Assessing the Crosstalk between CD47 and SIRPa in Modulating Tumor cell growth

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Rationale:

In past research, the protein CD47 has been found to be overexpressed in multiple types of tumor cells. When overexpressed, CD47 is able to bind with SIRPa, commonly present on phagocytes such as macrophages, to avoid phagocytosis by avoiding innate immune surveillance. In many tumor cells, blocking the CD47 and SIRPa interaction has the ability to enhance phagocytosis. However, in many melanoma cells,this was not the case, and these same melanoma cells were found to express SIRPa themselves. So, the question arose as to if there was a biological function of the CD47 and SIRPa interaction within the cells that allows for the cell to remain viable, as well as avoid innate immune surveillance. This research is important because it expands on the growing field of cancer immunotherapy. If cells that overexpress CD47 are found to respond to a CD47 blockade, it can be one step towards removing the toxic aspects of chemotherapy from cancer treatment.

Research Question(s), Hypothesis(ES), Engineering Goal(s), Expected Outcomes:

Based on the background research of the effectiveness of a CD47 blockade of enhancing phagocytosis, the hypothesis is that our research will reveal a biological function of the CD47 and SIRPa blockade in maintaining cell viability and modulating tumor cell growth.

The procedure will entail three main parts, which are cell culturing, a cell viability assay, and flow cytometry. Firstly for cell culturing, there are three cell lines planned to be used: B16 (melanoma cell line), MC38 (colorectal carcinoma cell line) and RAW264.7 (macrophage cell line). They will be grown in a combination of RPMI media and FCS in order to optimize cell growth. After being grown, they will then be plated for three time conditions in 3 96 well plates. The time conditions that they will be treated for are 0 hours, 24 hours, as well as 48 hours. They will be treated with either no treatment, gemcitabine (a concentration of 1.6 µl/ml as the positive control), a 1µg/ml, 10µg/ml, or 20µg/ml dose of MIAP301 (a clone CD47 blocking antibody), or a 1µg/ml, 10µg/ml, or 20µg/ml dose of IGg2a (the isotype control drug) .

Following being dosed with the treatment, we plan to assess the cell viability using an MTT assay. CCK8 will be used to assess the viability of the cells. When performing the assays, we will follow the protocol for an MTT assay, but will use WST8 as a substitute for MTT. WST-8 will produce formazan dye on the presence of an electron carrier, which allows for the dehydrolization activity of the cells to be assessed. The shade of the dye will be directly proportional to cell viability. This can be measured by assessing the shade of dye using a Molecular Devices spectraMaxS i3 spectrophotometer set at a wavelength of 45o nanometers. This will provide an accurate assessment of cell viability which we can use to measure the change in cell viability from

the isotype control to the experimental groups.

Lastly, flow cytometry will be utilized as a second assessment of cell viability, and to examine the expression of some of the cells. Cells harvested from each time point after treatment will be stained for CD47, SIRPa, and Live/dead cells. They will be stained for 30 minutes, then fixed using the Foxp3-fixation kit (eBioscience, Cat 00-5521-00) for 25 minutes. They will later be stained with intracellular Ki-67 for half an hour to help measure the growth factor of cell proliferation. In order to effectively analyze our results, they will be run through a manual analysis using an LSR II machine, and then analyzed again using the FlowJo software.

Because this project contains some biological hazards, all of the project will be done in a laboratory setting, under a lab hood. All people working on the experiment will be constantly required to wear a lab coat, lab goggles, and gloves. Furthermore, they will be subject to research grade protocols for disposal of any dangerous materials.

Potentially hazardous biological agents research:

The two cell lines that have to be obtained will be B16-F10 (ATCC, Cat# CRL-6475) as well as RAW264.7 (ATCC, Cat# TIB-71). They will be obtained and disposed of in accordance to the Memorial Sloan Kettering BSL1 safety handbook.

Hazardous chemicals, activities, and devices:

The only hazardous chemical that will be utilized through this experiment is the chemotherapy compound gemcitabine. It will be used inside the lamina hood, and users will wear disposable gloves and use pipetting tips/tubes. This chemical can be used inside a BSL 1 laboratory, and the lab is set with all categories of biological hazardous disposal procedures and bins.

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