovacs A, Courtois M, Muslin AJ. Akt1 is required for physiological cardiac growth. Circulation. 2006 May 2;113(17):2097-104.

Deyle KM, Farrow B, Hee YQ, Work J, Wong M, Lai B, Umeda A, Millward SW, Nag A, Das S, Heath JR. A protein-targeting strategy used to develop a selective inhibitor of the E17K point mutation in the PH domain of Akt1. Nature chemistry. 2015 May 1;7(5):455-62.

Di Cello F, Flowers VL, Li H, Vecchio-Pagán B, Gordon B, Harbom K, Shin J, Beaty R, Wang W, Brayton C, Baylin SB. Cigarette smoke induces epithelial to mesenchymal transition and increases the metastatic ability of breast cancer cells. Molecular cancer. 2013 Aug 6;12(1):90.

Di Lorenzo A, Fernández-Hernando C, Cirino G, Sessa WC. Akt1 is critical for acute inflammation and histamine-mediated vascular leakage. Proceedings of the National Academy of Sciences. 2009 Aug 25;106(34):14552-7.

Ding Z, Xu F, Li G, Tang J, Tang Z, Jiang P, Wu H. Knockdown of Akt2 expression by shRNA inhibits proliferation, enhances apoptosis, and increases chemosensitivity to paclitaxel in human colorectal cancer cells. Cell biochemistry and biophysics. 2015 Jan 1;71(1):383-8.

DiPaola RS. To arrest or not to G2-M Cell-cycle arrest: commentary re: AK Tyagi et al., Silibinin strongly synergizes human prostate carcinoma DU145 cells to doxorubicin-induced growth inhibition, G2-M arrest, and apoptosis. Clin. Cancer Res., 8: 3512–3519, 2002. Clinical cancer research. 2002 Nov 1;8(11):3311-4.

Do H, Salemi R, Murone C, Mitchell PL, Dobrovic A. Rarity of AKT1 and AKT3 E17K mutations in squamous cell carcinoma of lung. Cell cycle. 2010 Nov 1;9(21):4411-2.

Do H, Solomon B, Mitchell PL, Fox SB, Dobrovic A. Detection of the transforming AKT1 mutation E17K in non-small cell lung cancer by high resolution melting. BMC research notes. 2008 Dec;1(1):14.

Dobashi Y, Kimura M, Matsubara H, Endo S, Inazawa J, Ooi A. Molecular alterations in AKT and its protein activation in human lung carcinomas. Human pathology. 2012 Dec 1;43(12):2229-40.

Dose AM. The symptom experience of mucositis, stomatitis, and xerostomia. InSeminars in oncology nursing 1995 Nov 1 (Vol. 11, No. 4, pp. 248-255). WB Saunders.

Dost F, Le Cao K, Ford PJ, Ades C, Farah CS. Malignant transformation of oral epithelial dysplasia: a real-world evaluation of histopathologic grading. Oral surgery, oral medicine, oral pathology and oral radiology. 2014 Mar 1;117(3):343-52.

Dummler B, Tschopp O, Hynx D, Yang ZZ, Dirnhofer S, Hemmings BA. Life with a single isoform of Akt: mice lacking Akt2 and Akt3 are viable but display impaired glucose homeostasis and growth deficiencies. Molecular and cellular biology. 2006 Nov 1;26(21):8042-51.

Easton RM, Cho H, Roovers K, Shineman DW, Mizrahi M, Forman MS, Lee VM, Szabolcs M, de Jong R, Oltersdorf T, Ludwig T. Role for Akt3/protein kinase Bγ in attainment of normal brain size. Molecular and cellular biology. 2005 Mar 1;25(5):1869-78.

Ehsan S, Ball G, Choke E, Molyneux KM, London NJ, Herbert KE, Barratt J, Sayers RD, Bown MJ. Disease specific biomarkers of abdominal aortic aneurysms detected by surface enhanced laser desorption ionization time of flight mass spectrometry. European Journal of Vascular and Endovascular Surgery. 2012 Jul 1;44(1):52-4.

Eke I, Koch U, Hehlgans S, Sandfort V, Stanchi F, Zips D, Baumann M, Shevchenko A, Pilarsky C, Haase M, Baretton GB. PINCH1 regulates Akt1 activation and enhances radioresistance by inhibiting PP1α. The Journal of clinical investigation. 2010 Jul 1;120(7):2516-27.

Elango JK, Sundaram KR, Gangadharan P, Subhas P, Peter S, Pulayath C, Kuriakose MA. Factors affecting oral cancer awareness in a high-risk population in India. Asian Pac J Cancer Prev. 2009 Oct-Dec;10(4):627-30.

Endersby R, Zhu X, Hay N, Ellison DW, Baker SJ. Nonredundant functions for Akt isoforms in astrocyte growth and gliomagenesis in an orthotopic transplantation model. Cancer research. 2011 Jun 15;71(12):4106-16.

Ericson K, Gan C, Cheong I, Rago C, Samuels Y, Velculescu VE, Kinzler KW, Huso DL, Vogelstein B, Papadopoulos N. Genetic inactivation of AKT1, AKT2, and PDPK1 in human colorectal cancer cells clarifies their roles in tumor growth regulation. Proceedings of the National Academy of Sciences. 2010 Feb 9;107(6):2598-603.

Eswar N, John B, Mirkovic N, Fiser A, Ilyin VA, Pieper U, Stuart AC, Marti-Renom MA, Madhusudhan MS, Yerkovich B, Sali A. Tools for comparative protein structure modeling and analysis. Nucleic acids research. 2003 Jul 1;31(13):3375-80.

Fang M, Zhang W, Chen Y, He Z. Malignant transformation of oral lichen planus: A retrospective study of 23 cases. Quintessence International. 2009 Mar 1;40(3).

Fohlin H, Pérez-Tenorio G, Fornander T, Skoog L, Nordenskjöld B, Carstensen J, Stål O. Akt2 expression is associated with good long-term prognosis in oestrogen receptor positive breast cancer. European Journal of Cancer. 2013 Apr 1;49(6):1196-204.

Foley NH, Bray IM, Tivnan A, Bryan K, Murphy DM, Buckley PG, Ryan J, O'Meara A, O'Sullivan M, Stallings RL. MicroRNA-184 inhibits neuroblastoma cell survival through targeting the serine/threonine kinase AKT2. Molecular cancer. 2010 Dec;9(1):83.

Fong D, Spizzo G, Gostner JM, Gastl G, Moser P, Krammel C, Gerhard S, Rasse M, Laimer K. TROP2: a novel prognostic marker in squamous cell carcinoma of the oral cavity. Modern pathology. 2008 Feb 1;21(2):186-91.

Fotedar V, Fotedar S, Seam RK, Gupta MK. Oral cancer and chemoprevention. Int Jof Pharmaceutical Science Invention. 2013 Feb;2(2):16-20.

Franceschi S, Bidoli E, Herrero R, Munoz N. Comparison of cancers of the oral cavity and pharynx worldwide: etiological clues. Oral oncology. 2000 Jan 1;36(1):106-15.

Franken NA, Rodermond HM, Stap J, Haveman J, Van Bree C. Clonogenic assay of cells in vitro. Nature protocols. 2006 Dec;1(5):2315.

Frazzetto M, Suphioglu C, Zhu J, Schmidt-Kittler O, Jennings IG, Cranmer SL, Jackson SP, Kinzler KW, Vogelstein B, Thompson PE. Dissecting isoform selectivity of PI3K inhibitors: the role of non-conserved residues in the catalytic pocket. Biochemical journal. 2008 Sep 15;414(3):383-90.

Friesner RA, Murphy RB, Repasky MP, Frye LL, Greenwood JR, Halgren TA, Sanschagrin PC, Mainz DT. Extra precision glide: Docking and scoring incorporating a model of hydrophobic enclosure for protein− ligand complexes. Journal of medicinal chemistry. 2006 Oct 19;49(21):6177-96.

Fu M, Wang C, Li Z, Sakamaki T, Pestell RG. Minireview: Cyclin D1: normal and abnormal functions. Endocrinology. 2004 Dec 1;145(12):5439-47.

Gagnon V, Mathieu I, Sexton É, Leblanc K, Asselin E. AKT involvement in cisplatin chemoresistance of human uterine cancer cells. Gynecologic oncology. 2004 Sep 1;94(3):785-95.

Gallo O, Bianchi S. p53 expression: a potential biomarker for risk of multiple primary malignancies in the upper aerodigestive tract. European Journal of Cancer Part B: Oral Oncology. 1995 Jan 1;31(1):53-7.

Gantner BN, Jin H, Qian F, Hay N, He B, Richard DY. The Akt1 isoform is required for optimal IFN-β transcription through direct phosphorylation of β-catenin. The Journal of Immunology. 2012 Sep 15;189(6):3104-11.

Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, Cerami E. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Science signaling. 2013 Apr 2;6(269):pl1.

Geurink KV. Community Oral Health Practice for the Dental Hygienist-E-Book. Elsevier Health Sciences; 2014 Apr 14.

Gillenwater A, Papadimitrakopoulou V, Richards-Kortum R. Oral premalignancy: new methods of detection and treatment. Current oncology reports. 2006 Apr 1;8(2):146-54.

Girouard J, Lafleur MJ, Parent S, Leblanc V, Asselin E. Involvement of Akt isoforms in chemoresistance of endometrial carcinoma cells. Gynecologic oncology. 2013 Feb 1;128(2):335-43.

Giudice FS, Squarize CH. The determinants of head and neck cancer: Unmasking the PI3K pathway mutations. Journal of carcinogenesis & mutagenesis. 2013 Aug 2.
Goldenberg D, Harden S, Masayesva BG, Ha P, Benoit N, Westra WH, Koch WM, Sidransky D, Califano JA. Intraoperative molecular margin analysis in head and neck cancer. Archives of otolaryngology–head & neck surgery. 2004 Jan 1;130(1):39-44.

Gonzalez E, McGraw TE. The Akt kinases: isoform specificity in metabolism and cancer. Cell cycle. 2009 Aug 15;8(16):2502-8.

Grabinski N, Bartkowiak K, Grupp K, Brandt B, Pantel K, Jücker M. Distinct functional roles of Akt isoforms for proliferation, survival, migration and EGF-mediated signalling in lung cancer derived disseminated tumor cells. Cellular signalling. 2011 Dec 1;23(12):1952-60.

Grando SA. Connections of nicotine to cancer. Nature Reviews Cancer. 2014 Jun 1;14(6):419-29.

Green BD, Jabbour AM, Sandow JJ, Riffkin CD, Masouras D, Daunt CP, Salmanidis M, Brumatti G, Hemmings BA, Guthridge MA, Pearson RB. Akt1 is the principal Akt

isoform regulating apoptosis in limiting cytokine concentrations. Cell death and differentiation. 2013 Oct;20(10):1341.

Green BD, Jabbour AM, Sandow JJ, Riffkin CD, Masouras D, Daunt CP, Salmanidis M, Brumatti G, Hemmings BA, Guthridge MA, Pearson RB. Akt1 is the principal Akt isoform regulating apoptosis in limiting cytokine concentrations. Cell death and differentiation. 2013 Oct;20(10):1341.

Gupta PC, Bhonsle RB, Murti PR, Daftary DK, Mehta FS, Pindborg JJ. An epidemiologic assessment of cancer risk in oral precancerous lesions in India with special reference to nodular leukoplakia. Cancer. 1989 Jun 1;63(11):2247-52.

Häggblad Sahlberg S, Mortensen AC, Haglöf J, Engskog MK, Arvidsson T, Pettersson C, Glimelius B, Stenerlöw B, Nestor M. Different functions of AKT1 and AKT2 in molecular pathways, cell migration and metabolism in colon cancer cells. International journal of oncology. 2017 Jan 1;50(1):5-14.

Hamakawa H, Nakashiro KI, Sumida T, Shintani S, Myers JN, Takes RP, Rinaldo A, Ferlito A. Basic evidence of molecular targeted therapy for oral cancer and salivary gland cancer. Head & neck. 2008 Jun 1;30(6):800-9.

Han CP, Kok LF, Wang PH, Wu TS, Tyan YS, Cheng YW, Lee MY, Yang SF. Scoring of p16 INK4a immunohistochemistry based on independent nuclear staining alone can sufficiently distinguish between endocervical and endometrial adenocarcinomas in a tissue microarray study. Modern Pathology. 2009 Jun;22(6):797.

Han CP, Lee MY, Tzeng SL, Yao CC, Wang PH, Cheng YW, Chen SL, Wu TS, Tyan YS, Kok LF. Nuclear Receptor Interaction Protein (NRIP) expression assay using human tissue microarray and immunohistochemistry technology confirming nuclear localization. Journal of Experimental & Clinical Cancer Research. 2008 Aug;27(1):25.

Hao W, Hu Y, Niu C, Huang X, Chang CP, Gibbons J, Xu J. Discovery of the catechol structural moiety as a Stat3 SH2 domain inhibitor by virtual screening. Bioorganic & medicinal chemistry letters. 2008 Sep 15;18(18):4988-92.

Hara S, Nakashiro KI, Goda H, Hamakawa H. Role of Akt isoforms in HGF-induced invasive growth of human salivary gland cancer cells. Biochemical and biophysical research communications. 2008 May 23;370(1):123-8.

Hartnett JC, Barnett SF, Bilodeau MT, Defeo-Jones D, Hartman GD, Huber HE, Jones RE, Kral AM, Robinson RG, Wu Z. Optimization of 2, 3, 5-trisubstituted pyridine derivatives as potent allosteric Akt1 and Akt2 inhibitors. Bioorganic & medicinal chemistry letters. 2008 Mar 15;18(6):2194-7.

Hashibe M, Brennan P, Chuang SC, Boccia S, Castellsague X, Chen C, Curado MP, Dal Maso L, Daudt AW, Fabianova E, Fernandez L. Interaction between tobacco and alcohol

use and the risk of head and neck cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. Cancer Epidemiology and Prevention Biomarkers. 2009 Feb 1;18(2):541-50.

Hemmings BA, Restuccia DF. Pi3k-pkb/akt pathway. Cold Spring Harbor perspectives in biology. 2012 Sep 1;4(9):a011189.

Herrero R, Castellsagué X, Pawlita M, Lissowska J, Kee F, Balaram P, Rajkumar T, Sridhar H, Rose B, Pintos J, Fernández L. Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. Journal of the National Cancer Institute. 2003 Dec 3;95(23):1772-83.

Hewitt R, Forero A, Luncsford PJ, Martin FL. Enhanced micronucleus formation and modulation of BCL-2: BAX in MCF-7 cells after exposure to binary mixtures. Environmental health perspectives. 2007 Dec;115(Suppl 1):129.

Hittelman WN, Liao Y, Wang L, Milas L. Are cancer stem cells radioresistant?. Future Oncology. 2010 Oct;6(10):1563-76.

Ho PS, Chen PL, Warnakulasuriya S, Shieh TY, Chen YK, Huang IY. Malignant transformation of oral potentially malignant disorders in males: a retrospective cohort study. Bmc Cancer. 2009 Dec;9(1):260.

Hollander MC, Maier CR, Hobbs EA, Ashmore AR, Linnoila RI, Dennis PA. Akt1 deletion prevents lung tumorigenesis by mutant K-ras. Oncogene. 2011[a] Apr;30(15):1812.

Hollander MC, Zhou X, Maier CR, Patterson AD, Ding X, Dennis PA. A Cyp2a polymorphism predicts susceptibility to NNK-induced lung tumorigenesis in mice. Carcinogenesis. 2011[b] May 30:bgr097.

How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease. A Report of the Surgeon General 2010 U.S. Dept. of Health and Human Services. Public Health Service Office of the Surgeon General Rockville, MD.

Huang SH. Oral cancer: Current role of radiotherapy and chemotherapy. Medicina oral, patologia oral y cirugia bucal. 2013 Mar;18(2):e233.

Huang X, Begley M, Morgenstern KA, Gu Y, Rose P, Zhao H, Zhu X. Crystal structure of an inactive Akt2 kinase domain. Structure. 2003 Jan 31;11(1):21-30.

Huang X, Beullens M, Zhang J, Zhou Y, Nicolaescu E, Lesage B, Hu Q, Wu J, Bollen M, Shi Y. Structure and function of the two tandem WW domains of the pre-mRNA splicing factor FBP21 (formin-binding protein 21). Journal of Biological Chemistry. 2009 Sep 11;284(37):25375-87.

Hung CS, Peng YJ, Wei PL, Lee CH, Su HY, Ho YS, Lin SY, Wu CH, Chang YJ. The alpha9 nicotinic acetylcholine receptor is the key mediator in nicotine-enhanced cancer metastasis in breast cancer cells. Journal of Experimental & Clinical Medicine. 2011 Dec 31;3(6):283-92.

Iamaroon A, Krisanaprakornkit S. Overexpression and activation of Akt2 protein in oral squamous cell carcinoma. Oral oncology. 2009 Oct 31;45(10):e175-9.

Indian Council of Medical Research. 2009.

Islam MR, Ellis IR, Macluskey M, Cochrane L, Jones SJ. Activation of Akt at T308 and S473 in alcohol, tobacco and HPV-induced HNSCC: is there evidence to support a prognostic or diagnostic role?. Experimental hematology & oncology. 2014 Oct 17;3(1):25.

Jain P, Kumar P, Pai VR, Parikh PM. Neoadjuvant chemotherapy or chemoradiotherapy in head and neck cancer. Indian journal of cancer. 2008 Jul 1;45(3):83.

Jane C, Nerurkar AV, Karjodkar FR. Circulating Immune complexes (CIC) as marker for disease progress in oral cancer. Indian Journal of Clinical Biochemistry. 2007 Sep 1;22(2):114-7.

Jeng JH, Chang MC, Hahn LJ. Role of areca nut in betel quid-associated chemical carcinogenesis: current awareness and future perspectives. Oral oncology. 2001 Sep 1;37(6):477-92.

Jenkins DJ, Popovich DG, Kendall CW, Vidgen E, Tariq N, Ransom TP, Wolever TM, Vuksan V, Mehling CC, Boctor DL, Bolognesi C. Effect of a diet high in vegetables, fruit, and nuts on serum lipids. Metabolism. 1997 May 1;46(5):530-7.

Jensen PJ, Gunter LB, Carayannopoulos MO. Akt2 modulates glucose availability and downstream apoptotic pathways during development. Journal of Biological Chemistry. 2010 Jun 4;285(23):17673-80.

Jones PF, Jakubowicz T, Pitossi FJ, Maurer F, Hemmings BA. Molecular cloning and identification of a serine/threonine protein kinase of the second-messenger subfamily. Proceedings of the National Academy of Sciences. 1991 May 15;88(10):4171-5.

Joy A, Kapoor M, Georges J, Butler L, Chang Y, Li C, Crouch A, Smirnov I, Nakada M, Hepler J, Marty M. The role of AKT isoforms in glioblastoma: AKT3 delays tumor progression. Journal of neuro-oncology. 2016 Oct 1;130(1):43-52.

Jung IH, Leem GL, Jung DE, Kim MH, Kim EY, Kim SH, Park HC, Park SW. Glioma is formed by active Akt1 alone and promoted by active Rac1 in transgenic zebrafish. Neuro-oncology. 2013 Jan 16;15(3):290-304.

Jung SH, Kim MS, Lee SH, Park HC, Choi HJ, Maeng L, Min KO, Kim J, Park TI, Shin OR, Kim TJ. Whole-exome sequencing identifies recurrent AKT1 mutations in sclerosing hemangioma of lung. Proceedings of the National Academy of Sciences. 2016 Sep 6:201606946.

Juntilla MM, Wofford JA, Birnbaum MJ, Rathmell JC, Koretzky GA. Akt1 and Akt2 are required for αβ thymocyte survival and differentiation. Proceedings of the National Academy of Sciences. 2007 Jul 17;104(29):12105-10.

Jurel SK, Gupta DS, Singh RD, Singh M, Srivastava S. Genes and oral cancer. Indian journal of human genetics. 2014 Jan;20(1):4.

Kamoi S, AlJuboury MI, Akin MR, Silverberg SG. Immunohistochemical staining in the distinction between primary endometrial and endocervical adenocarcinomas: another viewpoint. International journal of gynecological pathology. 2002 Jul 1;21(3):217-23.

Kandel ES, Hay N. The regulation and activities of the multifunctional serine/threonine kinase Akt/PKB. Experimental cell research. 1999 Nov 25;253(1):210-29.

Kandel ES, Skeen J, Majewski N, Di Cristofano A, Pandolfi PP, Feliciano CS, Gartel A, Hay N. Activation of Akt/protein kinase B overcomes a G2/M cell cycle checkpoint induced by DNA damage. Molecular and cellular biology. 2002 Nov 15;22(22):7831-41.

Karemore TV, Karemore VA. Etiopathogenesis and treatment strategies of oral submucous fibrosis. Journal of Indian Academy of Oral Medicine and Radiology. 2011 Oct 1;23(4):598.

KassimKH, Daley TD. Herpes simplex virus type 1 proteins in human oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol. 1988 Apr;65(4):445-8.

Khan Z, Tönnies J, Müller S. Smokeless tobacco and oral cancer in South Asia: a systematic review with meta-analysis. Journal of cancer epidemiology. 2014 Jul 6;2014.

Kim JK, Jeon HY, Kim H. The molecular mechanisms underlying the therapeutic resistance of cancer stem cells. Archives of pharmacal research. 2015 Mar 1;38(3):389-401.

Kirkegaard T, Witton CJ, Edwards J, Nielsen KV, Jensen LB, Campbell FM, Cooke TG, Bartlett J. Molecular alterations in AKT1, AKT2 and AKT3 detected in breast and prostatic cancer by FISH. Histopathology. 2010 Jan 1;56(2):203-11.

Kishor Roy N, Bordoloi D, Monisha J, Padmavathi G, Kotoky J, Golla R, B Kunnumakkara A. Specific targeting of Akt kinase isoforms: Taking the precise path for prevention and treatment of cancer. Current drug targets. 2017 Mar 1;18(4):421-35.

Kometani T, Yoshino I, Miura N, Okazaki H, Ohba T, Takenaka T, Shoji F, Yano T, Maehara Y. Benzo[a]pyrene promotes proliferation of human lung cancer cells by accelerating the epidermal growth factor receptor signaling pathway. Cancer Lett. 2009 Jun 8;278(1):27-33.

Kong KA, Lee JY, Oh JH, Lee Y, Kim MH. Akt1 mediates the posterior Hoxc gene expression through epigenetic modifications in mouse embryonic fibroblasts. Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms. 2014 Sep 30;1839(9):793-9.

Krebs BB, De Mesquita JF. Amyotrophic lateral sclerosis type 20-In Silico analysis and molecular dynamics simulation of hnRNPA1. PloS one. 2016 Jul 14;11(7):e0158939.

Krześlak A, Pomorski L, Lipińska A. Expression, localization, and phosphorylation of Akt1 in benign and malignant thyroid lesions. Endocrine pathology. 2011 Dec 1;22(4):206-11.

Kuck D, Singh N,Lyko F, Medina-Franco JL. Novel and selective DNA methyltransferase inhibitors: docking-based virtual screening and experimental evaluation. Bioorganic & medicinal chemistry. 2010 Jan 15;18(2):822-9.

Kwon O, Kim KA, He L, Jung M, Jeong SJ, Ahn JS, Kim BY. Complex formation of p65/RelA with nuclear Akt1 for enhanced transcriptional activation of NF-κB. Biochemical and biophysical research communications. 2008 Jan 25;365(4):771-6.

Kyung HY, Lauring J. Recurrent AKT mutations in human cancers: functional consequences and effects on drug sensitivity. Oncotarget. 2016 Jan 26;7(4):4241.

Lapthanasupkul P, Poomsawat S, Punyasingh J. A clinicopathologic study of oral leukoplakia and erythroplakia in a Thai population. Quintessence International. 2007 Sep 1;38(8).

LarondeDM(1), Hislop TG, Elwood JM, Rosin MP. Oral cancer: just the facts. J Can Dent Assoc. 2008 Apr;74(3):269-72.

Laskowski RA, MacArthur MW, Moss DS, Thornton JM. PROCHECK: a program to check the stereochemical quality of protein structures. Journal of applied crystallography. 1993 Apr 1;26(2):283-91.

Lazarus P, Sheikh SN, Ren Q, Schantz SP, Stern JC, Richie Jr JP, Park JY. p53, but not p16 mutations in oral squamous cell carcinomas are associated with specific CYP1A1 and GSTM1 polymorphic genotypes and patient tobacco use. Carcinogenesis. 1998 Mar 1;19(3):509-14.

Lee D, Do IG, Choi K, Sung CO, Jang KT, Choi D, Heo JS, Choi SH, Kim J, Park JY, Cha HJ. The expression of phospho-AKT1 and phospho-MTOR is associated with a favorable prognosis independent of PTEN expression in intrahepatic cholangiocarcinomas. Modern pathology. 2012 Jan;25(1):131.

Lee HJ, Guo HY, Lee SK, Jeon BH, Jun CD, Lee SK, Park MH, Kim EC. Effects of nicotine on proliferation, cell cycle, and differentiation in immortalized and malignant oral keratinocytes. J Oral Pathol Med. 2005 Aug;34(7):436-43.

Lee MW, Kim DS, Lee JH, Lee BS, Lee SH, Jung HL, Sung KW, Kim HT, Yoo KH, Koo HH. Roles of AKT1 and AKT2 in non-small cell lung cancer cell survival, growth, and migration. Cancer science. 2011 Oct 1;102(10):1822-8.

Lee MY, Luciano AK, Ackah E, Rodriguez-Vita J, Bancroft TA, Eichmann A, Simons M, Kyriakides TR, Morales-Ruiz M, Sessa WC. Endothelial Akt1 mediates angiogenesis by phosphorylating multiple angiogenic substrates. Proceedings of the National Academy of Sciences. 2014 Sep 2;111(35):12865-70.

Leemans CR, Braakhuis BJ, Brakenhoff RH. The molecular biology of head and neck cancer. Nature reviews cancer. 2011 Jan;11(1):9.

Levi F, Te VC, Randimbison L, Maspoli M, La Vecchia C. Second primary oral and pharyngeal cancers in subjects diagnosed with oral and pharyngeal cancer. International journal of cancer. 2006 Dec 1;119(11):2702-4.

Li B, Li J, Xu WW, Guan XY, Qin YR, Zhang LY, Law S, Tsao SW, Cheung AL. Suppression of esophageal tumor growth and chemoresistance by directly targeting the PI3K/AKT pathway. Oncotarget. 2014 Nov;5(22):11576.

Li J, Chen Y, Zhao J, Kong F, Zhang Y. miR-203 reverses chemoresistance in p53-mutated colon cancer cells through downregulation of Akt2 expression. Cancer letters. 2011 May 1;304(1):52-9.

Li J, Su W, Zhang S, Hu Y, Liu J, Zhang X, Bai J, Yuan W, Hu L, Cheng T, Zetterberg A. Epidermal growth factor receptor and AKT1 gene copy numbers by multi-gene fluorescence in situ hybridization impact on prognosis in breast cancer. Cancer science. 2015 May 1;106(5):642-9.

Liang CC, Park AY, Guan JL. In vitro scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro. Nature protocols. 2007 Feb;2(2):329.

Liby TA, Spyropoulos P, Buff Lindner H, Eldridge J, Beeson C, Hsu T, Muise-Helmericks RC. Akt3 controls vascular endothelial growth factor secretion and angiogenesis in ovarian cancer cells. International journal of cancer. 2012 Feb 1;130(3):532-43.

Lin HP, Lin CY, Huo C, Jan YJ, Tseng JC, Jiang SS, Kuo YY, Chen SC, Wang CT, Chan TM, Liou JY. AKT3 promotes prostate cancer proliferation cells through regulation of Akt, B-Raf & TSC1/TSC2. Oncotarget. 2015 Sep 29;6(29):27097.

Lin Y, Cheng K, Wang T, Xie Q, Chen M, Chen Q, Wen Q. miR-217 inhibits proliferation, migration, and invasion via targeting AKT3 in thyroid cancer. Biomedicine & pharmacotherapy= Biomedecine & pharmacotherapie. 2017 Sep 25;95:1718.

Linnerth-Petrik NM, Santry LA, Moorehead R, Jücker M, Wootton SK, Petrik J. Akt isoform specific effects in ovarian cancer progression. Oncotarget. 2016 Nov;7(46):74820-33.

Linnerth-Petrik NM, Santry LA, Petrik JJ, Wootton SK. Opposing functions of Akt isoforms in lung tumor initiation and progression. PloS one. 2014 Apr 10;9(4):e94595.

Liu J, Sun SH, Sun SJ, Huang C, Hu HH, Jin YB, Qiu ZJ. Phosph-Akt1 expression is associated with a favourable prognosis in pancreatic cancer. Annals Academy of Medicine Singapore. 2010 Jul 1;39(7):548.

Liu J, Wei XL, Huang WH, Chen CF, Bai JW, Zhang GJ. Cytoplasmic Skp2 expression is associated with p-Akt1 and predicts poor prognosis in human breast carcinomas. PloS one. 2012 Dec 27;7(12):e52675.

Liu SA, Tsai WC, Wong YK, Lin JC, Poon CK, Chao SY, Hsiao YL, Chan MY, Cheng CS, Wang CC, Wang CP. Nutritional factors and survival of patients with oral cancer. Head & neck. 2006 Nov 1;28(11):998-1007.

Longley DB, Johnston PG. Molecular mechanisms of drug resistance. The Journal of pathology. 2005 Jan 1;205(2):275-92.

Macha MA, Matta A, Chauhan SS, Siu KW, Ralhan R. Guggulsterone targets smokeless tobacco induced PI3K/Akt pathway in head and neck cancer cells. PLoSOne. 2011 Feb 24;6(2):e14728.

Madani AH, Dikshit M, Bhaduri D. Risk for oral cancer associated to smoking, smokeless and oral dip products. Indian journal of public health. 2012 Jan 1;56(1):57.

Malanga D, Scrima M, De Marco C, Fabiani F, De Rosa N, De Gisi S, Malara N, Savino R, Rocco G, Chiappetta G, Franco R. Activating E17K mutation in the gene encoding the protein kinase AKT in a subset of squamous cell carcinoma of the lung. Cell cycle. 2008 Mar 1;7(5):665-9.

Manjari M, Popli R, Paul S, Gupta VP, Kaholon SK. Prevalence of oral cavity, pharynx, larynx and nasal cavity malignancies in Amritsar, Punjab. Indian Journal of Otolaryngology and Head and Neck Surgery. 1996 Jul 1;48(3):191-5.

Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. Cell. 2007 Jun 29;129(7):1261-74.

Manoharan N, Tyagi BB, Raina V. Cancer incidences in rural Delhi-2004-05. Asian Pac J Cancer Prev. 2010 Jan 1;11(1):73-7.

Martini M, De Santis MC, Braccini L, Gulluni F, Hirsch E. PI3K/AKT signaling pathway and cancer: an updated review. Annals of medicine. 2014 Sep 1;46(6):372-83.

Massano J, Regateiro FS, Januário G, Ferreira A. Oral squamous cell carcinoma: review of prognostic and predictive factors. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics. 2006 Jul 1;102(1):67-76.

Massarelli E, Liu DD, Lee JJ, El-Naggar AK, Lo Muzio L, Staibano S, De Placido S, Myers JN, Papadimitrakopoulou VA. Akt activation correlates with adverse outcome in tongue cancer. Cancer. 2005 Dec 1;104(11):2430-6.

Matos LL, Stabenow E, Tavares MR, Ferraz AR, Capelozzi VL, Pinhal MA. Immunohistochemistry quantification by a digital computer-assisted method compared to semiquantitative analysis. Clinics. 2006 Oct;61(5):417-24.

McBride SM, Rothenberg SM, Faquin WC, Chan AW, Clark JR, Ellisen LW, Wirth LJ. Mutation frequency in 15 common cancer genes in high-risk head and neck squamous cell carcinoma. Head & neck. 2014 Aug 1;36(8):1181-8.

McCurdy CE, Cartee GD. Akt2 is essential for the full effect of calorie restriction on insulin-stimulated glucose uptake in skeletal muscle. Diabetes. 2005 May 1;54(5):1349-56.

Mehta FS, Hamner JE. Tobacco-related Oral Mucosal Lesions and Conditions in India: A Guide for Dental Students, Dentists, and Physicians. Basic Dental Research Unit, Tata Institute of Fundamental Research; 1993.

Memmott RM, Dennis PA. The role of the Akt/mTOR pathway in tobacco carcinogen–induced lung tumorigenesis. Clinical Cancer Research. 2010 Jan 1;16(1):4-10.

Menges CW, Sementino E, Talarchek J, Xu J, Chernoff J, Peterson JR, Testa JR. Group I p21-activated kinases (PAKs) promote tumor cell proliferation and survival through the AKT1 and Raf–MAPK pathways. Molecular cancer research. 2012 Sep 1;10(9):1178-88.

Merhi F, Tang R, Piedfer M, Mathieu J, Bombarda I, Zaher M, Kolb JP, Billard C, Bauvois B. Hyperforin inhibits Akt1 kinase activity and promotes caspase-mediated apoptosis involving Bad and Noxa activation in human myeloid tumor cells. PloS one. 2011 Oct 6;6(10):e25963.

Miao X, Song Y, Lv T, Zhan P, Lv Y, Yuan D. Expression and prognostic value of AKT2 in non-small cell lung cancer. Zhongguo fei ai za zhi= Chinese Journal of Lung Cancer. 2011 May;14(5):396-9.

Miao X, Song Y, Lv T, Zhan P, Lv Y, Yuan D. Expression and prognostic value of AKT2 in non-small cell lung cancer. Zhongguo fei ai za zhi= Chinese Journal of Lung Cancer. 2011 May;14(5):396-9.

Monatsschrift fur Zahnmedizin= Revue mensuelle suisse d'odonto-stomatologie= Rivista mensile svizzera di odontologia e stomatologia. 2008;118(5):390-7.

Mukherjee A, Wilson EM, Rotwein P. Selective signaling by Akt2 promotes bone morphogenetic protein 2-mediated osteoblast differentiation. Molecular and cellular biology. 2010 Feb 15;30(4):1018-27.

Mure H, Matsuzaki K, Kitazato KT, Mizobuchi Y, Kuwayama K, Kageji T, Nagahiro S. Akt2 and Akt3 play a pivotal role in malignant gliomas. Neuro-oncology. 2009 Dec 21;12(3):221-32.

Muslin AJ. Akt2: a critical regulator of cardiomyocyte survival and metabolism. Pediatric cardiology. 2011 Mar 1;32(3):317-22.

Mydlarz WK, Hennessey PT, Califano JA. Advances and perspectives in the molecular diagnosis of head and neck cancer. Expert opinion on medical diagnostics. 2010 Jan 1;4(1):53-65.

Nakashiro KI, Tanaka H, Goda H, Iwamoto K, Tokuzen N, Hara S, Onodera J, Fujimoto I, Hino S, Hamakawa H. Identification of Akt1 as a potent therapeutic target for oral squamous cell carcinoma. International journal of oncology. 2015 Oct 1;47(4):1273-81.

Nakatani K, Sakaue H, Thompson DA, Weigel RJ, Roth RA. Identification of a human Akt3 (protein kinase B γ) which contains the regulatory serine phosphorylation site. Biochemical and biophysical research communications. 1999 Apr 21;257(3):906-10.

Nakatani K, Thompson DA, Barthel A, Sakaue H, Liu W, Weigel RJ, Roth RA. Up-regulation of Akt3 in estrogen receptor-deficient breast cancers and androgen-independent prostate cancer lines. Journal of Biological Chemistry. 1999 Jul 30;274(31):21528-32.

Nakayama K, Nakayama N, Kurman RJ, Cope L, Pohl G, Samuels Y, Velculescu VE, Wang TL, Shih IM. Sequence mutations and amplification of PIK3CA and AKT2 genes in purified ovarian serous neoplasms. Cancer biology & therapy. 2006 Jul 1;5(7):779-85.

Neasta J, Hamida SB, Yowell QV, Carnicella S, Ron D. AKT signaling pathway in the nucleus accumbens mediates excessive alcohol drinking behaviors. Biological psychiatry. 2011 Sep 15;70(6):575-82.

Neville BW, Damm DD, Allen CM, Bouquot JE. Pernicious anemia. Oral and Maxillofacial Pathology. 3rd ed. St. Louis, Mo.: Saunders Elsevier. 2009:829-31.

Neville BW, Day TA. Oral cancer and precancerous lesions. CA: a cancer journal for clinicians. 2002 Jul 1;52(4):195-215.

Nisar MI, Iqbal R. Letter to the Editor-Smokeless tobacco use prevention and cessation (S-TUPAC): A need of the time. JPMA-Journal of the Pakistan Medical Association. 2011;61(7):711.

Nishikawa M, Miyake H, Fujisawa M. Enhanced sensitivity to sunitinib by inhibition of Akt1 expression in human castration-resistant prostate cancer PC3 cells both in vitro and in vivo. Urology. 2015 May 1;85(5):1215-e1.

Nooshinfar E, Bashash D, Abbasalizadeh M, Safaroghli-Azar A, Sadreazami P, Akbari ME. The Molecular Mechanisms of Tobacco in Cancer Pathogenesis. Iranian Journal of Cancer Prevention. 2017;10(1).

Noske A, Kaszubiak A, Weichert W, Sers C, Niesporek S, Koch I, Schaefer B, Sehouli J, Dietel M, Lage H, Denkert C. Specific inhibition of AKT2 by RNA interference results in reduction of ovarian cancer cell proliferation: increased expression of AKT in advanced ovarian cancer. Cancer letters. 2007 Feb 8;246(1):190-200.

O'Brien KA, Stojanovic-Terpo A, Hay N, Du X. An important role for Akt3 in platelet activation and thrombosis. Blood. 2011 Oct 13;118(15):4215-23.

Ogden GR. Alcohol and oral cancer. Alcohol. 2005 Apr 1;35(3):169-73.

Ohno A, Jee J, Fujiwara K, Tenno T, Goda N, Tochio H, Kobayashi H, Hiroaki H, Shirakawa M. Structure of the UBA domain of Dsk2p in complex with ubiquitin: molecular determinants for ubiquitin recognition. Structure. 2005 Apr 30;13(4):521-32.

Omura K. Current status of oral cancer treatment strategies: surgical treatments for oral squamous cell carcinoma. International journal of clinical oncology. 2014 Jun 1;19(3):423-30.

Pal I, Mandal M. PI3K and Akt as molecular targets for cancer therapy: current clinical outcomes. Acta Pharmacologica Sinica. 2012 Dec;33(12):1441.

Pearson WR. Flexible sequence similarity searching with the FASTA3 program package. InBioinformatics methods and protocols 2000 (pp. 185-219). Humana Press, Totowa, NJ. Petti S. Lifestyle risk factors for oral cancer. Oral oncology. 2009 Apr 1;45(4):340-50.

Phung TL, Du W, Xue Q, Ayyaswamy S, Gerald D, Antonello Z, Nhek S, Perruzzi CA, Acevedo I, Ramanna-Valmiki R, Rodriguez-Waitkus P. Akt1 and Akt3 exert opposing roles in the regulation of vascular tumor growth. Cancer research. 2015 Jan 1;75(1):40-50.

Pickhard A, Gröber S, Haug AK, Piontek G, Wirth M, Straßen U, Rudelius M, Reiter R. Survivin and pAkt as potential prognostic markers in squamous cell carcinoma of the

head and neck. Oral surgery, oral medicine, oral pathology and oral radiology. 2014 Jun 1;117(6):733-42.

Pieper U, Eswar N, Webb BM, Eramian D, Kelly L, Barkan DT, Carter H, Mankoo P, Karchin R, Marti-Renom MA, Davis FP. MODBASE, a database of annotated comparative protein structure models and associated resources. Nucleic acids research. 2008 Oct 23;37(suppl_1):D347-54.

Pore N, Jiang Z, Shu HK, Bernhard E, Kao GD, Maity A. Akt1 Activation Can Augment Hypoxia-Inducible Factor-1α Expression by Increasing Protein Translation through a Mammalian Target of Rapamycin–Independent Pathway. Molecular cancer research. 2006 Jul 1;4(7):471-9.

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

Prijić S, Ugrina I, Labar B, Nemet D, Batinić J, Zadro R, Ries S, Gjadrov-Kuvedžić K, Davidović S, Batinić D. Prognostic significance of constitutive phosphatidylinositol 3-kinase/Akt and mitogen-activated protein kinase phosphorylation in acute myeloid leukemia. Leukemia & lymphoma. 2015 Aug 3;56(8):2281-8.

Pu P, Kang C, Li J, Jiang H, Cheng J. The effects of antisense AKT2 RNA on the inhibition of malignant glioma cell growth in vitro and in vivo. Journal of neuro-oncology. 2006 Jan 1;76(1):1.

Pu P, Kang C, Li J, Jiang H. Antisense and dominant-negative AKT2 cDNA inhibits glioma cell invasion. Tumor Biology. 2004;25(4):172-8.

Pu R, Li WM, Liu D, Mo XM, Tang Y, Liu LX, Chen BJ. The expression and clinical significance of AKT2, phosphorylated AKT2 in non-small cell lung cancer. Sichuan da xue xue bao. Yi xue ban= Journal of Sichuan University. Medical science edition. 2010 Jul;41(4):586-9.

Pugazhenthi S, Nesterova A, Sable C, Heidenreich KA, Boxer LM, Heasley LE, Reusch JE. Akt/protein kinase B up-regulates Bcl-2 expression through cAMP-response element-binding protein. Journal of Biological Chemistry. 2000 Apr 14;275(15):10761-6.

Puliyappadamba VT, Cheriyan VT, Thulasidasan AK, Bava SV, Vinod BS, Prabhu PR, Varghese R, Bevin A, Venugopal S, Anto RJ. Nicotine-induced survival signaling in lung cancer cells is dependent on their p53 status while its down-regulation by curcumin is independent. Molecular cancer. 2010 Aug 20;9(1):220.

Qi Q, He K, Liu X, Pham C, Meyerkord C, Fu H, Ye K. Disrupting the PIKE-A/Akt interaction inhibits glioblastoma cell survival, migration, invasion and colony formation. Oncogene. 2013 Feb;32(8):1030.

Qiao J, Kang J, Ishola TA, Rychahou PG, Evers BM, Chung DH. Gastrin-releasing peptide receptor silencing suppresses the tumorigenesis and metastatic potential of neuroblastoma. Proceedings of the National Academy of Sciences. 2008 Sep 2;105(35):12891-6.

Queyrat L. Erythroplasie du gland. Bull Soc Franc Derm Syph. 1911;22:378-82.

Rajalalitha P, Vali S. Molecular pathogenesis of oral submucous fibrosis–a collagen metabolic disorder. Journal of oral pathology & medicine. 2005 Jul 1;34(6):321-8.

Reagan JW, Hamonic MJ. The cellular pathology in carcinoma in situ. A cytohistopathological correlation. Cancer. 1956 Mar 1;9(2):385-402.

Remmele W, Schicketanz KH. Immunohistochemical determination of estrogen and progesterone receptor content in human breast cancer: computer-assisted image analysis (QIC score) vs. subjective grading (IRS). Pathology-Research and Practice. 1993 Sep 1;189(8):862-6.

Riggio M, Perrone MC, Polo ML, Rodriguez MJ, May M, Abba M, Lanari C, Novaro V. AKT1 and AKT2 isoforms play distinct roles during breast cancer progression through the regulation of specific downstream proteins. Scientific reports. 2017 Mar 13;7:44244.

Rodriguez LG, Wu X, Guan JL. Wound-healing assay. InCell Migration 2005 (pp. 23-29). Humana Press.

Rodu B, Jansson C. Smokeless tobacco and oral cancer: a review of the risks and determinants. Crit Rev Oral Biol Med. 2004 Sep 1;15(5):252-63. Review.

Rudolph M, Anzeneder T, Schulz A, Beckmann G, Byrne AT, Jeffers M, Pena C, Politz O, Köchert K, Vonk R, Reischl J. AKT1 E17K mutation profiling in breast cancer: prevalence, concurrent oncogenic alterations, and blood-based detection. BMC cancer. 2016 Dec;16(1):622.

Ruggeri BA, Huang L, Wood M, Cheng JQ, Testa JR. Amplification and overexpression of the AKT2 oncogene in a subset of human pancreatic ductal adenocarcinomas. Molecular carcinogenesis. 1998 Feb 1;21(2):81-6.

Rychahou PG, Kang J, Gulhati P, Doan HQ, Chen LA, Xiao SY, Chung DH, Evers BM. Akt2 overexpression plays a critical role in the establishment of colorectal cancer metastasis. Proceedings of the National Academy of Sciences. 2008 Dec 23;105(51):20315-20.

Sahlberg SH, Gustafsson AS, Pendekanti PN, Glimelius B, Stenerlöw B. The influence of AKT isoforms on radiation sensitivity and DNA repair in colon cancer cell lines. Tumor Biology. 2014 Apr 1;35(4):3525-34.

Saji M, Narahara K, McCarty SK, Vasko VV, La Perle KM, Porter K, Jarjoura D, Lu C, Cheng SY, Ringel MD. Akt1 deficiency delays tumor progression, vascular invasion, and distant metastasis in a murine model of thyroid cancer. Oncogene. 2011 Oct;30(42):4307.

Šali A, Blundell TL. Comparative protein modelling by satisfaction of spatial restraints. Journal of molecular biology. 1993 Dec 5;234(3):779-815.

SAnchez RO, Sali A. Large-scale protein structure modeling of the Saccharomyces cerevisiae genome. Proceedings of the National Academy of Sciences. 1998 Nov 10;95(23):13597-602.

Sankaranarayanan R, Ramadas K, Thomas G, Muwonge R, Thara S, Mathew B, Rajan B, Trivandrum Oral Cancer Screening Study Group. Effect of screening on oral cancer mortality in Kerala, India: a cluster-randomised controlled trial. The Lancet. 2005 Jun 4;365(9475):1927-33.

Santi SA, Douglas AC, Lee H. The Akt isoforms, their unique functions and potential as anticancer therapeutic targets. Biomolecular concepts. 2010 Dec 1;1(5-6):389-401.

Sasaki H, Hikosaka Y, Kawano O, Moriyama S, Yano M, Fujii Y. MEK1 and AKT2 mutations in Japanese lung cancer. Journal of Thoracic Oncology. 2010 May 1;5(5):597-600.

Sasaki H, Okuda K, Kawano O, Yukiue H, Yano M, Fujii Y. AKT1 and AKT2 mutations in lung cancer in a Japanese population. Molecular medicine reports. 2008 Sep 1;1(5):663-6.

Schaal C, Chellappan SP. Nicotine-mediated cell proliferation and tumor progression in smoking-related cancers. Molecular Cancer Research. 2014 Jan 1;12(1):14-23.

Schmidt M, Hövelmann S, Beckers TL. A novel form of constitutively active farnesylated Akt1 prevents mammary epithelial cells from anoikis and suppresses chemotherapy-induced apoptosis. British journal of cancer. 2002 Oct;87(8):924.

Schrödinger (2013) LLC: New York, NY. Glide, version 6.1

Schwimmer E. Die idopathisches Schleimhaut-plaques der Mundhohle (leukoplakia buccalis). Arch Dermatol Syphilol. 1877;9:511-70.

Sen S, Carnelio S. Expression of epithelial cell adhesion molecule (EpCAM) in oral squamous cell carcinoma. Histopathology. 2016 May 1;68(6):897-904.

Shah JP, Gil Z. Current concepts in management of oral cancer–surgery. Oral oncology. 2009 Apr 1;45(4):394-401.

Shah KV. Do human papillomavirus infections cause oral cancer?. Journal of the National Cancer Institute. 1998 Nov 4;90(21):1585-6.

Shearn CT, Reigan P, Petersen DR. Inhibition of hydrogen peroxide signaling by 4-hydroxynonenal due to differential regulation of Akt1 and Akt2 contributes to decreases in cell survival and proliferation in hepatocellular carcinoma cells. Free Radical Biology and Medicine. 2012 Jul 1;53(1):1-1.

Shen MY, Sali A. Statistical potential for assessment and prediction of protein structures. Protein science. 2006 Nov 1;15(11):2507-24.

Shi H, Hong A, Kong X, Koya RC, Song C, Moriceau G, Hugo W, Clarissa CY, Ng C, Chodon T, Scolyer RA. A novel AKT1 mutant amplifies an adaptive melanoma response to BRAF inhibition. Cancer discovery. 2014 Jan 1;4(1):69-79.

Shi Y, Chen L, Li J, Lv YL, Sun Q, Wang LX, Jiao SC. Prognostic and predictive values of pERK1/2 and pAkt-1 expression in non-small cell lung cancer patients treated with adjuvant chemotherapy. Tumor Biology. 2011 Apr 1;32(2):381-90.

Shimakage M, Horii K, Tempaku A, Kakudo K, Shirasaka T, Sasagawa T. Association of Epstein-Barr virus with oral cancers. Hum Pathol. 2002 Jun;33(6):608-14.

Shimura T, Noma N, Oikawa T, Ochiai Y, Kakuda S, Kuwahara Y, Takai Y, Takahashi A, Fukumoto M. Activation of the AKT/cyclin D1/Cdk4 survival signaling pathway in radioresistant cancer stem cells. Oncogenesis. 2012 Jun;1(6):e12.

Shukla D, Dinesh Kale A, Hallikerimath S, Vivekanandhan S, Venkatakanthaiah Y. Genetic polymorphism of drug metabolizing enzymes (GSTM1 and CYP1A1) as risk factors for oral premalignant lesions and oral cancer. Biomedical Papers. 2012 Sep 1;156(3):253-9.

Shukla S, MacLennan GT, Hartman DJ, Fu P, Resnick MI, Gupta S. Activation of PI3K-Akt signaling pathway promotes prostate cancer cell invasion. International journal of cancer. 2007 Oct 1;121(7):1424-32.

Simossis VA, Heringa J. PRALINE: a multiple sequence alignment toolbox that integrates homology-extended and secondary structure information. Nucleic acids research. 2005 Jul 1;33(suppl_2):W289-94.

Slaughter DP, Southwick HW, Smejkal W. "Field cancerization" in oral stratified squamous epithelium. Clinical implications of multicentric origin. Cancer. 1953 Sep 1;6(5):963-8.

Smith EM, Hoffman HT, Summersgill KS, Kirchner HL, Turek LP, Haugen TH. Human papillomavirus and risk of oral cancer. The laryngoscope. 1998 Jul 1;108(7):1098-103.

Sobolewski C, Cerella C, Dicato M, Ghibelli L, Diederich M. The role of cyclooxygenase-2 in cell proliferation and cell death in human malignancies. International journal of cell biology. 2010;2010.

Sobus SL, Warren GW. The biologic effects of cigarette smoke on cancer cells. Cancer. 2014 Dec 1;120(23):3617-26.

Song G, Ouyang G, Bao S. The activation of Akt/PKB signaling pathway and cell survival. Journal of cellular and molecular medicine. 2005 Jan 1;9(1):59-71.

Speight PM. Update on oral epithelial dysplasia and progression to cancer. Head and neck pathology. 2007 Sep 1;1(1):61-6.

Staal SP. Molecular cloning of the akt oncogene and its human homologues AKT1 and AKT2: amplification of AKT1 in a primary human gastric adenocarcinoma. Proceedings of the National Academy of Sciences. 1987 Jul 1;84(14):5034-7.

Stahl JM, Sharma A, Cheung M, Zimmerman M, Cheng JQ, Bosenberg MW, Kester M, Sandirasegarane L, Robertson GP. Deregulated Akt3 activity promotes development of malignant melanoma. Cancer research. 2004 Oct 1;64(19):7002-10.

Stål O, Pérez-Tenorio G, Åkerberg L, Olsson B, Nordenskjöld B, Skoog L, Rutqvist LE. Akt kinases in breast cancer and the results of adjuvant therapy. Breast Cancer Research. 2003 Apr;5(2):R37.

Stemke-Hale K, Gonzalez-Angulo AM, Lluch A, Neve RM, Kuo WL, Davies M, Carey M, Hu Z, Guan Y, Sahin A, Symmans WF. An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. Cancer research. 2008 Aug 1;68(15):6084-91.

St-Germain ME, Gagnon V, Mathieu I, Parent S, Asselin E. Akt regulates COX-2 mRNA and protein expression in mutated-PTEN human endometrial cancer cells. International journal of oncology. 2004 May 1;24(5):1311-24.

Stopper H, Schmitt E, Gregor C, Mueller SO, Fischer WH. Increased cell proliferation is associated with genomic instability: elevated micronuclei frequencies in estradiol-treated human ovarian cancer cells. Mutagenesis. 2003 May 1;18(3):243-7.

Stottrup C, Tsang T, Chin YR. Upregulation of AKT3 confers resistance to the AKT inhibitor MK2206 in breast cancer. Molecular cancer therapeutics. 2016 Aug 1;15(8):1964-74.

Stout TJ, Tondi D, Rinaldi M, Barlocco D, Pecorari P, Santi DV, Kuntz ID, Stroud RM, Shoichet BK, Costi MP. Structure-based design of inhibitors specific for bacterial thymidylate synthase. Biochemistry. 1999 Feb 2;38(5):1607-17.

Sugatani T, Hruska KA. Akt1/Akt2 and mammalian target of rapamycin/Bim play critical roles in osteoclast differentiation and survival, respectively, whereas Akt is dispensable for cell survival in isolated osteoclast precursors. Journal of Biological Chemistry. 2005 Feb 4;280(5):3583-9.

Sugerman PB, Sabage NW. Oral lichen planus: causes, diagnosis and management. Australian dental journal. 2002 Dec 1;47(4):290-7.

Sui GQ, Fei D, Guo F, Zhen X, Luo Q, Yin S, Wang H. MicroRNA-338-3p inhibits thyroid cancer progression through targeting AKT3. American journal of cancer research. 2017;7(5):1177.

Sumitani S, Goya K, Testa JR, Kouhara H, Kasayama S. Akt1 and Akt2 differently regulate muscle creatine kinase and myogenin gene transcription in insulin-induced differentiation of C2C12 myoblasts. Endocrinology. 2002 Mar 1;143(3):820-8.

Sun M, Paciga JE, Feldman RI, Yuan ZQ, Coppola D, Lu YY, Shelley SA, Nicosia SV, Cheng JQ. Phosphatidylinositol-3-OH kinase (PI3K)/AKT2, activated in breast cancer, regulates and is induced by estrogen receptor α (ERα) via interaction between ERα and PI3K. Cancer research. 2001 Aug 15;61(16):5985-91.

Surviladze Z, Sterk RT, DeHaro SA, Ozbun MA. Cellular entry of human papillomavirus type 16 involves activation of the phosphatidylinositol 3-kinase/Akt/mTOR pathway and inhibition of autophagy. Journal of virology. 2013 Mar 1;87(5):2508-17.

Suter VG, Morger R, Altermatt HJ, Spieler P, Bornstein MM. Oral erythroplakia and erythroleukoplakia: red and red-white dysplastic lesions of the oral mucosa--part 1: epidemiology, etiology, histopathology and differential diagnosis. Schweizer

Taga M, Hirooka E, Ouchi T. Essential roles of mTOR/Akt pathway in Aurora-A cell transformation. International journal of biological sciences. 2009;5(5):444.

Tanaka T, Ishigamori R. Understanding carcinogenesis for fighting oral cancer. Journal of oncology. 2011;2011.

Tanaka T, Tanaka M, Tanaka T. Oral carcinogenesis and oral cancer chemoprevention: a review. Pathology research international. 2011;2011.

Tei H, Miyake H, Fujisawa M. Enhanced sensitivity to sorafenib by inhibition of Akt1 expression in human renal cell carcinoma ACHN cells both in vitro and in vivo. Human cell. 2015 Jul 1;28(3):114-21.

Testa JR, Tsichlis PN. AKT signaling in normal and malignant cells. Oncogene. 2005 Nov;24(50):7391.

Thomas GJ. Oral Squamous Cell Carcinoma. Encyclopedia of Cancer (2009-01-01): 2184-2188.

Toker A. Achieving specificity in Akt signaling in cancer. Advances in biological regulation. 2012 Jan;52(1):78.

Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA: a cancer journal for clinicians. 2015 Mar 1;65(2):87-108.

Tripathi SK, Muttineni R, Singh SK. Extra precision docking, free energy calculation and molecular dynamics simulation studies of CDK2 inhibitors. Journal of theoretical biology. 2013 Oct 7;334:87-100.

Tsurutani J, Castillo SS, Brognard J, Granville CA, Zhang C, Gills JJ, Sayyah J, Dennis PA. Tobacco components stimulate Akt-dependent proliferation and NFκB-dependent survival in lung cancer cells. Carcinogenesis. 2005 Jul 1;26(7):1182-95.

Valkov A, Kilvaer TK, Sorbye SW, Donnem T, Smeland E, Bremnes RM, Busund LT. The prognostic impact of Akt isoforms, PI3K and PTEN related to female steroid hormone receptors in soft tissue sarcomas. Journal of translational medicine. 2011 Dec;9(1):200.

Veeriah S. Opposing roles of the oncogene Akt isoforms in tumour progression: is there a dark side to Akt pathway inhibition?. Journal of chemical biology. 2012 Jul 1;5(3):115-7.

Vermorken JB, Specenier P. Optimal treatment for recurrent/metastatic head and neck cancer. Annals of oncology. 2010 Oct 1;21(suppl_7):vii252-61.

Villagrasa P, Diaz VM, Vinas-Castells R, Peiro S, Del Valle-Perez B, Dave N, Rodriguez-Asiain A, Casal JI, Lizcano JM, Dunach M, De Herreros AG. Akt2 interacts with Snail1 in the E-cadherin promoter. Oncogene. 2012 Sep;31(36):4022.

Virtakoivu R, Pellinen T, Rantala JK, Perälä M, Ivaska J. Distinct roles of AKT isoforms in regulating β1-integrin activity, migration, and invasion in prostate cancer. Molecular biology of the cell. 2012 Sep 1;23(17):3357-69.

Virtakoivu R, Pellinen T, Rantala JK, Perälä M, Ivaska J. Distinct roles of AKT isoforms in regulating β1-integrin activity, migration, and invasion in prostate cancer. Molecular biology of the cell. 2012 Sep 1;23(17):3357-69.

Vogiatzi P, Giordano A. Following the tracks of AKT1 gene. Cancer biology & therapy. 2007 Oct 1;6(10):1521-4.

Vogt N, Gibaud A, Almeida A, Ourliac-Garnier I, Debatisse M, Malfoy B. Relationships linking amplification level to gene over-expression in gliomas. PloS one. 2010 Dec 8;5(12):e14249.

Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Munoz N. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. The Journal of pathology. 1999 Sep 1;189(1):12-9.

Wanebo HJ, Jun MY, Strong EW, Oettgen H. T-cell deficiency in patients with squamous cell cancer of the head and neck. The American Journal of Surgery. 1975 Oct 1;130(4):445-51.

Wang B, Zhang S, Yue K, Wang XD. The recurrence and survival of oral squamous cell carcinoma: a report of 275 cases. Chinese journal of cancer. 2013 Nov;32(11):614.

Wang C, Xu X, Jin H, Liu G. Nicotine may promote tongue squamous cell carcinoma progression by activating the Wnt/β-catenin and Wnt/PCP signaling pathways. Oncology Letters. 2017 May 1;13(5):3479-86.

Wang G, Kang C, Pu P. Increased expression of Akt2 and activity of PI3K and cell proliferation with the ascending of tumor grade of human gliomas. Clinical neurology and neurosurgery. 2010 May 1;112(4):324-7.

Wang H, Wang H, Zhang W, Huang HJ, Liao WS, Fuller GN. Analysis of the activation status of Akt, NFκB, and Stat3 in human diffuse gliomas. Laboratory investigation. 2004 Aug;84(8):941.

Wang J, Miao LJ, Wu YM, Wu YJ, Wang XC. Expression of AKT2, cyclin D1, and MMP-9 and their correlations to clinicopathologic features of non-small cell lung cancer. Ai zheng= Aizheng= Chinese journal of cancer. 2006 Jan;25(1):69-72.

Wang L, Yao J, Zhang X, Guo B, Le X, Cubberly M, Li Z, Nan K, Song T, Huang C. miRNA-302b suppresses human hepatocellular carcinoma by targeting AKT2. Molecular cancer research. 2014 Feb 1;12(2):190-202.

Wang Q, Fan SY, Qian J, Wang JY, Lu YC, Hu GH, Luo C. AKT2 expression in histopathologic grading and recurrence of meningiomas. European Journal of Surgical Oncology. 2014 Sep 1;40(9):1056-61.

Wang Q, Gao P, Wang X, Duan Y. The early diagnosis and monitoring of squamous cell carcinoma via saliva metabolomics. Scientific reports. 2014 Oct 30;4:6802.

Wang W, Wen Q, Xu L, Xie G, Li J, Luo J, Chu S, Shi L, Huang D, Li J, Fan S. Activation of Akt/mTOR pathway is associated with poor prognosis of nasopharyngeal carcinoma. PloS one. 2014 Aug 28;9(8):e106098.

Wang YW, Lin KT, Chen SC, Gu DL, Chen CF, Tu PH, Jou YS. Overexpressed-eIF3I interacted and activated oncogenic Akt1 is a theranostic target in human hepatocellular carcinoma. Hepatology. 2013 Jul 1;58(1):239-50.

Warnakulasuriya KA, Johnson NW. Sensitivity and specificity of OraScan® toluidine blue mouthrinse in the detection of oral cancer and precancer. Journal of oral pathology & medicine. 1996 Mar 1;25(3):97-103.

Warren GW, Singh AK. Nicotine and lung cancer. Journal of carcinogenesis. 2013 Jan 1;12(1):1.

Watanabe S, Sato K, Okazaki Y, Tonogi M, Tanaka Y, Yamane GY. Activation of PI3K-AKT pathway in oral epithelial dysplasia and early cancer of tongue. The Bulletin of Tokyo Dental College. 2009;50(3):125-33.

Watkins III A. The role of Akt isoforms in BCR-ABL induced chronic myeloid leukemia. Thomas Jefferson University; 2012.

Wei PL, Kuo LJ, Huang MT, Ting WC, Ho YS, Wang W, An J, Chang YJ. Nicotine enhances colon cancer cell migration by induction of fibronectin. Annals of surgical oncology. 2011 Jun 1;18(6):1782-90.

Wei Y, Zhao L, He W, Yang J, Geng C, Chen Y, Liu T, Chen H, Li Y. Benzo[a]pyrene promotes gastric cancer cell proliferation and metastasis likely through the Aryl hydrocarbon receptor and ERK-dependent induction of MMP9 and c-myc. Int J Oncol. 2016 Nov;49(5):2055-2063.

West KA, Brognard J, Clark AS, Linnoila IR, Yang X, Swain SM, Harris C, Belinsky S, Dennis PA. Rapid Akt activation by nicotine and a tobacco carcinogen modulates the phenotype of normal human airway epithelial cells. The Journal of clinical investigation. 2003 Jan 1;111(1):81-90.

Williams HK. Molecular pathogenesis of oral squamous carcinoma. Molecular Pathology. 2000 Aug;53(4):165.

Wilson TR, Longley DB, Johnston PG. Chemoresistance in solid tumours. Annals of Oncology. 2006 Sep 1;17(suppl_10):x315-24.

Wright GL, Maroulakou IG, Eldridge J, Liby TL, Sridharan V, Tsichlis PN, Muise-Helmericks RC. VEGF stimulation of mitochondrial biogenesis: requirement of AKT3 kinase. The FASEB Journal. 2008 Sep 1;22(9):3264-75.

Xing H, Weng D, Chen G, Tao W, Zhu T, Yang X, Meng L, Wang S, Lu Y, Ma D. Activation of fibronectin/PI-3K/Akt2 leads to chemoresistance to docetaxel by regulating survivin protein expression in ovarian and breast cancer cells. Cancer letters. 2008 Mar 8;261(1):108-19.

Xu X, Sakon M, Nagano H, Hiraoka N, Yamamoto H, Hayashi N, Dono K, Nakamori S, Umeshita K, Ito Y, Matsuura N. Akt2 expression correlates with prognosis of human hepatocellular carcinoma. Oncology reports. 2004 Jan 1;11(1):25-32.

YamazakI H, Yoshida K, Yoshioka Y, Shimizutani K, Furukawa S, Koizumi M, Ogawa K. High dose rate brachytherapy for oral cancer. Journal of radiation research. 2012 Nov 23;54(1):1-7.

Yang W, Ju JH, Lee KM, Nam K, Oh S, Shin I. Protein kinase B/Akt1 inhibits autophagy by down-regulating UVRAG expression. Experimental cell research. 2013 Feb 1;319(3):122-33.

Yu H, Littlewood T, Bennett M. Akt isoforms in vascular disease. Vascular pharmacology. 2015 Aug 1;71:57-64.

Yu MA, Kiang A, Wang-Rodriguez J, Rahimy E, Haas M, Yu V, Ellies LG, Chen J, Fan JB, Brumund KT, Weisman RA. Nicotine promotes acquisition of stem cell and epithelial-to-mesenchymal properties in head and neck squamous cell carcinoma. PLoS One. 2012 Dec 26;7(12):e51967.

Yu SM, Gweon EJ, Chung KW, Kim KH, Cho HS, Kim SJ. Gallotannin regulates apoptosis and COX-2 expression via Akt and p38kinase pathway in human lung cancer cell line, A549. Animal Cells and Systems. 2012 Oct 1;16(5):366-75.

Yu W, Chu L, Zhao K, Chen H, Xiang J, Zhang Y, Li H, Zhao W, Sun M, Wei Q, Fu X. A nomogram based on phosphorylated AKT1 for predicting locoregional recurrence in patients with oesophageal squamous cell carcinoma. Journal of Cancer. 2017;8(18):3755.

Yu Y, Savage RE, Eathiraj S, Meade J, Wick MJ, Hall T, Abbadessa G, Schwartz B. Targeting AKT1-E17K and the PI3K/AKT pathway with an allosteric AKT inhibitor, ARQ 092. PLoS One. 2015 Oct 15;10(10):e0140479.

Yuan ZQ, Feldman RI, Sussman GE, Coppola D, Nicosia SV, Cheng JQ. Akt2 Inhibition of Cisplatin-induced JNK/p38 and Bax Activation by Phosphorylation of ASK1 Implication of AKT2 in Chemoresistance. Journal of Biological Chemistry. 2003 Jun 27;278(26):23432-40.

Yuan ZQ, Sun M, Feldman RI, Wang G, Ma XL, Jiang C, Coppola D, Nicosia SV, Cheng JQ. Frequent activation of AKT2 and induction of apoptosis by inhibition of phosphoinositide-3-OH kinase/Akt pathway in human ovarian cancer. Oncogene. 2000 May;19(19):2324.

Zaini ZM, Karen-Ng LP, Cheong SC, Gurdeep SM, Yuen KM, Tay KK, Jalil N, Ismail SM, Jallaluddin A, Zain RB. Genetic polymorphisms and risk of oral cancer. Oral Diseases. 2010;16(6):522-3.

Zhang B, Gu F, She C, Guo H, Li W, Niu R, Fu L, Zhang N, Ma Y. Reduction of Akt2 inhibits migration and invasion of glioma cells. International journal of cancer. 2009 Aug 1;125(3):585-95.

Zhang J, Chang L, Jin H, Xia Y, Wang L, He W, Li W, Chen H. Benzopyrene promotes lung cancer A549 cell migration and invasion through up-regulating cytokine IL8 and chemokines CCL2 and CCL3 expression. Experimental Biology and Medicine. 2016 Aug;241(14):1516-23.

Zhang L, Sun S, Zhou J, Liu J, Lv JH, Yu XQ, Li C, Gong L, Yan Q, Deng M, Xiao L. Knockdown of Akt1 promotes Akt2 upregulation and resistance to oxidative-stress-induced apoptosis through control of multiple signaling pathways. Antioxidants & redox signaling. 2011 Jul 1;15(1):1-7.

Zhang Y, Guo X, Yang M, Yu L, Li Z, Lin N. Identification of AKT kinases as unfavorable prognostic factors for hepatocellular carcinoma by a combination of expression profile, interaction network analysis and clinical validation. Molecular BioSystems. 2014;10(2):215-22.

Zhou B, Shan H, Su Y, Xia K, Zou R, Shao Q. Let-7a inhibits migration, invasion and tumor growth by targeting AKT2 in papillary thyroid carcinoma. Oncotarget. 2017 Sep 19;8(41):69746.

Zhou Z, Yang F, Jiang G, Huang Y, Fu J, Wang J. Phophorylated Akt protein expression in non-small cell lung cancer. Zhongguo fei ai za zhi= Chinese journal of lung cancer. 2007 Oct;10(5):376-80.

Zhu J, Liu Y, Zhu Y, Zeng M, Xie J, Lei P, Li K, Hu Y. Role of RANK and Akt1 activation in human osteosarcoma progression: A clinicopathological study. Experimental and Therapeutic Medicine. 2017 Jun 1;13(6):2862-6.

Zhu Y, Zhou J, Ji Y, Yu B. Elevated expression of AKT2 correlates with disease severity and poor prognosis in human osteosarcoma. Molecular medicine reports. 2014 Aug 1;10(2):737-42.

Zhu Z, Yu W, Fu X, Sun M, Wei Q, Li D, Chen H, Xiang J, Li H, Zhang Y, Zhao W. Phosphorylated AKT1 is associated with poor prognosis in esophageal squamous cell carcinoma. Journal of Experimental & Clinical Cancer Research. 2015 Sep 4;34(1):95.

Zhuang J, Ye Y, Wang G, Ni J, He S, Hu C, Xia W, Lv Z. MicroRNA-497 inhibits cellular proliferation, migration and invasion of papillary thyroid cancer by directly targeting AKT3. Molecular Medicine Reports. 2017 Nov 1;16(5):5815-22.

Zitzmann K, Vlotides G, Brand S, Lahm H, Spöttl G, Göke B, Auernhammer CJ. Perifosine-mediated Akt inhibition in neuroendocrine tumor cells: role of specific Akt isoforms. Endocrine-related cancer. 2012 Jun 1;19(3):423-34.

Zlotolow IM. General consideration in prevention and treatment of oral manifestation of cancer therapies. InPrinciples and Practice of Supportive Oncology 1998 (p. 237). Lippincott Raven, Philadelphia, PA.

ABBREVIATIONS

µg	: Microgram
µM	: Micromolar
4EBP1	: 4E binding protein 1
ACC	: Adenoid cystic carcinoma
ANOVA	: Analysis of variance
ATP	: Adenosine triphosphate
BCC	: basal cell carcinoma
Bcl-2	: B-cell lymphoma 2
CAT	: Cancer adjacent tissues
CEBPβ	: CCAAT-enhancer binding protein β
COX-2	: cyclooxygenase-2
cyclin D1	: CCND1 gene encodes the cyclin D1 protein
DAB	: 3,3'-diaminobenzidine
DFS	: Disease free survival
DMEM	: Dulbecco's Modified Eagle Medium
DMSO	: Dimethyl sulfoxide
DPX	: distyrene, plasticizer (tricresyl phosphate), and xylene
DTT	: Dithiothreitol
EBRT	: External beam radiotherapy
EBV	: Epstein barr virus
EDTA	: Ethylenediaminetetraacetic acid
EGFR	: Epidermal growth factor receptor
EMT	: Epithelial-mesenchymal transition
EpCAM	: Epithelial adhesion molecule
ER	: estrogen-receptor
ER-α	: estrogen receptor alpha
ESCC	: Esophageal squamous cell carcinoma
FACS	: Fluorescence-activated cell sorting
FAK	: Focal adhesion kinase
FBS	: Fetal bovine serum
FDA	: Food and Drug Administration
GAP	: GTPase-activating protein
GAPDH	: Glyceraldehyde-3-Phosphate Dehydrogenase
HCC	: Hepatocellular carcinoma
HEPES	: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HER2	: Human epidermal growth factor receptor 2
HGF	: Hepatocyte growth factor
HIF-1α	: Hypoxia inducible factor 1alpha Subunit
HNSCC	: Head and neck squamous cell carcinoma
HPV	: Human papillomavirus
HSV	: Herpes simplex viruses
ICMR	: Indian Council for Medical Research
IFN-β	: Interferon beta
IHC	: Immunohistochemistry
IRS	: Insulin receptor substrate

JNK	: c-Jun NH2-terminal kinase
LPFS	: Locoregional-progression free survival
LRC	: Loco-regional control
LSCC	: Laryngeal squamous cell carcinoma
miR	: MicroRNA
ml	: Milliliter
mM	: Millimolar
mRNA	: Messenger RNA
mTOR	: Mammalian target of rapamycin
MTSS1	: Metastasis suppressor 1
MTT	: (3-[4,5-dimethylthiazol-2yl]-2,5-diphenyl tetrazolium bromide)
nAChR	: Nicotinic acetylcholine receptors
NF-κB	: Nuclear factor kappa-light-chain-enhancer of activated B cells
NIH	: National Institutes of Health
nm	: Nanometer
nM	: Nanomolar
NNK	: 4 [methylnitrosoamino]-1-[3- pyridyl]-1-butanone
NNN	: N-nitrosonornicotine
NSCLC	: Non-small-cell lung carcinoma
OLP	: Oral lichen planus
OSCC	: Oral squamous cell carcinoma
OSMF	: Oral submucous fibrosis
PAH	: Polycyclic aromatic hydrocarbons
PBS	: Phosphate Buffer Saline
PCNA	: Proliferating Cell Nuclear Antigen
PDB	: Protein Data Bank
PFS	: Progression free survival
PH	: Pleckstrin-homology
pH	: potential of hydrogen
PHLPP1/2	: PH-domain and Leucine-rich Repeat Protein Phosphatase ½
PI	: Propidium iodide
PI3K	: Phosphoinositide 3-kinase
PIP2	: Phosphatidylinositol (3,4)-bisphosphate
PIP3	: Phosphatidylinositol (3,4,5)-trisphosphate
PKB	: Protein kinase B
PMSF	: Phenylmethane sulfonyl fluoride
PP2A	: Protein phosphatase 2
PRAS40	: Proline-rich Akt substrate of 40 kDa
PSI-BLAST	: Position-Specific Iterative Basic Local Alignment Search Tool
PTEN	: Phosphatase and tensin homolog
Ras	: Rat Sarcoma
RGCB	: Rajiv Gandhi Centre for Biotechnology
RMSD	: Root mean square deviation
ROS	: Reactive oxygen species
RTKs	: Receptor tyrosine kinases
S6K1	: Ribosomal protein S6 kinase, 70 kDa, polypeptide 1

SCC	: Squamous cell carcinomas
SCLC	: Small cell lung cancer
SDS	: Sodium dodecyl sulfate
SGK	: Serum- and glucocorticoid-inducible kinase
shRNA	: Short hairpin RNA
siRNA	: Short interference RNA
STAT3	: Signal transducer and activator of transcription 3
TBS	: Tris buffer saline
TBST	: Tris-buffered saline (TBS) and Polysorbate 20 (also known as Tween 20)
TCGA	: The Cancer Genome Atlas
TCRP1	: Tongue cancer chemotherapy resistant-associated protein 1
TE	: Tobacco extract
TMA	: Tissue microarray
TNM	: Tumor lymph node metastasis
TROP2	: Trophoblast cell-surface antigen
TSNA	: Tobacco-specific-nitrosamines
VEGF	: Vascular endothelial growth factor

LIST OF FIGURES

LISTS OF TABLES

LIST OF PUBLICATIONS, CONFERENCES, AND WORKSHOPS

1. **Roy NK**, Monisha J, singh AK, Kunnumakkara AB: Isoform-specific role of Akt isoforms in development of oral squamous cell carcinoma. (Communicated)

2. **Roy NK**, Monisha J, AK singh, Kunnumakkara AB: Evaluating the Significance of Promoter Sequences in Isoform-specificity of Akt Kinase and Selective Targeting by demarcation of Natural Agents and General Akt Kinase Inhibitors: An In Silico Approach. (Communicated)

3. **Roy NK,** Bordoloi D, Monisha J, Padmavathi G, Kotoky J, Golla R, B Kunnumakkara A. Specific targeting of Akt kinase isoforms: Taking the precise path for prevention and treatment of cancer. *Current drug targets. 2017 Mar 1;18(4):421-35.*

4. **Roy NK,** Monisha J, Padmavathi G, Das A, Gupta S, Ramakrishnan E, Kotoky J, Kunnumakkara AB. Rapid Biosynthesis of Gold Nanoparticles Using Aqueous-ethanoic Leaf Extract of Heartleaf Moonseed: Characterization and Effect of pH on its Synthesis. *Current Nanomaterials. 2017 Apr 1;2(1):3-10.*

5. **Roy NK**, Deka A, Bordoloi D, Mishra S, Kumar AP, Sethi G, Kunnumakkara AB. The potential role of boswellic acids in cancer prevention and treatment. *Cancer letters. 2016 Jul 10;377(1):74-86.*

6. Monisha J, **Roy NK**, Padmavathi G, Banik K, Bordoloi D, Khwairakpam AD, Arfuso F, Chinnathambi A, Alahmadi TA, Alharbi SA, Sethi G. NGAL is downregulated in oral squamous cell carcinoma and leads to increased survival, proliferation, migration, and chemoresistance. *Cancers (Basel). 2018 Jul 10;10(7).*

7. Ranaware AM, Banik K, Deshpande V, Padmavathi G, **Roy NK**, Sethi G, Fan L, Kumar AP, Kunnumakkara AB. Magnolol: A Neolignan from the Magnolia Family for the Prevention and Treatment of Cancer. *International journal of molecular sciences. 2018 Aug 10;19(8).*

8. Kunnumakkara AB, Banik K, Bordoloi D, Choudhary H, Sailo BL, **Roy NK**, Gupta SC, Aggarwal BB. Googling the Guggul (Commiphora & Boswellia) for Prevention of Chronic Diseases. *Frontiers in Pharmacology. 2018;9:686.*

9. Khwairakpam AD, Damayenti YD, Deka A, Monisha J, Roy NK, Padmavathi G, Kunnumakkara AB. Acorus calamus: a bio-reserve of medicinal values. *Journal of basic and clinical physiology and pharmacology. 2018 Mar 28;29(2):107-22.*

10. Kunnumakkara AB, Bordoloi D, Padmavathi G, Monisha J, **Roy NK**, Prasad S, Aggarwal BB. Curcumin, the golden nutraceutical: multitargeting for multiple chronic diseases. *British journal of pharmacology. 2017 Jun 1;174(11):1325-48.*

11. Padmavathi G, **Roy NK**, Bordoloi D, Arfuso F, Mishra S, Sethi G, Bishayee A, Kunnumakkara AB. *Butein in health and disease: A comprehensive review. Phytomedicine. 2017 Feb 15;25:118-27.*

12. Monisha J, Padmavathi G, **Roy NK**, Deka A, Bordoloi D, Anip A, B Kunnumakkara A. NF-κB blockers gifted by mother nature: Prospectives in cancer cell chemosensitization. *Current pharmaceutical design. 2016 Aug 1;22(27):4173-200.*

13. Monisha J, **Roy NK**, Bordoloi D, Kumar A, Golla R, Kotoky J, Padmavathi G, B Kunnumakkara A. Nuclear factor kappa B: a potential target to persecute head and neck cancer. *Current drug targets. 2017 Feb 1;18(2):232-53.*

14. D Bordoloi, **NK Roy**, J Monisha, P Ganesan, AB Kunnumakkara: Multi-Targeted Agents in Cancer Cell Chemosensitization: What We Learnt from Curcumin Thus Far. *11/2015; 10(999)., DOI:10.2174/1574892810666151020101706*

15. Padmavathi G, Rathnakaram SR, Monisha J, Bordoloi D, Roy NK, Kunnumakkara AB. Potential of butein, a tetrahydroxychalcone to obliterate cancer. *Phytomedicine. 2015 Dec 1;22(13):1163-71.*

16. Monisha J, Padmavathi G, Bordoloi D, **Roy NK**, Kunnumakkara AB. Neutrophil gelatinase-associated lipocalin (NGAL): a promising biomarker for cancer diagnosis and a potential target for cancer therapeutics. *J Cell Sci Molecul Biol. 2014;1(2):106.*

17. Bakshi S, **Roy NK**, Sahoo L. Seedling preconditioning in thidiazuron enhances axillary shoot proliferation and recovery of transgenic cowpea plants. *Plant Cell, Tissue and Organ Culture (PCTOC). 2012 Jul 1;110(1):77-91.*

18. Bakshi S, Saha B, **Roy NK**, Mishra S, Panda SK, Sahoo L. Successful recovery of transgenic cowpea (Vigna unguiculata) using the 6-phosphomannose isomerase gene as the selectable marker. *Plant cell reports. 2012 Jun 1;31(6):1093-103.*

19. **Roy NK**, Sharma A, Singh AK, Bordoloi D, Sailo BL, Monisha J, Kunnumakkara AB. Bladder Cancer: *Chemoresistance and Chemosensitization. Cancer Cell Chemoresistance And Chemosensitization. 2018 Feb 13:51.*

20. Thakur KK, Bordoloi D, Prakash J, Monisha J, **Roy NK**, Kunnumakkara AB. Different Chemosensitization Approaches for the Effective Management of

HNSCC. *Cancer Cell Chemoresistance And Chemosensitization. 2018 Feb 13:399.*

21. Singh AK, **Roy NK**, Anip A, Banik K, Monisha J, Bordoloi D, Kunnumakkara AB. Different Methods to Inhibit Chemoresistance in Hepatocellular Carcinoma. *Cancer Cell Chemoresistance And Chemosensitization. 2018 Feb 13:373.*

22. Choudhary H, Thakur KK, Sharma A, **Roy NK**, Khwairakpam AD, Bordoloi D, Kunnumakkara AB. Strategies to Overcome Chemoresistance in Ovarian Cancer. *Cancer Cell Chemoresistance And Chemosensitization. 2018 Feb 13:373.*

23. Sailo BL, Bordoloi D, Banik K, Khwairakpam AD, **Roy NK**, Prakash J, Kunnumakkara AB. Therapeutic Strategies for Chemosensitization of Renal Cancer. *Cancer Cell Chemoresistance And Chemosensitization. 2018 Feb 13:373.*

24. Sailo BL, Monisha J, Jaiswal A, Prakash J, **Roy NK**, Thakur KK, Banik K, Bordoloi D, Kunnumakkara AB. Molecular Alterations Involved in Pancreatic Cancer Chemoresistance and Chemosensitization Strategies. *Cancer Cell Chemoresistance And Chemosensitization. 2018 Feb 13:373.*

25. Monisha J, **Roy NK**, Sharma A, Banik K, Padmavathi G, Bordoloi D, Kunnumakkara AB. Chemoresistance and Chemosensitization in Melanoma. *Cancer Cell Chemoresistance And Chemosensitization. 2018 Feb 13:373.*

26. Padmavathi G, Bordoloi D, Monisha J, **Roy NK**, Harsha C, Kunnumakkara AB. Recently Discovered Fusion Genes and Their Implications in Cancer. *InFusion Genes And Cancer 2017 (pp. 315-348).*

27. **Roy NK**, Bordoloi D, Padmavathi G, Kunnumakkara AB. Targeting Fusion Genes for Cancer Therapy. *InFusion Genes And Cancer 2017 (pp. 349-371).*

28. Padmavathi G, Banik K, **Roy NK**, Monisha J, Kunnumakkara AB. Role of BCR-ABL Fusion Kinase in the Development of Leukemia. *InFusion Genes And Cancer 2017 (pp. 111-127).*

29. **Roy NK**, Padmavathi G, Bordoloi D, Kunnumakkara AB. Techniques to Identify Novel Fusion Genes and to Detect Known Fusion Genes. *InFusion Genes And Cancer 2017 (pp. 59-79).*

30. Padmavathi G, **Roy NK**, Bordoloi D, Monisha J, Kunnumakkara AB. Basic Concepts of Fusion Genes and Their Classification. *InFusion Genes And Cancer 2017 (pp. 17-58).*

31. **Roy NK**, Bordoloi D, Monisha J, Anip A, Padmavathi G, Kunnumakkara AB. Cancer—An Overview and Molecular Alterations in Cancer. *InFusion Genes And Cancer 2017 (pp. 1-15).*

Oral Presentations

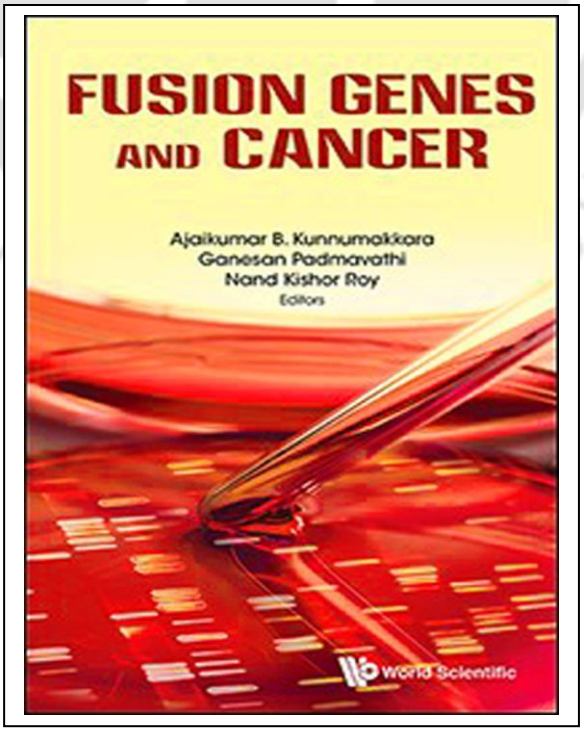
1. **NK Roy**, M Javadi, D Bordoloi, P Ganesan, A Singh and AB Kunnumakkara. Dissecting the Specificity of Akt Kinase Isoforms in Oral Cancer and an in silico approach for Selective Targeting by Natural Agents, at National conference on "Ethno-medicine and Traditional Health Practices in Northeast region of India, NIPER Guwahati, Mirza Campus, Assam India 2018.

2. **NK Roy**, M Javadi, D Bordoloi, A Singh and AB Kunnumakkara. Distinct role of Akt isoforms in oral cancer and tobacco-induced aggressiveness, at Trends in Biochemical and Biomedical Research (TBBR-2018) organized by Banaras Hindu University (BHU), Varansi, Assam India, 2018.

3. **NK Roy**, AB Kunnumakkara. Studies on the Expression of Different Proteins, Genes and microRNAs for the Development of Diagnosis, Prognosis and Predictive Biomarkers for Head and Neck in North-East India, at 2nd Institutional Ethics Committee Meeting organized by North East Cancer Hospital and Research Institute, Guwahati, Assam India, 2016.

Book

Fusion Genes and Cancer. AB Kunnumakkara, G Padmavathi, **NK Roy**. World Scientific 2016.
(http://www.worldscientific.com/worldscibooks/10.1142/10306) Published in April 2017.



Abstracts in conference proceedings

1. NK Roy, J Monisha, G Padmavati, D Bordoloi, AB Kunnumakkara: Precise Distinction of general Akt Inhibitors into Akt Isoform-specific Inhibitors. Journal of Carcinogenesis 2016; 15:278. ISSN: 0974-6773.

2. **NK Roy**, J Monisha, DBordoloi, A Singh, AB Kunnumakkara: Distinct role of Akt isoforms in oral cancer and tobacco-induced aggressiveness. Research Reports 2: e1-e203. doi:10.9777/rr.2018.1001. ISSN: 2471-5689.

3. **NK Roy**, AB Kunnumakkara. Isoform specific role of akt in oral squamous cell carcinoma. Cancer Medicine 2018; 7:11, 111 River St, Hoboken 07030-5774, NJ USA: Wiley. ISSN: 2045-7634.

Abstracts presented in conferences

1. **NK Roy**, J Monisha, G Padmavathi, D Bordoloi**,** AB Kunnumakkara, "Precise distinction of general Akt Inhibitors into Akt Isoform specific inhibitors", Indian Association of Cancer Research-2016 (IACR-2016), New Delhi, India, 2016.

2. **NK Roy**, J Monisha, G Padmavathi, D Bordoloi, A Singh, AB Kunnumakkara. "Isoform-specific Targeting of Akt Kinase by Natural Inhibitors: An In Silico Approach" in First International Conference on Nutraceuticals and Chronic Diseases, 2016, Kerala, India, in the year 2017.

3. **NK Roy**, BL Sailo, AB Kunnumakkara, "Isoform-specificity of Akt kinase in Cancer: Does Static Sequence Embraces the Dynamism?" at Research Conclave organized by the PhD Council of the Students' Academic Board (SAB), Indian Institute of Technology Guwahati in the year 2017.

4. J Monisha, KF Sajin, **NK Roy**, G Padmavathi, MS Nair, AB Kunnumakkara, "Insights into anticancer activity and mechanism of action of azadiradione against triple negative breast cancer, in First International Conference on Nutraceuticals and Chronic Diseases, 2016, Kerala, India, in the year 2016.

5. **NK Roy**, J Monisha, G Padmavathi, D Bordoloi, AB Kunnumakkara, "Precise distinction of general Akt Inhibitors into Akt Isoform-specific inhibitors" in the 12th International Conference of the Asian Clinical Oncology Society and 35th Annual Convention of the Indian Association of Cancer Research (IACR) and Mid-Term Conference of IASO held at New Delhi in the year 2016.

6. **NK Roy**, T Usham, D Thingujam, AB Kunnumakkara, "Tobacco usage and cancer" at Research Conclave organized by the PhD Council of the Students' Academic Board (SAB), Indian Institute of Technology Guwahati in the year 2016.

7. **NK Roy**, J Monisha, G Padmavathi, D Bordoloi, AB Kunnumakkara, "Isoform

specific action of Akt kinase inhibitors for better efficacy: An in silico approach" at Translational Cancer Research at Ahmedabad in the year 2016.

8. **NK Roy,** A Das, S Gupta, J Monisha, G Padmavathi, E Ramakrishnan, J Kotoky and AB Kunnumakkara, "Rapid Synthesis of Gold Nanoparticles using Tinospora cordifolia leaf extract and its Characterization" at Research Conclave organized by the PhD Council of the Students' Academic Board (SAB), Indian Institute of Technology Guwahati in the year 2015.

9. **NK Roy**, A Das, S Gupta, J Monisha, G Padmavathi, E Ramakrishnan, J Kotoky and AB Kunnumakkara, "Green Synthesis of Gold Nanoparticles and Assessment of Their Cytotoxicity" at National Conference on Recent Advances in Cancer Biology and Therapeutics, IIT Guwahati, India, 2014.

10. **NK Roy**, A Das, S Gupta, J Monisha, G Padmavathi, E Ramakrishnan, J Kotoky and AB Kunnumakkara, "Biosynthesis of Gold Nanoparticles and Evaluation of Their Cytotoxicity against Cancer Cells" International Conference on Disease Biology and Therapeutics, IASST Guwahati, India, 2014.

Conferences, workshops and trainings attended

1. Participated in Indo-japan symposium on "**Hope from Herbs: research based Care and cure Potentials**" jointly organized by IIT Guwahati and AIST, Japan, held on 8th May, 2017.

2. Actively participated in the National Conference on "**Recent Developments in Medical Biotechnology and Structure Based Drug Designing (RDMBSBDD)**" held at IIT Guwahati, organized by BSBE in the year 2015.

3. Actively participated in the symposium cum workshop on "**Advances in Computational Biology and Computer Aided Drug Design**" held at IIT Guwahati, organized by the Bioinformatics Infrastructure Facility (BIF), Dept of BSBE in the year 2015.

4. Actively participated in National Course on "**Theoretical and Pratical Aspects of Cancer Research**" conducted under the Technical Education Quality Improvement Programme sponsored by Minis try of Human Resource Development, Government of India in the year 2015.

5. Participated in "**34th Annual Convention of Indian Association for Cancer Research (IACR2015)**" for which Rajasthan Medical Council has granted 8 CME credit hours, held at Jaipur, India in the year 2015.

6. Actively participated in the workshop "**Flow cytometry data analysis**" organized by Dept of Biotechnology, IIT Guwahati in the year 2015.

7. Attended two day Indo-US clinical cytometry symposium and wet labs on "**Haematological Malignancies: Challenges and Management Strategies**" held at Dr S. Krishnamurthi Centre for Research and Education in Cancer, Cahchar Cancer Hospital and Research Centre, Silchar, Assam in the year 2014.

8. Participated in a two day workshop on "**Next Generation Sequencing and Data Analysis**" organized by Biotech Hub, Centre of Environment, IIT Guwahati in the year 2014.

9. Actively participated in the "**Indo-Japan DBT workshop for Fostering Research, Collaboration and Innovation in Translational Bioresources in Northeast India**" organized by DBT Program Support Center at Dept of BSBE, IIT Guwhati in the year 2012.

10. Actively participated in the DBT Program Support Project sponsored Short-term Corse on "**Advanced Techniques in Cellular and Molecular Biology**" held at IIT Guwahati organized by Dept of BSBE, in the year 2010.

Awards

Received '**Best Poster Presentation Award**' for the paper entitled "Isoform-specific Targeting of Akt Kinase by Natural Inhibitors: An *In Silico* Approach" in Second International Conference on Nutraceuticals and Chronic Diseases, at Goa, India 2017.

Received '**Best Oral Presentation Award**' for the paper entitled "Dissecting the Specificity of Akt Kinase Isoforms in Oral Cancer and an *in silico* approach for Selective Targeting by Natural Agents" in "Ethno-medicine and Traditional Health Practices in Northeast region of India", at NIPER Guwahati, India 2018.

Annexure I

1. https://en.wikipedia.org/wiki/Eucalyptus_globulus.
2. http://www.bcrc.firdi.org.tw/fungi/fungal_detail.jsp?id=FU200802270008
3. https://en.wikipedia.org/wiki/Sarcomelicope_simplicifolia#/media/File:Sarcomelicope_simplicifolia_leaves.jpg
4. http://circleforwards.blogspot.com/2013/01/garlic-allium-sativum-l-exterminate.html
5. http://resizeme.club/picresize-234_9.html
6. https://en.wikipedia.org/wiki/Vaccinium_corymbosum#/media/File:Vaccinium_corymbosum(01).jpg
7. http://all-free-download.com/free-photos/download/genuine-chamomile-matricaria-chamomilla-chamomile_272522.html
8. https://en.wikipedia.org/wiki/Pomelo#/media/File:Pomelo_.jpg
9. https://en.wikipedia.org/wiki/Scutellaria_baicalensis
10. http://www.eda-land.ru/specii/barbaris/yagody/
11. http://www.eda-land.ru/specii/barbaris/yagody/
12. http://www.fruitsvegetablecinnamonsuppliersrilanka.com/Sri_Lanka/Beetroot_Beta_vulgaris
13. https://economictimes.indiatimes.com/industry/cons-products/food/climate-change-may-hit-milk-products-by-3-million-tonnes-per-year-by-2020/articleshow/57187203.cms
14. https://commons.wikimedia.org/wiki/File:Brassica_napus_003.JPG
15. https://en.wikipedia.org/wiki/Toxicodendron_vernicifluum
16. https://en.wikipedia.org/wiki/Propolis
17. . http://all-free-download.com/free-photos/download/capsicum-pa-who-did-this-to-you_553049.html
18. http://www.innerpath.com.au/matmed/herbs/Alpinia~katsumadai.htm
19. https://en.wikipedia.org/wiki/Caraway
20. https://en.wikipedia.org/wiki/Cinnamomum_verum#/media/File:Cinnamomum_verum_spices.jpg

21. http://tropical.theferns.info/image.php?id=Hedychium+coronarium
22. https://en.wikipedia.org/wiki/Galium_odoratum
23. https://en.wikipedia.org/wiki/Physalis_peruviana
24. https://en.wikipedia.org/wiki/File:Curcuma_longa_roots.jpg
25. https://ast.wikipedia.org/wiki/Lonchocarpus_urucu
26. https://en.wikipedia.org/wiki/Viola_odorata
27. https://en.wikipedia.org/wiki/Onion
28. https://en.wikipedia.org/wiki/Dioscorea_villosa
29. https://en.wikipedia.org/wiki/Fragaria_vesca
30. https://en.wikipedia.org/wiki/Rheum_(plant)
31. https://en.wikipedia.org/wiki/Camellia_sinensis
32. https://en.wikipedia.org/wiki/Clove
33. https://en.wikipedia.org/wiki/Strawberry
34. http://dogalhayat.org/property/quercus-infectoria-mazi-mesesi-2/#prettyPhoto[iframes]/0/
35. http://www.biodiversityexplorer.org/plants/rosaceae/prunus_dulcis.htm
36. https://en.wikipedia.org/wiki/Garcinia_indica
37. https://en.wikipedia.org/wiki/Genista_tinctoria
38. https://en.wikipedia.org/wiki/Ginseng
39. https://en.wikipedia.org/wiki/Magnolia_grandiflora
40. https://en.wikipedia.org/wiki/Hypericum_perforatum
41. http://commons.hortipedia.com/wiki/File:Brassica_oleracea_var._italica_PDB.jpg
42. https://en.wikipedia.org/wiki/Tomato
43. https://en.wikipedia.org/wiki/Myristica_fragrans
44. https://en.wikipedia.org/wiki/Abronia_villosa
45. https://en.wikipedia.org/wiki/File:Starr_070313-5645_Trifolium_repens.jpg

46. https://naturesbuggy.com/mango-mangifera-indica-amba-aam-live-plant
47. https://en.wikipedia.org/wiki/Citron
48. https://en.wikipedia.org/wiki/Nerium
49. http://gernot-katzers-spice-pages.com/engl/Olea_eur.html
50. https://en.wikipedia.org/wiki/Watercress\
51. https://en.wikipedia.org/wiki/Maize
52. https://en.wikipedia.org/wiki/Black_pepper
53. https://en.wikipedia.org/wiki/Black_pepper
54. https://en.wikipedia.org/wiki/Plumbago_zeylanica
55. https://en.wikipedia.org/wiki/Apple
56. http://www.apuliaplants.it/en/prodotti/vitis-vinifera-vite/
57. https://en.wikipedia.org/wiki/Basil
58. https://en.wikipedia.org/wiki/File:Bloodroot_(Sanguinaria_canadensis).jpeg
59. https://en.wikipedia.org/wiki/Silybum_marianum
60. https://en.wikipedia.org/wiki/Brassica_oleracea
61. https://en.wikipedia.org/wiki/Mandarin_orange
62. https://en.wikipedia.org/wiki/Nigella_sativa
63. https://en.wikipedia.org/wiki/Vanilla_planifolia
64. https://en.wikipedia.org/wiki/Humulus_lupulus
65. http://www.circulating-oils-library.com/en/essential-oils/ginger-essential-oil-zingiber-officinale
66. https://en.wikipedia.org/wiki/Carrot
67. http://localqueen.com/seedstores-precious-curcuma-aromatica-kasturimanjal-fresh-root-cuttings-tubers-for-growing-5-rhizomes/az/B00LMFOVZC

8/23/2018 Rightslink® by Copyright Clearance Center

Copyright Clearance Center RightsLink®

Home | Account Info | Help

Title: Verticillium balanoides, a nematode endoparasite associated with pine needles of collapsing Japanese red pine trees in Tsukuba

Author: Tsuneo Watanabe

Publication: Mycoscience

Publisher: Elsevier

Date: 2000

Logged in as:
nand kishor roy
IIT Guwahati
LOGOUT

Order Completed

Thank you for your order.

This Agreement between IIT Guwahati -- nand kishor roy ("You") and Elsevier ("Elsevier") consists of your license details and the terms and conditions provided by Elsevier and Copyright Clearance Center.

Your confirmation email will contain your order number for future reference.

printable details

License Number	4414641180049
License date	Aug 23, 2018
Licensed Content Publisher	Elsevier
Licensed Content Publication	Mycoscience
Licensed Content Title	Verticillium balanoides, a nematode endoparasite associated with pine needles of collapsing Japanese red pine trees in Tsukuba
Licensed Content Author	Tsuneo Watanabe
Licensed Content Date	Jan 1, 2000
Licensed Content Volume	41
Licensed Content Issue	3
Licensed Content Pages	3
Type of Use	reuse in a thesis/dissertation
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
Format	both print and electronic
Are you the author of this Elsevier article?	No
Will you be translating?	No
Original figure numbers	1
Title of your thesis/dissertation	Understanding the isoform-specific role of Akt kinase in the development of oral squamous cell carcinoma (OSCC)
Expected completion date	Aug 2018
Estimated size (number of pages)	150
Requestor Location	IIT Guwahati Dept of BSBE, IIT Guwahati guwahati, assam 781039

https://s100.copyright.com/AppDispatchServlet

8/23/2018 Rightslink® by Copyright Clearance Center

	India Attn: nand kishor roy
Publisher Tax ID	GB 494 6272 12
Total	0.00 USD

ORDER MORE CLOSE WINDOW

BIOGRAPHY

Nand Kishor Roy was born and brought up in Shillong (Meghalaya) on 20th August, 1987. He completed his Matric examination (10th) in 2002 and Intermediate (12th) education in 2004 from KV Happy Valley, Shillong affiliated to Central Board of Secondary Education (CBSE). Later, he joined Sankardev College, Shillong affiliated to North Eastern Hill University (NEHU) and graduated in the year 2007 with Zoology as an honors subject. After qualifying university entrance test, he joined NEHU for his Master's in Biotechnology and Bioinformatics and completed in 2009. Subsequently, he worked as a trainee in the Indian Council of Agricultural Research (ICAR) and JK Agri Genetics Limited (Biotech Industrial Training Programme; BITP) during the year 2009. Successively, he joined PhD at IIT Guwahati under the supervision of Dr. Ajaikumar B Kunnumakkara in July 2010 in Dept of Biosciences and Bioengineering. His PhD work was mainly focused on the understanding the isoform-specific role of Akt kinase in the development of oral squamous cell carcinoma (OSCC). He successfully defended his thesis on 20th August 2018. He also shares his research interest in evaluating the anticancer potential of natural agents and exploring the chromosomal rearrangements in cancer. He has published in many peer-reviewed international journals and also edited a monograph "Fusion Gene and Cancer". He has also qualified national level competitive exams such as CSIR-NET (conducted by Council of Scientific & Industrial Research) and GATE (Conducted jointly by IISc and 7 IITs on behalf of the National Co-ordination Board– GATE, Department of Higher Education, Ministry of Human Resource Development, Government of India).