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14. ABSTRACT The adhesion receptor CD44 is known to decrease the metastatic potential of breast cancer cells in vivo. This study focuses on understanding the mechanisms by which CD44 inhibits breast cancer cell invasion. We have found that the interaction between CD44 and Hyaluronan leads to decreased phosphorylation of FAK. Additionally, this interaction also leads to decreased transcription of the metalloprotease MMP9. Together, these mechanisms provide significant insight into how CD44 inhibits the movement of breast cancer cells away from their primary site.					
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Introduction

The interactions that occur between tumor epithelial cells and the surround stroma are important in determining the disease progression of various cancers, including breast cancer. Recent studies have found that the interactions that occur between the epithelial adhesion receptor, CD44, and the extracellular sugar, Hyaluronan (HA) can decrease the tumor epithelium's ability to invade and metastasize[1]. Our current research focuses on understanding the mechanisms by which CD44-HA interactions lead to decreased tumor invasion.

Body

Our first goal was to understand how CD44/HA interactions decrease the activation of pro-invasive signaling pathways within breast cancer cells. We proposed to immunoprecipitate CD44 to visualize proteins that associate with this adhesion receptor when it is bound or unbound to HA. We have successfully performed this experiment and found CD44 to associate with Focal Adhesion Kinase (FAK), a protein known to influence the invasive behavior of cells[2], only in the absence of HA in gels (Fig 1A). Using densitometric analysis, we found that there was a greater association of FAK with CD44 in the absence of HA (Fig 1B). In collagen gels that were cast with HA, there was a decreased level of CD44 associated FAK. We looked at the activation status of FAK by probing with a phospho-specific antibody, and found greater activation of FAK in absence of HA in the gels (Fig 2). The presence of HA in Collagen I gels decreases the activation of FAK, in a CD44 dependant manner. These finding may provide a significant insight into how CD44 may suppress invasion in breast cancer cells by inhibiting the activation of FAK.

Our second goal is establish whether CD44/HA interactions may lead to decreased MMP9 transcription. MMP9 is a protease that has been widely implicated in the disease progression of breast cancer as it allows cells to degrade their surrounding matrix so that they may move away from their primary site[3]. We have performed a number of studies using a MMP9 promoter reporter construct. We found that HA embedded into Collagen I gels do not have any effect on MMP9 transcription levels (data not shown). However, we also found that HA/CD44 interactions decreased the transcription of MMP9 in Collagen IV gels in a CD44 dependant manner (Figure 3). Collagen I is a collagen that is primarily deposited in the stroma surrounding tumors while Collagen IV is primarily deposited in the basal lamina adjacent to the tumor epithelium at the primary site. Decreased expression of MMP9 when cells are adjacent to the basal lamina may be yet another mechanism by which CD44 may decrease breast cancer cell invasion.

Key research accomplishments

- **Identified FAK as a binding partner for CD44.**
- **Established decreased FAK phosphorylation as a mechanism by which CD44 inhibits breast cancer cell invasion.**
- **Ruled out the importance of HA in Collagen I in the transcriptional repression of MMP9**
- **Established that Collagen IV/HA may serve to inhibit the expression of MMP9.**

Reportable Outcomes

None.

Conclusion

Understanding the underlying mechanisms that control breast cancer cell invasion is important to decreasing mortality rates for this disease. CD44 is a protein that can decrease the metastatic potential of breast

cancer cells, however, the mechanisms by which it achieves this are not understood. In our current work we have identified FAK as a binding partner for CD44. FAK is a kinase that localizes to the invasive front of breast cancer cells where it organized the machinery necessary for cellular movement. The interaction between CD44 and HA leads to decreased activation of FAK through phosphorylation at tyrosine 397. Decreased activation of FAK may be one of the mechanisms that CD44 employs to decrease the invasion of breast cancer cells.

Additionally, we have found that CD44 interactions with HA lead to decreased MMP9 transcription in Collagen IV gels but not in Collagen I gels. MMP9 is a protease responsible for opening up spaces in the extracellular matrix that cells employ to invade away from their primary site. The identification that the transcriptional repression of MMP9 occurs in Collagen IV but not Collagen I gels suggest that this repression may occur while the mammary epithelium is still at its primary site and before invasion.

Both of these findings are significant as they provide insight into mechanisms that cells may employ to prevent mammary epithelium from migrating away from their primary sites. CD44 dependant inactivation of FAK and decreased MMP9 transcription may need to be overcome by malignant cells in disease progression. Further understanding of these mechanisms may lead to the development of a therapy to prevent cellular invasion in the future.

Figure 1.

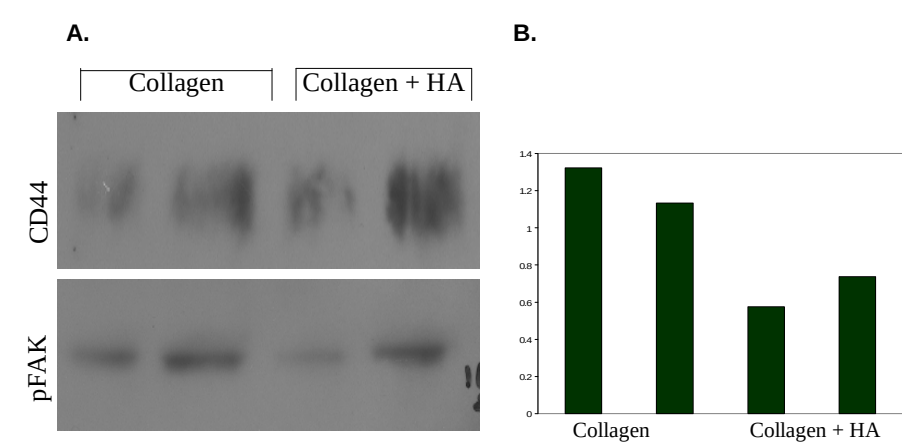
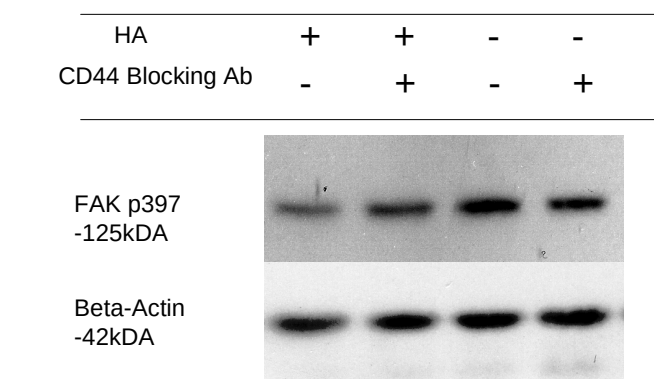


Figure 2.



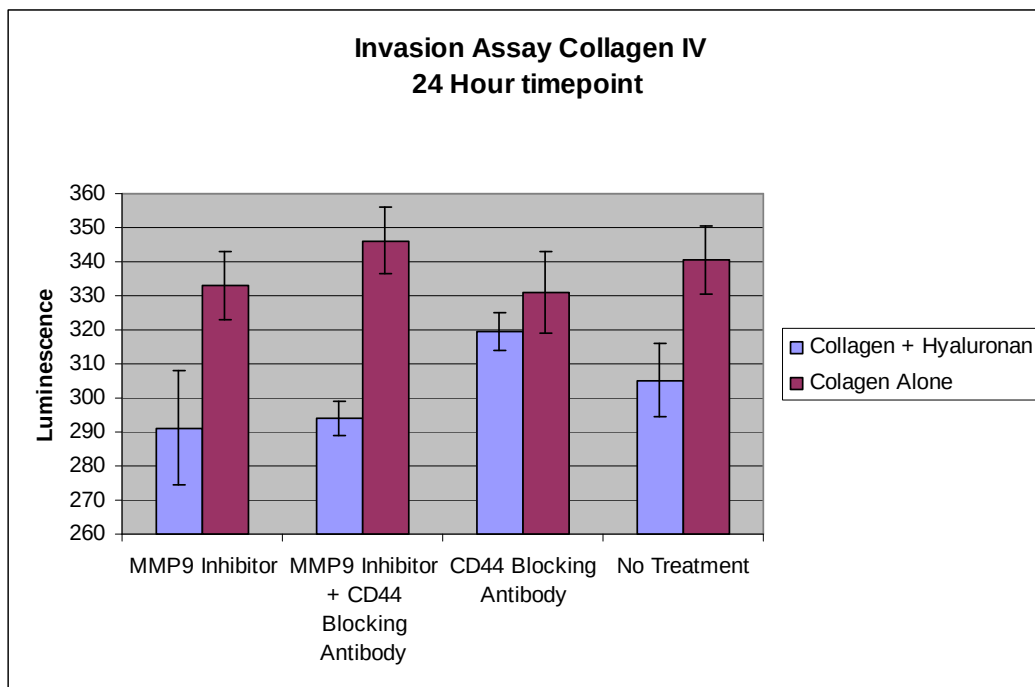


Figure 3.

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Appendices

None.