



INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

Name of the Student : Archita Ghoshal
Roll Number : 11610607
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Name of Thesis Supervisor(s) : Prof. Siddhartha Sankar Ghosh
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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. One such pathway is the Wnt signal cascade, which is one of the multiple facets responsible for the upregulation of several pro-proliferative genes in cancer. In non-cancerous cells, this Wnt pathway is blocked by a family of secretory glycoproteins playing a role in cell growth arrest, called the secreted frizzled-related proteins (sFRPs). However, these sFRPs are typically silenced in cancer due to promoter hypermethylation.

The current thesis aims to exploit the anti-proliferative role of sFRPs to regulate cancer cell proliferation *in vitro* by targeted protein therapeutics. More specifically, the two most promising sFRPs, viz., sFRP1 and sFRP4, were selected for the development of novel co-therapeutic and theranostic models for combating cancer in cell culture model. In **Chapter 1**, the burgeoning field of recombinant protein therapeutics has been delved into. Essentially, the role of recombinant proteins in regulating signal networks, their potential clinical usage as well as the advantages they hold over current modes of cancer therapy, have been encompassed in this chapter. More specifically, the role of sFRPs in regulating cell growth by modulation of the Wnt pathway has been deliberated. The use of nanomaterials for stability and sustained release of the recombinant proteins, while enabling their tracking and delivery to desired sites, has also been discussed herein. In **Chapter 2**, the cloning, expression, and purification of recombinant human sFRP1 using *Escherichia coli* has been reported. Further, the therapeutic implications of the GST tagged sFRP1, alone and in combination with conventional chemotherapeutic drugs, viz. cisplatin and doxorubicin, in two different cancer cell lines were deciphered by cell viability assay and cell cycle analysis. In **Chapter 3**, the fabrication of a versatile novel sFRP1 bound composite nanoparticles has been demonstrated. In this approach, gold nanoclusters-embedded nanoparticles were utilized for analysis of binding, tracking, and sustained release of sFRP1 from the nanoparticles. Inferences were drawn based on luminescence detection using fluorescence spectrometry, flow cytometry, and high-end deconvolution microscopy. The stability imparted by the nanoparticles to the protein resulted in enhanced anti-tumor efficacy. Most importantly, this method implied targeted cancer therapy, as the protein component sFRP1 ensured the targeting of the Wnt pathway in cancer cells. Studies using Western blotting and semi-quantitative PCR-based expression of

essential molecules of the Wnt pathway validated the molecular mechanisms. A co-therapy module with cisplatin was also exhibited by extensive cell-based assays for further augmentation of anti-cancer activity. In **Chapter 4**, the cloning of human sFRP4 from a novel source- ACHN renal carcinoma cell line has been reported, along with its bacterial expression, purification, characterization, and anti-proliferative effect. Expression analysis of downstream Wnt pathway molecules by Western blotting and quantitative real-time PCR showed that the functional recombinant sFRP4 inhibited the canonical Wnt signaling. Improved cellular responses upon combination therapy with cisplatin/doxorubicin were revealed by cell cycle analysis and dual staining-based assays. In **Chapter 5**, the theranostic potential of a sFRP4 bound silver nanoclusters-embedded nanoparticle platform has been illustrated. While the extraordinary luminescence properties of the nanoclusters enabled binding, imaging, and uptake studies, Western blotting documented the targeting of Wnt signaling by the GST-sFRP4 released from the NPs. Furthermore, co-therapeutic benefits of sFRP4 and silver clusters were examined. The effects on cancer cells were elaborately delineated by cell viability assays, flow cytometry-based cell cycle, apoptosis detection assays as well as microscopy-based experiments. Time dependent uptake of luminescent silver clusters was demonstrated by confocal microscopy and flow cytometry. In the final section on **Conclusion and Future Prospects**, the thrust areas of this study have been highlighted and the importance of these findings has been emphasized.

In brief, human sFRP1 and sFRP4 have been cloned and expressed in bacterial system. The expression and purification procedure was extensively optimized to obtain the proteins in soluble form. Glutathione agarose-based affinity chromatography was used to purify both the recombinant proteins. Thereafter, the therapeutic efficacy of the proteins on mammalian cancer cells was determined, alone as well as in co-therapy module. Binding with noble metal nanoclusters enhanced the efficacy of the proteins and enabled luminescence-based binding, imaging, and uptake studies. Further the functionality of the proteins was evaluated by their role in inhibiting the Wnt/ β -catenin signaling pathway and induction of apoptosis of the cells was investigated by various assays. The current therapeutic approach holds immense promise in the field of *in vivo* cancer theranostics.