Summary of Doctoral Thesis

Therapeutic polymeric nanoparticles for targeting and destruction of cancer stem cells (CSCs)

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The discovery of cancer stem cells (CSCs) in acute myeloid leukemia paved the foundation for detailed investigation of its presence in many solid tumors of the brain, breast, and pancreas among the few. These stem cells in the long run have the opportunity to accumulate the mutations required for malignant transformation owing to their unlimited division potential. CSCs display certain properties such as high expression of drug efflux transporters, abnormal cellular metabolism, deregulated SRPs, acquisition of epithelial-mesenchymal transition (EMT) and extensive DNA repair mechanisms. Since CSCs are associated with chemo, radio resistance and cancer recurrence, it prompted us to investigate the possible therapeutic strategies for targeting and destruction of CSCs. One of the current advancements in this direction is the nanoparticle mediated targeting of CSCs as a novel treatment modality. Nanotechnology in the past few decades have witnessed tremendous development in cancer diagnosis and therapy. It enables the creation and manipulation of the biological and physicochemical properties of materials at nanoscale level (in the size range of 10nm to 100nm) to facilitate more efficient drug targeting, drug delivery, diagnosis and imaging in the arsenal of new age cancer therapy. Looking into various facets of nano technological advancements in the field of cancer therapy, it can be harnessed to the best of its ability for targeting the drug resistant CSCs, the major cause behind cancer relapse and failure of conventional cancer treatments.

The essence of this thesis entitled "**Therapeutic polymeric nanoparticles for targeting and destruction of cancer stem cells (CSCs)**" underlies the approach of development of a prospective strategy to target CSCs with the aid of nanotechnology. Since CSCs possess certain properties specific to them we intend to exploit three aspects in order to target these fraction of cells. We have divided our thesis into six chapters that is described as below:

Chapter 1 is entitled "**Therapeutic Strategies and Application of Nanotechnology for Elimination of Cancer Stem Cells: Introduction & Review of Literature**" and discusses regarding the various aspects of CSCs that are being targeted currently to develop anti-CSC therapies and application of nanotechnology for the improvement of such therapies. Extensive cancer research in the past few decades has identified the existence of a rare subpopulation of stem cells in the grove of cancer cells. These cells are known as the cancer stem cells (CSCs) marked by the presence of surface biomarkers, Multi drug resistance (MDR) pumps and deregulated self-renewal pathways (SRPs). They play a crucial role in provoking cancer cells leading to tumorigenesis, its progressive metastasis, relapse and drug resistance. Current treatment strategies in cancer, fails to detect and differentiate the CSCs from their non-tumorigenic progenies due to absence of specific biomarkers. Now it has become imperative to understand complex functional biology and characteristics of CSCs in order to design improved treatment strategies to target them. This chapter is divided into sections: Section 1.1 reviews the potential therapeutic targets of CSCs with thorough experimental findings. Section 1.2 discusses the application of nanotechnology thus adding another dimension for the improvement of the existing anti-CSC therapies.

Chapter 2 comprise of "**Instrumentation**" techniques that we have utilized in our work. Characterization of nanomaterials is the basic study to gain insight of the material's composition, structure and properties including its physical, chemical, electrical, magnetic to name a few. The various characterization techniques not only provide in-depth analysis on the dynamic nature of different nanomaterial's but also to some extent how their properties change in different environments. Two fundamental types of characterization methods generally exist: imaging by

microscopy and analysis by spectroscopy. This chapter deals with the different instrumentation techniques such as microscopy, spectroscopy, which are used to analyze the surface morphology, surface chemistry, size and charge and other properties of the nanomaterials. Other analytical instruments needed to study the application of nanomaterials in biological studies are also described in this chapter. We have incorporated various techniques such as SEM, TEM, phase contrast microscopy, confocal laser scanning microscopy, x-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy (FTIR), UV-visible spectroscopy, dynamic light scattering (DLS) to characterize the nanoparticles.

Chapter 3 is entitled "**Poly-lactic-co-glycolic acid Nanoformulation of Small Molecule Antagonist GANT61 for Cancer Annihilation by Modulating Hedgehog Pathway**" where we have targeted the Hedgehog pathway reported to be aberrantly active in most of the cancers and CSCs by encapsulating a small molecule antagonist GANT61 inside PLGA NPs. GANT61 is a small molecule GLI antagonist known to show superior anticancer effects by blocking the Hedgehog pathway in most of the cancers reported. However it is poorly soluble in water. So, DMSO is mostly used as a vehicle, which hinders its clinical translation. We have reported the nanoformulation of GANT61 by using PLGA as its carrier for the first time as an alternative drug delivery system for anticancer therapy. Our GANT61 PLGA NPs synthesized through single emulsion solvent evaporation method are completely soluble in water compared to free GANT61 with high encapsulation efficiencies and the results are highly reproducible. Single emulsion solvent evaporation is a simple and efficient technique employed to encapsulate many hydrophobic anti-cancer drugs and can be easily scaled up. We have performed physicochemical characterization on the GANT61 PLGA NPs by using various techniques including SEM, TEM, XPS and

UV-vis spectroscopy. In vitro drug release studies revealed sustained release of GANT61 from the PLGA NPs. In vitro cytotoxicity assay of the GANT61 PLGA NPs elucidate an increased killing of the cancer cells than normal cells, which is an advantage over the free drug as it kills normal cells to some extent. GANT61 PLGA NPs also induced apoptosis in both the cancer cells in comparison to the normal cells. Since GANT61 is an effective GLI1 inhibitor, immunofluorescence analysis depicts the inhibition of GLI1 nuclear translocation in cancer cells when treated with GANT61 PLGA NPs. The GANT61 PLGA NPs were a novel approach to deliver the drug without raising any toxic side effects that is accompanied while using DMSO as a carrier. GANT61 NPs were successful in imparting cytotoxicity to cancer cells while posing no side effects to the normal cells and also reduced the CSCs proportion in cancer cell lines (HT-29 and MCF-7), as evaluated from tumorsphere formation assay and migration assay. From our results we inferred that PLGA is a more suitable nano carrier for delivering GANT61 to cancer stem cells as a novel anti-CSC therapy.

In Chapter 4 "**CD133 Aptamer Targeted Delivery of GANT61 PLGA NPs to Colorectal Cancer Cells**" the synthesized GANT61 PLGA NPs were further modified to mediate aptamer based targeting of CSCs where we have conjugated A15 aptamer onto the surface of GANT61 PLGA NPs that specifically targets AC133 epitope of the CD133 protein expressed by the CSCs in various solid tumors as reported. The conjugation of these aptamer with delivery vehicles mediate specificity towards tumor cells resulting in rapid internalization and improve therapeutic efficacy of the drug. This strategy could be exploited for targeting and identification of specific biomarkers that are expressed by cancer stem cells (CSCs) as well. In this work, we have developed CD133 aptamer GANT61 PLGA NPs to target colorectal cancer cells, since colorectal cancer is reported to be one of the lethal cancers

worldwide and the discovery of colorectal CSCs has added another dimension to the disease complexities. The GANT61 PLGA NPs were prepared by single emulsion-solvent evaporation method and further functionalized with CD133 aptamer (A15) by EDC/NHS technique. SEM, DLS and XPS techniques characterized the functionalized NPs for their size, morphology and surface chemistry. We also evaluated the in vitro cytotoxicity of the NPs in colon cancer cells by alamar blue cytotoxicity assay. The A15-GANT61 PLGA NPs showed successful conjugation of the aptamer on the surface of the GANT61 PLGA NPs. The in vitro cytotoxicity assay reported that the A15-GANT61 PLGA NPs imparted enhanced toxicity towards the colon cancer cells at a mid range concentration of 250 μg/ml, much lesser than the non-targeted GANT61 PLGA NPs. Further studies needs to be carried out with A15-GANT61 PLGA NPs to check their specificity against CD133+ colon cancer stem cells for future cancer therapy.

Chapter 5 titled "**PLGA Nanoparticles Co-delivers GANT61 and Curcumin for Enhanced Anti-Tumor Activity in Breast Cancer**" discusses the final strategy that we have employed to develop dual drug combination therapy encapsulating GANT61 and curcumin in PLGA NPs and checked in vitro anti-tumor activity in breast cancer. Breast cancer is one of the most lethal cancers affecting women worldwide and often curbed by limited treatment options available. This study aims to develop PLGA nanoparticles (NPs) containing combination therapy of GANT61 and curcumin to provide enhanced anti-tumor activity in breast cancer cells and mediates less cytotoxic effects to the normal cells. The dual drugs PLGA NPs are characterized using SEM, DLS and ATR-FTIR for their surface morphology, size and chemical interactions respectively. In vitro drug release was performed in physiological pH to study the release pattern of both the drugs. Following this the

single drug and dual drug NPs will be analyzed for their in vitro cytotoxicity in MCF-7 breast adenocarcinoma cell line and L929 normal cell line, and also by optical microscopy. Since MCF-7 breast cancer cells have the ability to form colonies in soft agar matrix, colony formation assay was carried out to check the inhibition on the formation of colonies by dual drug NPs. The dual drug nanoformulation had an average size of 256 d.nm with a smooth surface and spherical morphology. GANT61 had a faster release profile than curcumin. Dual drug nanoformulation reportedly showed more cytotoxicity towards the MCF-7 breast cancer cells compared to both the single drug nanoformulations suggesting the synergistic effects of both the anti-cancer drugs. The MCF-7 cells when treated with dual drug nanoformulation showed no formation of colonies on soft agar matrix, which could be due to inhibition of the molecular targets of GANT61 and curcumin resulting into death of the cancer cells. Single drug & dual drug nanoformulation did not elicit any toxic effects on the L929 fibroblast cells during the study period. These dual drug nanoformulation offer a combination therapeutic strategy that could kill the CSC and bulk tumor cells in a single shot, eradicating the tumor mass and thus may prevent cancer relapse in the near future.

In Chapter 6 " **Conclusion**" we have compiled the concluding remarks of our research work. Our findings reveal an effective in vitro nanoformulations of GANT61 that holds a great prospect for future in vivo studies.