TITLE

"The Influence of Amyloid Precursor-Like Protein 2 and Invariant Chain on the Cell Surface Expression of Major Histocompatibility Complex Class I Molecules"

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INTRODUCTION

I did my research at the Eppley Cancer Institute in Omaha, Nebraska. Eppley Cancer Institute is affiliated with the University of Nebraska Medical Center. The institute has many students and faculty that research various topics in medical science including cancer. My research involved looking at a specific mechanism and associating proteins in this mechanism in breast cancer cells. My research advisor was Dr. Joyce Solheim.

The mechanism I looked at was antigen presentation in breast cancer cells. Major histocompatibility complex (MHC) molecules are important in the presentation of antigens to be recognized by T lymphocytes. T lymphocytes survey cells to look for abnormalities presented by MHC molecules. If a cell is recognized as infected or tumorous, it is killed by the T lymphocytes. This mechanism helps to stop the growth of tumor cells. Two chaperone molecules found in the antigen presenting process are amyloid precursor-like protein 2 (APLP2) and invariant chain. APLP2 is a type I transmembrane protein that is ubiquitously expressed (1) and plays a variety of cellular

roles: in mitotic segregation, in neurite outgrowth, and in epithelial cell migration (2-6). APLP2 is found in higher than normal amounts in cancer cells and is therefore a molecule of interest in cancer research. In previous studies, APLP2 has been found to have a down-regulatory (decreasing) effect on the cell surface expression of the mouse MHC class I molecule K^d.

The second chaperone molecule, invariant chain, is a transmembrane type II glycoprotein. Over-expression (more than normal amounts) of invariant chain via transfection has previously been found to have an up-regulatory (increasing) effect on cell surface HLA class I expression in T47D and MCF-7 human breast tumor cells (unpublished data). Both APLP2 and invariant chain bind to folded MHC class I molecules and are dissociated in the presence of peptide ligand (8-9). They also require β2-microglobulin presence in order for the association with the MHC molecule to occur (10).

In this study we extended previous findings on the effect of APLP2 on K^d to the human MHC class I molecules expressed by the breast tumor cell line MDA-MB 435S. In addition, we sought to determine the influence of down-regulating the expression of the endogenous invariant chain in the MDA-MB 435S cells. This will allow comparison of down-regulating invariant chain to previous results obtained with over-expression of invariant chain in breast tumor cells T47D and MCF-7.

By obtaining information about the influence of APLP2 and invariant chain on antigen presentation, methods can be constructed to exploit these mechanisms in favor of better recognition of tumor cells by T cells of the immune system.

METHODS

APLP2 Over-expression

We over-expressed APLP2 in the human breast tumor cell line MDA-MB 435S to compare the results to previous results using K^d mouse molecules. pCMV-Tag4A vectors containing APLP2 and empty control vectors were grown in DH5α bacteria following transformation. Plasmid DNA was retrieved and added to the MDA-MB 435S tumor cells via transfection using two different reagents, TransFectin and Effectene. G418/neomycin selection was added to the MDA-MB 435S cells to eliminate negative, non-transfected cells. Single cell sorting of the positively transfected MDA-MB 435S cells was done. MDA-MB 435S cells containing APLP2 and MDA-MB 435S cells containing the empty vector were then pelleted for immunoprecipitation with an antibody specific for the FLAG tag attached to the transfected APLP2. (The FLAG tag is a section in the vector that can be tested for to insure the success of vector transfer.) The immunoprecipitation eluate was used to perform an APLP2 Western Blot to determine successful transference of the vector into the tumor cells. By flow cytometry, the expression of MHC class I molecules (and MHC class II molecules, for comparison) was examined on APLP2 transfected MDA-MB 435S cells and on control cell lines as well.

Invariant Chain Down-regulation

We down-regulated invariant chain in the tumor cell line MDA-MB 435S to compare with previous down-regulating results in other human tumor cells. pRS vector containing shRNA inserts (CD74-65, CD74-66, CD74-67, CD74-68) and controls, pRS empty vector and pRS non-effective GFP, were grown in DH5α bacteria following

transformation. Plasmid DNA was retrieved and developed into complete retroviruses via transfection into a Phoenix packaging cell line. The Phoenix cell line allowed for the pRS vector to enter the MDA-MB 435S cells. Retroviruses were then harvested from the Phoenix cells and introduced to the MDA-MB 435S tumor cell line via transduction. Puromycin selection was added to the MDA-MB 435S cells to eliminate the negative, non-transduced cells. Positive transduced cells were then prepared for FACS (fluorescent-activated cell sorting) analysis to determine the levels of invariant chain and MHC class I.

RESULTS

The Western Blot results for the APLP2 over-expression showed successful over-expression was achieved by presence of bands at 110 kDa (Figure 1). Row 1 contained MDA-MB 435S cells alone. Row 2 contained MDA-MB 435S cells with empty pCMV-Tag4A vectors. Rows 3 and 4 both contained MDA-MB 435S cells with pCMV-Tag4A vectors carrying the extra APLP2.

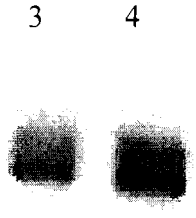

Figure 1. Western Blot of APLP2 over-expression in MDA-MB 435S cells. Over-expression is shown by the presence of bands at 110 kDa. Row 1 contained MDA-MB 435S cells alone. Row 2 contained MDA-MB 435S cells with empty pCMV-Tag4A vectors. Row 3 and 4 both contained MDA-MB 435S cells with pCMV-Tag4A vectors carrying the extra APLP2.

Evaluation by flow cytometry analysis revealed that APLP2 over-expression caused a decrease in cell surface expression of folded human MHC class I molecules (Table 1). The folded MHC class I molecule was detected by the W6/32 antibody. The HLA-DR antibody detected a subset of MHC class II molecules (for comparison).

Table 1. Flow cytometry analysis of MDA-MB 435S cells with over-expressed APLP2. MHC class I molecule expression was detected by W6/32 antibody. HLA-DR antibody detected MHC class II molecules.

Cell Lines	W6/32	HLA-DR
MDA-MB 435S	799.08	614.82
MDA-MB 435S pCMV-Tag4A	857.57	608.76
MDA-MB 435S pCMV-Tag4A-APLP2	678.23	628.81

Down-regulation of invariant chain and cell surface expressed MHC class I molecules in MDA-MB 435S cells was measured by flow cytometry analysis (Table 2). Cells were probed by an antibody against invariant chain to detect invariant chain levels and W6/32 antibody was used to detect MHC class I levels.

Table 2. Flow cytometry analysis of invariant chain and MHC class I molecule expression levels in MDA-MB 435S cells. MHC class I molecule expression was detected with W6/32 antibody.

Cell Lines	Invariant Chain	W6/32
MDA-MB 435S	23.08	1127.55
MDA-MB 435S pRS shRNA anti-CD74-65	7.82	583.41
MDA-MB 435S pRS shRNA anti-CD74-66	7.16	439.58
MDA-MB 435S pRS shRNA anti-CD74-67	8.94	662.88
MDA-MB 435S pRS shRNA anti-CD74-68	12.20	557.33

DISCUSSION

Over expression of APLP2 in human breast tumor MDA-MB 435S cells resulted in a decrease of cell surface expression of folded human MHC class I molecules. This coincides with previous findings of over expressed APLP2 down-regulating cell surface mouse MHC class I K^d molecules. An increase in cell surface expression of MHC class I molecules was observed in the MDA-MB 435S cells containing an empty vector. The cause of this is unknown.

Down regulation of invariant chain in human breast tumor MDA-MB 435S cells was achieved and resulted in down-regulation of cell surface expression of folded human MHC class I molecules. This coincides with previous results of over-expressed invariant chain causing up-regulation of MHC class I molecule expression.

By obtaining information about the influence of APLP2 and the invariant chain on antigen presentation, methods can be constructed to exploit these mechanisms in favor of better recognition and control of tumor cells by T cells.

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