Public Abstract

Distant metastases of the breast cancer can arise immediately following the diagnosis of the primary tumor or months/years afterwards. Distant metastasis to the liver are considered the terminal stage of the disease and found in 35% of patients with metastatic breast cancer and >50% of patients with distant metastasis. Liver metastasis of breast cancer are characterized by a very poor median survival of 1–14 months, as compared to metastases at other sites (e.g. isolated soft tissue metastases have a median survival of > 50 months, bone 33–48 months). In the majority of cases liver metastases cannot be resected and, currently, there are no efficient therapies that can guarantee a cure for the metastatic breast cancer. Thus, new therapeutic approaches are necessary. The failure to treat liver metastasis is related largely to the fact that the drug do not reach tumor cells in the liver in sufficient therapeutic concentrations. Since the liver itself is enriched with blood vessels, metastatic lesions may survive from the surrounding vessels, without growing their own. Therefore, unlike other tumor metastases, which are typically full of blood vessels, tumors in the liver lack functioning blood vessels, preventing efficient delivery of the drugs. On the other hand, macrophages (cells of the immune system) represent one of the main cell population in the liver (~10% of total cell) can play crucial role in modulating therapeutic responses.

Macrophages are a flexible cell population and their role and function can be modulated (or polarized) depending on the factors available in their surroundings. Typically, activated macrophages, or so-called M1 macrophages promote inflammatory responses that inhibit cancer growth. However, cancer cells release factors into their surroundings that cause polarization of the macrophages in the vicinity towards the tumor-supporting subset, so-called M2 macrophages. In our previous study, we have shown that when tumor-supporting M2 macrophages were reprogrammed into the M1 subset, they can cause the cancer cell killing.

In this study we will utilize gene modification to reprogram macrophages into the tumor-fighting M1 macrophages. Macrophage polarization is a reversible process that depends on their environment. In order to enable sustainable reprogramming, we need to induce stable genetic changes in this cell population. In this regard, the recently discovered clustered regularly interspaced short palindromic repeats (CRISPR) will be very useful in achieving our aims for macrophage reprogramming. CRISPR technology has enabled simple and specific gene targeting, however there are still limitations in their in vivo utilization due to non-specific cell targeting.

To enable CRISPR delivery towards macrophages we will utilize nanomedicine such as liposome. This technique will take advantage of the fact that macrophages also play significant role in processing of drug loaded into nanocarriers. Since macrophage's main task is to ingest foreign antigen, most of nanocarriers will be taken up by macrophages. We have previously utilized nanocarriers loading chemotherapies, which can accumulate in the liver macrophages. Our observation showed that nanocarriers enabled an increase in uptake by the surrounding macrophages in the liver metastases, increasing the concentration of the drug near the tumor cells. Both the increase of drug concentration in the lesion and macrophage reprogramming synergistically boosted the efficacy of the drug delivered via nanocarriers. Based on these results we concluded that macrophages are excellent targets for drug targeting in the liver metastasis.

The main advantages for utilizing nanomedicine for this purpose are: 1) nano-size enhances the particle uptake by professional phagocytes (macrophages); 2) nanocarriers enable protection of gene editing material from plasma and enzymes in circulation; 3) the proposed drug carrier class, liposomes, is currently used in the clinic and has been proven to be safe and efficient.

In this study we will verify the effect of gene editing in the 3D model of liver metastasis. We will evaluate and optimize the CRISPR system for macrophage reprogramming and their effect to the tumor growth before moving to a complex animal model. In the animal model, we will assess the accumulation of the nano-drug in the cancer lesions, as well as their efficacy in inhibiting tumor growth.

Our approach is to utilize the patient's own immune system and modulate it to fight the cancer cells, which, in combination with other means of immunotherapy will cause total eradication of cancer cells and prevent its recurrence. This approach can change the treatment strategy in breast cancer liver metastasis, and can be expanded to other metastases.