Utilizing CD24 as an indicator for tumor aggressiveness and metastasis

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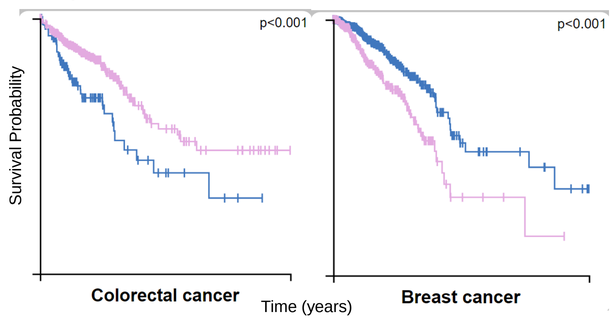
INTRODUCTION:

- CD24 is a complex and dynamic membrane protein that participates in cell adhesion and migration but also wields influence over biological processes like B-cell development and neurogenesis.[1]
- In the context of cancer, heightened expression contributes to oncogenic signaling and poses challenges to the immune system through phagocytosis evasion.[2]
- Particularly in breast cancer research, studies have shed light on a specific role of surface CD24 in facilitating cell movement within the bloodstream, leading to increased invasiveness to distant sites.[3]
- High CD24 signal, however, does not uniformly allow for same levels of metastasis and proliferation.[4]



- **Figure 1. CD24 Kaplan-Meier Curve.** Pink: High CD24 Blue: Low CD24. Found within the different longevities of survivability within the different tumor cell locations, this intriguing relationship underscores the enigmatic nature of the effects of CD24 on tumors, where high CD24 expression does not uniformly promote metastasis and tumor progression in all tumor histology.

HYPOTHESES

- High CD24 expression will be visable within paired human breast primary tumor and brain metastatic tumor.
- Utilizing CD24 overexpression would then allow for a foresight of the possibility for metastasis and increased tumor proliferation within specific cell lines and tumors.
- Within murine cell lines of high CD24 signal, anti-CD24 antibody blocking would increase phagocytosis.

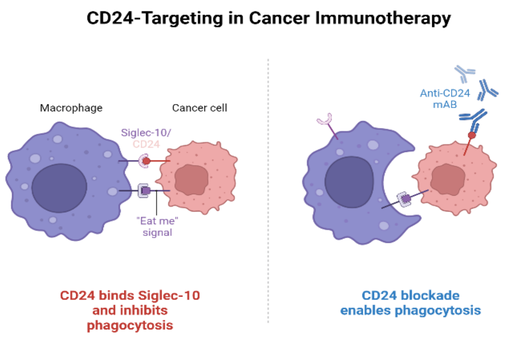


Figure 2. CD24 Blocking allowing for macrophage-mediated phagocytosis. SIGLEC-10 (Sialic Acid-Binding Immunoglobulin Lectin 10) is primarily known for its expression on certain immune cells, such as macrophages. CD24 on cancer cells have been shown to transmit inhibitory signals to dampen the macrophages' anti-tumor activities. By blocking or disrupting CD24-SIGLEC-10 interactions, it may be possible to restore macrophage-mediated phagocytosis and promote immune recognition, such as other “eat me signals” like SLAMF7 (signaling lymphocyte activation marker family member 7)

RESULTS

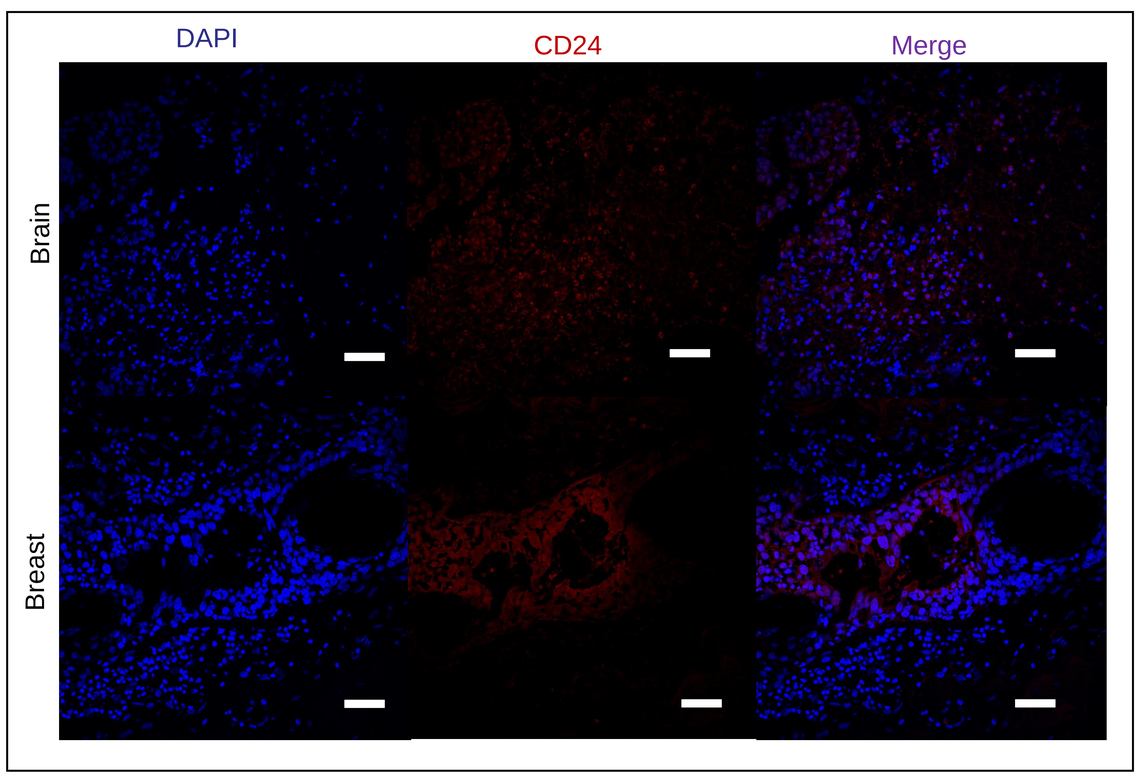


Figure 3. Immunofluorescence (IF) slides of breast and brain tumor stained for CD24. Scale bar: 50 µm. Human paired breast carcinoma primary and brain metastatic tumors were stained utilizing DAPI and a Polyclonal Anti-Rabbit antibody targeting CD24, and images were captured at 30x magnification. The IF images revealed a distinctive pattern of CD24 expression in both types of tumors. In both breast primary and brain metastatic tumors, a heightened signal of CD24 was observed on cell membranes. These IF images indicate a high expression of CD24 in the metastatic tumors, suggesting a potential role of CD24 in the metastatic process. (n=1, similar CD24 expression was found in other patient samples)

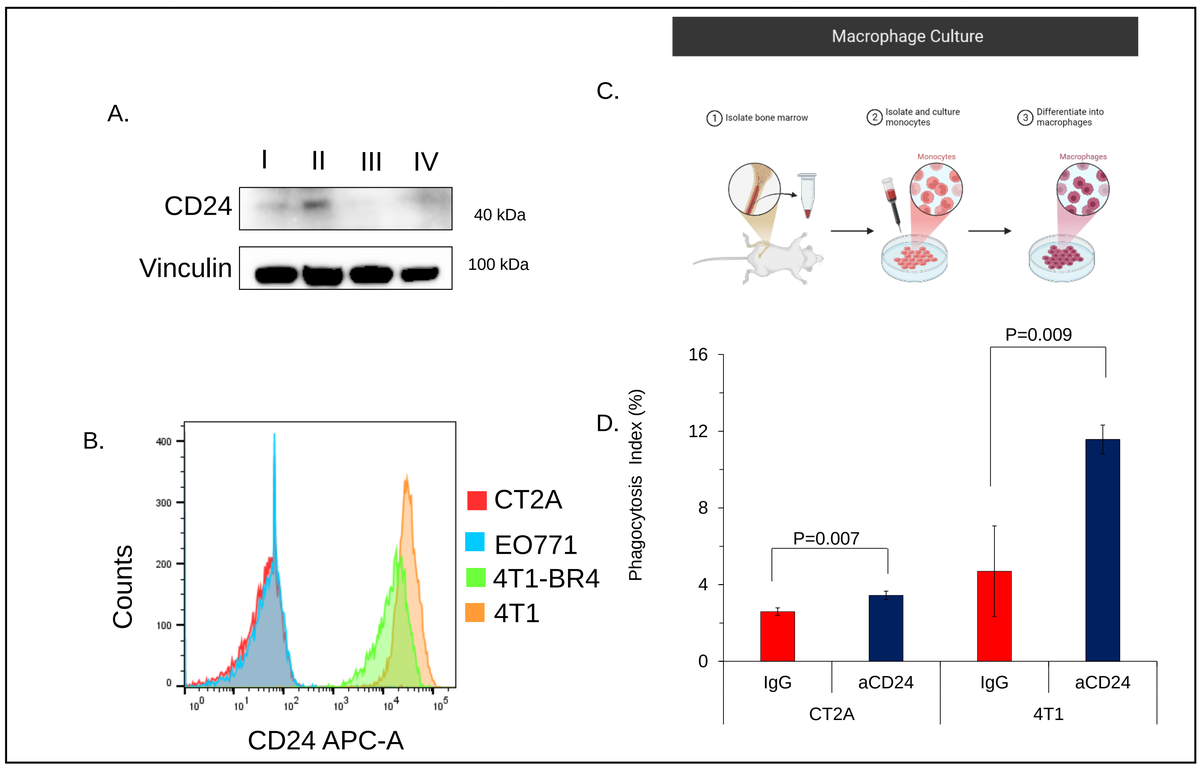


Figure 4. CD24 Expression within breast cancer cell lines. A. I. 4T1, II. 4T1 BR4, III. EO771, and IV. CT2A. CT2A is a murine glioma, utilized as a negative control as it has no to low expression CD24. Western blot analysis revealed distinct bands representing CD24 protein levels in the four cell lines. Notably, the 4T1 BR4 cell line exhibited the most intense signal of the CD24 band, indicating a higher abundance of CD24 protein compared to the other cell lines. **B.** Flow cytometry data further supported the findings, with 4T1 and 4T1 BR4 displaying high CD24 expression. On the other hand, EO771 and CT2A cell lines exhibited minimal CD24 expression, which was expected of CT2A but not EO771. **C.** Monocytes were harvested from femur and tibia of mouse samples. Cultured for 7 days within differentiation buffer, producing BMDM (bone marrow derived macrophages). **D.** A phagocytosis assay was performed with con-cultures of BMDM with CT2A and 4T1. Both macrophages and cell lines were stained with fluorescence dye. They were then cocultured for 4 hours, after having been introduced to aCD24 and IgG accordingly. Evident from the results, both IgG and aCD24 had minimal effects on phagocytosis rate in CT2A, while having significant increases of phagocytosis of 4T1 following aCD24 treatment.

CONCLUSION

- An upregulated expression of CD24 was observed in breast tumors that had metastasized to the brain as compared to primary site
- Heightened CD24 expression was particularly prominent in aggressive and highly metastatic murine breast cancer tumor cell lines, notably the 4T1 and 4T1 BR4 cell lines
- Cell lines exhibiting increased CD24 expression were subsequently susceptible to elevated levels of phagocytosis when utilizing aCD24
- These findings suggests that aCD24 antibodies may be utilized for immunotherapeutic purposes.

FUTURE DEVELOPMENTS

- Complete IF on different paired tumor metastatic sites from breast to view pattern of CD24 expression.
- Further explore changes in tumor microenvironment and associated interactions between CD24 presenting cells
- Utilize aCD24 antibodies and view whether it can halt or hinder the metastatic properties of aggressive and dispersive tumors.
- Investigate the molecular pathways downstream of CD24 that contribute to metastasis (e.g., transcriptomic analyses) utilizing single-cell mRNA sequencing.

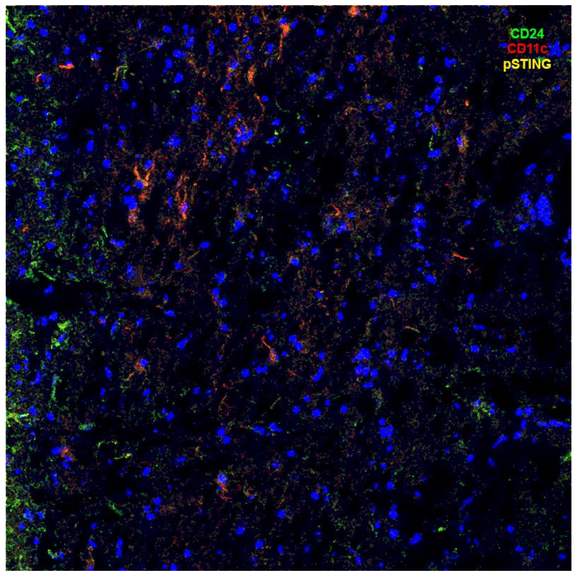


Figure 5. Demarcation of CD24+ tumor cells and CD11c+ antigen presenting. The IF images above show a clear and distinctive seperation of high CD24 and (in this example) CD11c expression. Investigating to why this seperation occurs may shed light on the affects of CD24 in the subcellular microenvironment.

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