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 provide evidence for CD22 being a potential biomarker for antibody treatment for lung cancer (Tuscano 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

Understanding of aberrant expression of CD22 can be used in determining whether antibody directed therapy is an appropriate treatment for CD22-expressing cancers.

Methods

Tissue Culture

Cell lines maintained with preferred media under sterile conditions with a 5% controlled 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 cells)

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 temporary negative control upon 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 .

Methods (Continued)

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 unattached 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.

Visualization

Figure 1: Gel Electrophoresis

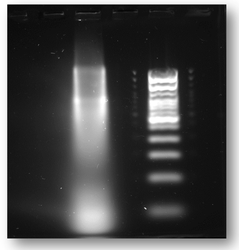


Figure 1a (left) is an example of a gel displaying intact RNA from PA-1 cells prior to RT PCR.

Figure 1b (right) displays the results of RT PCR with 3 different primer combinations. Each ovarian cell line presents similarly to Ramos cells.

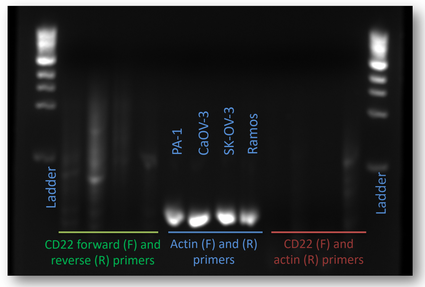
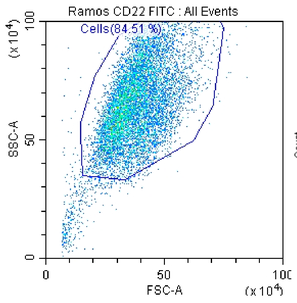
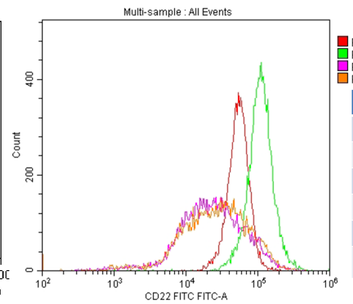


Figure 2: Flow Cytometry

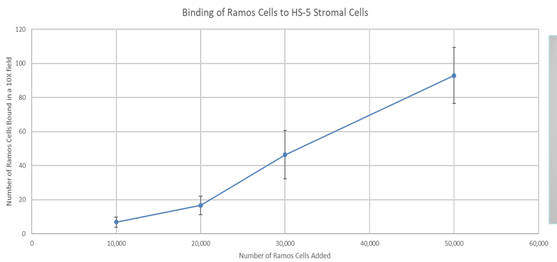


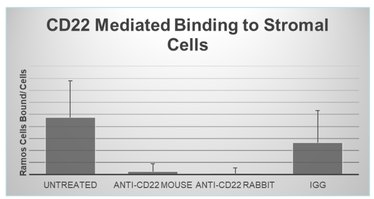


Population	Mean CD22 FITC FITC A
Ramos CD22 FITC	174143.5
Ramos IgG FITC	89540.4
PA-1 CD22 FITC	67676.0
PA-1 IgG FITC	40243.0
SK-OV-3 CD22 FITC	165736.0
SK-OV-3 IgG FITC	129423.4

- The two graphs depict examples of data obtained from flow cytometry. In the leftmost graph, the population selected is encircled in a solid blue line. In the rightmost graph, fluorescence (x-axis) is compared to the number of events (y-axis).
- The table summarizes information gathered from each population by listing the mean CD22 FITC fluorescence for each cell-line/antibody combination. In each cell line, CD22 antibody produced a greater mean (or right-shifted mean) in comparison to the control IgG antibody.
- CaOV-3 was unable to be analyzed.

Figure 3: CD22 Binding to Bone Stromal Cells





- **Figure 3a (left)** displays data indicating that increasing concentrations of B-lymphocytes added to increasing concentrations of HS-5 cells an upwards trend in binding occurred, with significant binding shown at 50,000 Ramos cells.
- **Figure 3b (right)** is a graph that demonstrates binding of Ramos to HS-5 bone marrow stromal cells, which is mediated by CD22 binding. Little to no binding occurred with anti-CD22 mouse and anti-CD22 rabbit antibodies, and an IgG kappa isotype was used to show specificity of binding.

Results

Figure 1b depicts a gel that was run following reverse transcription and PCR amplification. While fainter compared to the Ramos (B-cell) band, definite bands are present for PA-1, CaOV-3, and SK-OV-3 cDNA, and they present similarly to the positive control (Ramos cells). Prior gels not depicted here have repeatedly demonstrated this for PA-1 using actin 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.

Binding of Ramos cells to bone marrow stromal cells indicates that CD22 may play a role in the metastasis of certain cancers (Figure 3a). To rule out the possibility that sources of binding other than that caused by CD22, blocking of CD22 was performed with anti-CD22 antibodies. When CD22 was blocked from binding, there was little to no binding of Ramos and HS-5 cells. An IgG antibody was used as a negative control; significant binding still occurred in the presence of the non-CD22-specific antibody (Figure 3b).

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

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Disclosures

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