Activation of CD22; a Potential Novel Marker for Ovarian Cancer

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Background

- CD22 is a gene that codes for a protein by the same name, which is normally only expressed on the surface of B-lymphocytes, where it participates in cell survival.
- Various studies prove evidence for CD22 being a potential biomarker for antibody treatment for lung cancer (Tusciano et al., 2012).
- This research is controversial as one study (Pop et al., 2014) was unable to specifically target CD22 with their selected antibody.
- Expression of CD22 is suspected in ovarian and pancreatic cancers due to a chromosomal abnormality common to each of these cancers.
- This protein acts as a potential biomarker for lung, ovarian, and pancreatic cancers, in which CD22 may be inappropriately expressed.
- CD22 may also participate in metastasis of these cancers to bone and lymph through CD22 homing receptor activity, providing a metastatic advantage to the cell.

Objectives

The purpose of this study was to:
- Determine if inappropriate activation of CD22 occurs in ovarian cancer cell lines
- Determine the expression patterns of CD22 on the surface of these cell lines compared to B-lymphocytes
- Characterize normal and aberrant CD22 receptor substrate interactions on bone marrow stromal cells

Methods

Tissue Culture

Cell lines maintained with preferred media under sterile conditions with a 5% carbon dioxide atmosphere at 37°C:
- PA-1 (Ovarian cancer cell line)
- CaOV-3 (Ovarian cancer cell line)
- SK-OV-3 (Ovarian cancer cell line)
- Ramos (B-lymphocytes; positive control)
- HS-5 (Bone stromal) cell line

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

- PA-1, CaOV-3, SK-OV-3, and Ramos cells were harvested for RNA, which was purified, quantitated using a nanodrop, and assessed for quality via gel electrophoresis (Figure 1a).
- Four different primer combinations were used for each cell line to optimize PCR; one primer combination that did not produce amplified cDNA was utilized as a negative control after repeat runs (Figure 1b).

Flow Cytometry (Figure 2)

Flow cytometry involves a laser-based technology in cell counting, sorting, and biomarker detection analysis. The flow analysis was performed on a Beckman Coulter Cytoflex. Analysis was performed by suspending cells in isotonic solution and staining with fluorescently labeled antibodies, and then analyzing the cell distribution.

All cell lines except CaOV-3 were analyzed using the same detector settings.
- Use of CD22-FITC antibody in each cell line assessed the frequency of CD22 surface expression. Ramos cells were used as a positive control.
- Anti-mouse IgG kappa isotype-FITC acted as a control for nonspecific binding by the antibody.

Results

Binding of CD22 to Bone Marrow Stromal Cells

- HS-5 (bone marrow stromal cells) and Ramos cells (B-lymphocytes) were added to a 96-well in a checkerboard titration and allowed to bind.
- The plate was flipped upside down overnight to allow unsaturated Ramos cells to fall away.
- Extra media and unattached Ramos cells were removed before the plate was fixed and stained with Trypan Blue.
- Remaining Ramos cells were counted to determine the number of bound Ramos cells.
- CD22 was blocked using CD22-specific antibodies, then this process was repeated to compare binding to untreated Ramos cells. An IgG kappa isotype antibody was utilized as a negative control.

Flow Cytometry (Figure 2) supported that not only did CD22 antibody bind to PA-1 and SK-OV-3, which indicates surface expression of CD22 in these cells, but this binding was specific. In each cell line, there was a similar trend to that seen in Ramos cells; the mean CD22 FITC fluorescence for the cells stained with CD22 antibody was notably larger than that of the IgG kappa isotype negative control. PA-1 had the fewest events detected, which may be due to the relatively poor state of these cells.

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Conclusions

- The gene CD22 is inappropriately activated in multiple cancerous ovarian cell lines; however, it is not necessarily expressed to the extent that it is in Ramos cells.
- CD22 antibody binds specifically to PA-1 and SK-OV-3 cell lines, indicating surface expression of CD22 in these cells.
- CD22 demonstrates affinity for bone stromal cells, which could lend it to participation in metastasis in CD22-expressing cancers.

References


Disclosures

This funding was supported by a seed grant from the Ben and Marylee Fish College of Pharmacy.