A Multifaceted Approach for Cancer Therapeutics

A Thesis

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By

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ABSTRACT

Cellular, genetic and epigenetic modifications leading to uncontrolled proliferation results in tumor mass formation. At tumor hypoxic core, cells barely proliferate resulting in drug-resistance. Effective monotherapies, as well as combination therapies, are being employed for remission of such tumors. However, chemotherapeutic approaches have limitations mainly due to poor solubility of anticancer drugs, lack of selectivity and multi-drug resistance. Alternative therapeutic strategies including nanomedicine, may improve drug solubility, enhance stability in the circulatory system and reduce drug toxicity. Targeted delivery of anti-cancer drugs is of paramount importance for cancer treatment, as it helps in improving the overall therapeutic efficacy by minimizing killing of normal cells due to non-specific cytotoxicity of chemotherapeutic drugs. In the present study, investigations have persuaded to standardize the procedures for development and use of different nanocarriers (NCs) for drug delivery.

The present thesis aims to exploit the nanocarriers based treatment approaches for cancer therapeutics. In Chapter 1, the review of literature covers fundamentals of nanoparticle-mediated targeted drug delivery to cancer cells. Different types of nanoparticles such as liposomes, dendrimers, micelles, hydrogel, inorganic or polymeric nanoparticles with potential therapeutic applications have been mentioned. Tumor cells specific biomarkers mainly overexpressed in tumor cells such as, folic acid receptor (FAR) and transferrin receptor (TR) are mentioned for targeted delivery. Importantly, development of nanocarriers based on Selenium and RBC membrane have been discussed in this chapter. Finally, key research areas and salient features of the current thesis have been illustrated.

In Chapter 2, development of a novel nanocarrier (NC) based drug delivery system for chemotherapeutic drug paclitaxel (PTX), by employing pluronic F-127 stabilized selenium nanoparticles (SeNPs) has been reported.

Successful delivery of PTX, PTX-loaded SeNPs as well as anti-proliferative activity against lung, breast, cervical and colon cancer cells have been demonstrated. Flow-cytometry based cell cycle analyses of PTX-SeNPs treated cells revealed G_2/M phase arrest in a dose-dependent manner leading to apoptosis. This chapter also describes the cellular mechanism of apoptosis in treated conditions via induction of reactive oxygen species (ROS), disruption of mitochondrial membrane potential (MMP) and activation of caspases.

In Chapter 3, a more selective and targeted therapy have been reported for the tumor cells with high expression of FAR and mutations in the genes of the MAPK pathway. MDAMB231 (breast cancer) cells have G13D and G464V mutations in RAS and BRAF genes, respectively, while A375 (melanoma) cells possess V600E mutations in BRAF. These mutations cause constitutive activation of the MAPK signaling, which leads to uncontrolled proliferation and cancerous growth. The chapter describes use of combination therapy module involving FAR-targeted SeNPs (FA-SeNPs) and MAPK inhibitor PD98059 (PD98).

Chapter 4 demonstrates the use of red blood cell (RBC) membrane for anti-cancer drug delivery. The nanocarriers have been designed for the delivery of chemotherapeutic agent (Curcumin/Cur) and hypoxia activating molecule (Tirapazamine/TPZ). Initially, Cur and TPZ were loaded on biodegradable PLGA NPs, and these drug-loaded PLGA NPs were coated with RBC membrane by extrusion. This chapter describes characterization and the functional assays of the drug-loaded NPs on monolayer as well as hypoxic spheroids. It was found that the RBC membrane provided improved stability and biocompatibility. Functional assays on cells and multicellular spheroids (MCS) suggested facile uptake of these NPs in cancer cells resulting in synergistic activity. Finally, the mechanism of the action of cell death has been elucidated.

In Chapter 5, the therapeutic efficiency of the transferrin bound RBC membrane-coated PLGA NPs to deliver doxorubicin and methylene blue was studied for chemo- and photodynamic therapy. PLGA NPs loaded with doxorubicin and methylene blue were extruded with RBC membranes, forming membrane coated PLGA NPs. To achieve target-specific delivery to tumor cells, transferrin was conjugated onto RBC membranes before extrusion. Transferrin-bound, doxorubicin, and methylene blue loaded RB-NPs (TF-RB-NPs) were characterized and tested on the cells overexpressing transferrin receptors.

The final section on conclusion and future prospects summarizes the findings and major leads of the current thesis work. The experimental evidence demonstrated the importance of targeted delivery of selenium nanoparticles and RBC membrane coated nanocarriers in the modulation of cellular signaling leading to annihilation of cancer monolayer cells as well as spheroids. Inhibition of MAP kinase pathways, treatment of hypoxic spheroids will provide a better strategy for development of successful combination therapy to combat drug-resistant tumor.