1. **Introduction**

Endoplasmic Reticulum Aminopeptidase 2, ERAP2, is located on chromosome 5q15 (Andres et al, 2010). ERAP2 is responsible for peptide trimming and functions in N-terminal trimming. ERAP2 is responsible for trimming HLA class I-binding peptides, which makes it an ideal protein to help measure immune responses of specific cells (Saveanu et al, 2005). The ERAP2 protein is encoded by the ERAP2 gene. This has not been proven yet, but not having the ERAP2 protein present can lead cancer to grow (Lee, 2017). To induce the proper immune response it is very important to have this peptide processing components: a peptide transporter (TAP), and ERAP2 trimming HLA class I proteins in most cells (Saveanu et al, 2005). The ERAP2 gene has two isoforms, ERAP2-K and ERAP2-N. In most cells, ERAP2-K and ERAP-N are present but majority of ERAP2 expresses the K isoform.

Melanoma is a type of skin cancer and kills 10,130 people annually. Most cell lines have both the K and N isoform of ERAP2 but only ERAP2-K is expressed. The N isoform of ERAP2 is genetically present but the protein cannot be expressed. In figure 1, it shows melanoma human cell line MRN-1 expresses low amounts of ERAP2 (Fruci et al, 2006).

Since ERAP2-N is not expressed in the melanoma cell line, MRN-1, the purpose of this experiment is to introduce ERAP2-N into the cell line and see which isoform, between N and K, induces a better immune response.

Figure 1: Shows melanoma cell line MRN-1 expresses low amounts of ERAP2 (Fruci et al 2006)

1. **Experiment**

The cell line that is chosen for this experiment is MRN-1, a melanoma human cell line that expresses low amounts of ERAP2. An ideal cell line for this experiment would not express ERAP2, but according to Fruci et al, no cell lines without ERAP2 have been found, yet. This cell line is ideal for this experiment because it has low amounts of ERAP2 and has a peptide transporter, TAP, which helps makes sure that the ERAP2 gene functions the way it needs to by transporting the peptides to the endoplasmic reticulum to go through the final peptide processing prior to loading onto the HLA molecule.

The goal of this experiment is to measure the lymphocytes in the melanoma cell line, MRN-1, and determine which isoform, ERAP2-N or ERAP2-K, induces a better immune response. ERAP2-N is not expressed in the cell line so it will need to be introduced exogenously by transfection using a pTracer-ERAP2-N vector. The western blot analysis will confirm the ERAP2 expression in MRN-1 and will also determine the amounts of K so the same amount of N can be introduced. Lymphocyte activation assay will be used to measure and compare the natural killer cell/T-cell activation level against ERAP-N and ERAP2-K expressed in MRN-1. Flow cytometry analysis will quantify lymphocyte activation and count all the activated natural killer and T cells.

Part A. Introduction of ERAP2-N into MRN-1

Following the transfection protocol in Alistair et al (2001), ERAP2-N will be introduced exogenously by the method of transfection using a pTracer ERAP2-N vector. MRN-1 cells will be plated in the appropriate media and will be left overnight in temperature of 37º C. A pTracer that includes ERAP2-N plasmid will be used to transfect the MRN-1with the ERAP2-N isoform.

Part B. Western blot analysis

Andres et al (2010) use western blotting to see the expression of ERAP2 in lymphoblastoid cell lines. Following their procedure of western blotting, it will confirm the expression of the ERAP2 protein and will confirm the expression of the ERAP2-N isoform in MRN-1. This method will also determine the amounts of ERAP2-K so the same amount of ERAP2-N can be introduced.

Part C. Lymphocyte activation assay and flow cytometry analysis:

Lymphocytes are white blood cells that are part of the immune system. Lymphocytes include natural killer cells, T-cells, and B-cells. Natural killer cells are immune cells that that can kill cancer cells and T-cells help the body fight cancer. When the body is trying to fight off an infection, lymphocyte activation occurs.

 For the lymphocyte activation assay, only CD8+ T-cells and natural killer cells will be measured. Natural killer cells/T-cells in ERAP2-N will be measured and compared with the natural killer cells/T-cells in ERAP2-K. Flow cytometry will quantify lymphocyte activation and only measure the activated natural killer cells/T-cells.

1. **Discussion**

There will be three possible results of this experiment: exogenously introduced ERAP2-N induces more natural killer cells/T-cells, ERAP2-K induces more activated natural killer cells/T-cells, or ERAP2-N and ERAP2-K have the same amounts of activated natural killer cells/T-cells. The purpose of this experiment is to determine which isoform of ERAP2, K or N, induces a better immune response. This will be determined by counting the activated natural killer cells/T-cells in each isoform. More natural killer cells mean that more cancer cells are killed.

 Evnouchidou et al (2012) mentioned that ERAP2-N trims peptides at a fast rate, about 165 times more than ERAP2-K. This can alter with the activation of the natural killer cells and T-cells. If ERAP2-N trims peptides at a faster rate than ERAP2-K, it may have provide a better immune response than ERAP2-K because if peptides are being trimmed at a faster rate that may trigger more natural killer cells and T-cells.

Having ERAP2-K in MRN-1 could maybe skew the results because having a cell line that doesn’t express both the ERAP2 isoforms would have been more ideal. Having a cell line with neither K nor N would have been more ideal because then the cell line would go through each step for each isoform and the results would be more accurate.

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