INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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SHORT ABSTRACT

Cancer cells are known to lack regulation of cell proliferation due to the aberrant behavior of a myriad of signaling pathways. It is a disease of "abnormal homeostasis" mediated by defects in intra-, extra-, and intercellular forms of communications. Intercellular communication between cells is achieved with the help of gap junctional intercellular communication (GJIC). GJIC plays a crucial role in maintaining cell-cell homeostasis by keeping growth control signals at equilibrium among GJIC connected cells. The majority of neoplastic cells have less number of gap junctions, smaller in size, express less connexin (Cx), and have reduced GJIC as compared to normal cells. A Gap junction (GJ) channel consists of two juxtaposed Cx hexamers. Connexin-43 (Cx43), a tumor-suppressor gene, is one of the most abundant Cx proteins and ubiquitous in many tissues. A plethora of studies demonstrates the role of Cx43 in regulating tissue homeostasis through channel dependent as well as independent manner.

The present thesis aims to exploit the gap junction-dependent as well as independent anti-tumour property of Cx43 in combination with the plant based, semi-synthetic, anti-malarial drug called artesunate (ART). The GJIC deficient cell line was selected and the forced expression of Cx43 was performed by two different means – using gene therapy and by the inducible expression of Cx43 using drugs. In **Chapter 1** (review of literature), the burgeoning field of cancer gene therapy has been delved into. Essentially, the role of GJIC in cancer cells, their potential clinical usage as well as the advantages they hold over current modes of cancer therapy, have been encompassed in this chapter. Specifically, the GJ forming protein named Cx43 was discussed and the effect of Cx43 re-expression in Cx43

deficient cancer cells was evaluated. The GJ-dependent as well as independent mode of anti-tumour property mediated by Cx43 and the role of GJIC in mediating 'bystander effect' in combination with chemotherapeutic drugs was also discussed in this chapter. Gene therapy as well as histone deacetylase inhibitor (HDACi), more specifically 4-phenylbutyrate (4-PB) was used to established GJIC in cancer cells and combination therapy with the ART has also been discussed in this chapter.

In **Chapter 2,** the establishment of functional GJIC by cloning and expression of Cx43 has been reported. Further, the therapeutic implications of Cx43 in enhancing the tumor suppressing activity of artesunate via gap junction-dependent as well as independent pathways in human breast cancer cells were discussed. The pathway by which Cx43 showed the GJ independent anti-tumour activity has been deciphered. GJIC mediated bystander cell death after treatment with ART and the transfer of ROS between the neighbouring cancer cells not exposed to ART was demonstrated by performing co-culture experiment.

In **Chapter 3,** the use of 4-PB in forceful expression of Cx43 and its synergistic interaction with ART both in MCF-7 cells as well as in DLA bearing mice was demonstrated. Re-expression of Cx43 in MCF-7 cells leads to the regulation of anti-tumour proteins, which further enhanced the dose dependent cytotoxicity of ART. Moreover, 4-PB attenuated the mRNA and proteins expression of the crucial DNA damage response (DDR) elements of nonhomologous end-joining (NHEJ) pathway and the central DDR transducer proteins, consequently enhancing the DNA damaging effect of ART. Combination therapy showed improvement in the life expectancy of the treated mice and a prominent reduction in the tumour volume without interfering with the normal biochemical, haematological and histological parameters of the mice. Overall, in chapter 3, a novel potential combination therapy in which 4-PB potentiated the cytotoxicity of ART synergistically and provided a promising combination drug for an effective cancer therapy was explained.

Chapter 4 reported the sub-cloning of *E. coli* cytosine deaminase (CD) and its mutants in mammalian expression vector. The mutants have been previously designed in our lab using *in silico* mutagenesis. Out of several mutants, F186W mutant was selected based on *in vitro* experimental data. F186W mutant has previously showed enhanced binding affinity towards prodrug 5-FC as compared to the natural substrate cytosine, *in vitro.* The chapter 4 dealt with the expression and functional analysis of CD and F186W mutant in A549 cells after transfection. Experimental results of *E. coli* cytosine deaminase mutant in A549 cells had validated *in silico* and *in vitro* predicted data. Further, the potency of the mutant was improved by co-transfecting it in the MCF-7 cells expressing Cx43. The F186W-Cx43 mutant required a much lower dose of 5-FC to reach its IC50, thus minimizing the systemic side effects of large doses of 5-FC as required for wtCD and F186W alone. The overall improvement in the cytotoxic activity of the F186W mutant in conjunction with the Cx43 has been reported in this chapter.

In **Chapter 5,** the fabrication of a versatile novel 4-PB bound composite nanoparticles has been demonstrated. In this approach, silver nanoclusters (AgNCs) embedded chitosan nanocarrier were utilized for analysis of binding, tracking, and sustained release of 4-PB from the nanocarrier. Inferences were drawn based on luminescence detection using fluorescence spectrometry, flow cytometry, and high-end confocal microscopy. The stability imparted by the nanocarrier to the 4-PB resulted in enhanced anti-tumor efficacy. Most importantly, this method implied improved cancer therapy without dose-dependent side effects of the drug, as the AgNCs ensured the stability of the 4-PB in cancer cells. The efficacy of the 4-PB bound nanocarrier was also evaluated in spheroids based model. Time-dependent uptake of luminescent silver clusters was demonstrated by confocal microscopy and flow cytometry.

In the final section on **Conclusions and Future Prospects**, the anti-tumour mechanism of Cx43 has been delineated and its application with the other therapies have been highlighted. In brief, the functional GJIC has been established in GJ devoid cells using 4-PB drug or Cx43 gene cloning. The GJ-dependent as well as independent anti-tumour activity of the Cx43 gene has been investigated extensively in conjunction with the drug ART. The Cx43 also enhanced the CD/5-FC suicide gene therapy system and showed an effective gene therapy module. Binding with noble metal nanocarrier enhanced the efficacy of the 4-PB and enabled luminescence based binding, imaging, and uptake studies. The current therapeutic approach holds immense promise in the field of *in vivo* cancer therapeutics.