Abstract

Immunotherapy is a biological therapy that harness immune system to target, recognize, and kill the cells which is either transformed or infected. Immunotherapies are divided into passive or active based on their mechanism of action to activate the immune response. Active immunotherapy aims to induce host immunity against any disease or infectious agents. Few commonly used active immunotherapies are vaccines, cytokines etc. Furthermore, passive immunotherapy involves the administration of immune system components such as T-cell and monoclonal antibodies (mAbs) to target infected or cancerous cells. Various immunotherapy approaches including vaccines have successfully been used for the treatment of cancer and infectious diseases like Covid-19.

Despite the use of different strategies of immunotherapy in clinics or preclinical models, mortality related to cancer remain one of the leading cause of death worldwide. The failure of most of the treatment is associated with tumor promoting chronic inflammation (TPI) which supports the development and metastasis of malignant cells. Owing to the interesting connection between cancer and inflammation, neutralization of TPI seems to be an eminent approach for a more proficient anticancer treatment. There are different approaches to neutralize TPI- a. Boost of anticancer pathway, b. Reprogramming/depletion of immune cells, and c. Inhibition of pro-cancer inflammation. However, for an efficient neutralization, targeting one of them is not enough, hence a combinatorial approach is needed to combat TPI. To this end, in this thesis, various therapeutics were developed to neutralize TPI, where a. Dendritic cell (DC) based vaccine was developed to boost the anticancer pathway; b. Reprogramming/depletion of immune cells was accomplished by using CSF-1R inhibitor or a nano-formulation of recombinant IL-12; and c. Inhibition of pro-cancer inflammation was achieved by inhibiting induced PD-L1 expression. Specifically, this thesis describes three approaches of combinatorial immunotherapy for Cancer:

1. Development of tumor antigen presenting DC derived extracellular vesicles (Dex/DEV) as cancer vaccine and its synergistic effect with CSF-1R inhibitor, PLX-3397: Here we isolated Dex from bone marrow derived DCs which were activated with tumor antigen. We found that targeting CSF-1/CSF-1R signaling improves the in vivo efficacy of Dex where both Dex and PLX-3397 work in a collaborative manner by overcoming the disadvantages associated with monotherapies.

2. Use of DEVs as delivery carrier for IL-12: We encapsulated IL-12 into DEV and the nanoformulation (DEVIL) showed an improved plasma exposure, increased the tumor

accumulation of IL-12, which eventually averted immunosuppressive microenvironment and systemic toxicity. This nano-formulation can be further explored to deliver other cytokines to enhance the efficacy while minimizing the dose-limiting toxicities.

3. Repurposing of Ponatinib as PD-L1 inhibitor: Here we have shown that Ponatinib can bind to PD-L1, inhibit PD-1/PD-L1 interaction, and delay the tumor growth by modulating antitumor immunity. Further studies revealed that Ponatinib can also inhibit the induced PD-L1 overexpression by regulating HIF-1α.

4. Combinatorial approach: A combination treatment including Dex, PLX-3397, and Ponatinib was used to address all the three modalities for the neutralization of TPI. The combination treatment reduced the tumor growth by modulating the tumor microenvironment and systemic immunity which eventually resulted into a complete remission of tumors in murine colon carcinoma.

Similarly, for the prevention of Covid-19, several vaccines have been developed and got approved by FDA. However, most of the developed vaccine are mRNA or virus based which are associated with disadvantages such as poor stability and immunogenicity. Hence, to enhance the stability as well as the immunogenicity, a delivery vehicle can be used which will also add to the therapeutic value. Therefore, this thesis also describes the harnessing of immune system for the treatment of Covid-19. In this study, the extracellular vesicles isolated from DCs were used as cell-free vaccine. Here we demonstrated that Spike protein delivered by DEVs enhances the immunogenicity of free protein and induces humoral immunity by producing neutralizing antibodies. A one-tenth Spike protein equivalent dose of DEVs was able to induce a comparable level of humoral and cellular immunity.