PROJECT SUMMARY

The survival rate of colorectal cancer and breast cancer as measured by Mortality to incidence ratios (MIRs) is worse among American Indian and Alaskan Native (AI/AN) population as compared to the white population. AI/AN individuals are more likely to be diagnosed with late-stage disease and this leads to poor treatment outcomes and increased mortality. These disparities in cancer within the AI/AN population is compounded by resistance to existing therapies and a high relapse rates among patients. Thus, there is a need to develop novel therapies to cure treatment resistant breast cancer. This study aims to alleviate these disparities faced by the AI/AN population by testing out a new cellular therapeutic approach to treat cancer. NK cells are a key component of the innate immune system and play an important role in early immunity to cancer by killing a variety of tumors. Adoptive transfer of NK cells and NK cell lines is a significant mode of cellular therapy that is being tested in various clinical trials to treat cancers. Efforts are being made to enhance the cytotoxic effects of NK cells and NK cell lines by treating them ex-vivo with different modulating agents. In our recent study, we found that NK cells when treated with reovirus become activated and show an enhanced cytotoxicity against tumor cells. In this project, we will test the ability of reovirus to increase the cytotoxicity of a NK cell line, NK-92MI, against human colorectal cancer cell line-DLD-1. Subsequently, we will perform an adoptive transfer of the reovirus activated NK-92MI cells to treat mice bearing DLD-1 tumors. Results from this study will increase our understanding of NK cell mediated antitumor response and help us develop new strategies to enhance NK cell cytotoxic functions for cancer treatment. Successful completion of this study will provide us with a novel cellular anti-cancer therapy with a potential to be translated into the clinic.

SPECIFIC AIMS

Reovirus is a promising unmodified double-stranded RNA (dsRNA) oncolytic virus, which specifically targets tumor cells with activated Ras. Reovirus has been tested in a wide range of preclinical models and has entered various clinical trials [1-3]. In addition to its direct cytotoxic effects on tumors, recent studies have demonstrated that reovirus can enhance anti-tumor immune responses. Reovirus-infected dendritic cells enhance NK cytotoxicity towards tumor cells[4]. Interestingly, reovirus infected mast cells enhanced NK cell cytotoxicity against a tumor cell line, mediated by cytokines TNF-α and type I and type III IFNs[5] [6]. Natural Killer cells are important for host anti-tumor immune responses [7-10].In addition to direct tumor cytotoxicity, NK cells can kill tumor cells through antibody depend cytotoxicity (ADCC), by recognizing antibody coated tumor cells through their surface Fc receptors [11, 12] . In our recent studies, we show that reovirus can directly activate NK cells in in-vitro assays. Importantly, NK cells when treated with reovirus exhibit enhanced cytotoxicity against different types of tumor cells. Treating tumor bearing T cell deficient athymice nude mice with reovirus showed an increased tumor regression and a large infiltration of NK cells in regressing tumors. Though, our preliminary studies demonstrate an enhanced tumor specific cytotoxicity of NK cells on exposure to reovirus, ***we lack direct in-vivo evidence to demonstrate that reovirus activated NK cells can efficiently kill tumors in mice. Importantly, the mechanism behind the increased cytotoxicity of NK cells needs to be investigated to better understand the immunomodulatory effects of reovirus on NK cells.*** We propose to address these deficiencies in our knowledge by performing the following experiments stated in the two specific aims. Here, we propose to use the NK-92MI cells, a NK cell line derived from a human NK cell lymphoma, to achieve our aims.

1) **To assess the cytotoxicity of reovirus treated NK-92MI cells against cancer cell lines.**
   In this aim we will first test if NK-92MI cells, a human NK cell line, when treated with reovirus will increase their activation and cytotoxicity like human PBMC derived NK cells. The cytotoxicity of reovirus treated, NK-92MI cells and human PBMC derived NK cells against the colorectal cancer cell line DLD-1 will be compared by performing a lactate dehydrogenase release based cytotoxicity assay. Expression of NK cell specific activation and inhibitory receptors, cytokines- TNF-α and IFNγ, and cytolytic enzymes- granzyme B and perforin will be analyzed by flow cytometry post reovirus treatment. We will block, the release of perforin with concanamycin A and activity of FasL using anti- human FasL antibody, to investigate the contribution of perforin and Fas mediated pathways in tumor cell killing. The ability of reovirus treated NK cells to kill different human tumor cell lines will be tested.

2) **To evaluate the in-vivo anti-tumor response to the adoptive transfer of ex-vivo reovirus treated NK-92MI cells into NOD.SCIDγ mice implanted with human tumor cell lines.**
   We will answer this question by injecting reovirus treated NK-92MI cells into NOD.SCIDγ mice bearing DLD-1 tumors. Mice will be followed for tumor progression and compared with the mice injected with NK-92MI cells. Adoptively transferred NK-92MI cells in the tumors and spleens of mice will be analyzed for the expression of activation markers, cytokines- TNF-α and IFNγ and cytolytic enzymes granzyme B and perforin by flow cytometry.

We anticipate that this study will demonstrate that NK-92MI cells can be activated by reovirus and exhibit an improved cytotoxicity against tumor cells. We will identify markers of activation - cytokines and cytolytic enzymes expressed on NK-92MI cells that will shed light on the mechanism involved in the increased cytotoxicity of NK cell on reovirus treatment. Importantly, we will be able to demonstrate that adoptive transfer of reovirus treated NK-92MI cells can enhance tumor regression in mice indicating that reovirus activated NK-92MI cells can be used in cellular therapy to treat cancers.