CD82 Regulates TGF-β Signaling in Metastatic Prostate Cell Lines

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ABSTRACT

The metastasis suppressor CD82 and promoter CD151 are tetraspanins, a group of glycoproteins that collectively regulate various cellular processes, including cell differentiation, migration, adhesion, and signaling. These mechanisms are significant in nonpathological and pathological physiology; aberrant endothelial to mesenchymal transition permits cell migration and metastasis, and has been induced by TGF-β signaling. In renal cell carcinoma, CD151 has been shown to promote cell metastasis by activating TGF-β/SMAD signaling, whereas CD82 downregulates cell migration and invasion by inhibiting TGF-β/SMAD signaling. CD82 has been demonstrated to change CD151 surface expression and suppress invasion by regulating c-Met and Src signaling in metastatic prostate cell lines. The purpose of this experiment was to investigate if CD82's and CD151's abilities to respectively suppress and promote metastasis involve TGF-β signaling.

OBJECTIVES

- Identify if CD82 regulates c-Met via CD151 expression in the cells.
- Determine if this regulation involves or affects TGF-β signaling pathway.

LOCATION OF CD82 & ASSOCIATED PROTEINS

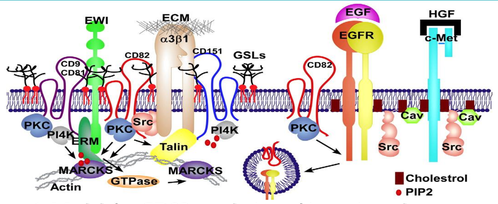
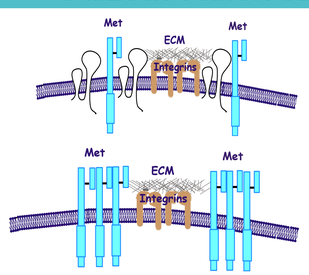


Figure 1. Model for CD82 regulation of integrin and receptor tyrosine kinase signaling. CD82 in association with other tetraspanins (CD9, CD81, CD151) within the ganglioside (GSLs) enriched microdomains control the signaling output from integrins (α3β1) and associated proteins (EWI). The ability of CD82 to inhibit EGFR signaling by its ligand EGF within caveolin (Cav) cholesterol rich lipid rafts (brown bars) is mediated by PKC-dependent phosphorylation of EGFR cytoplasmic tail and its subsequent internalization. Whether the same mechanism is true for regulating HGF-induced c-Met activity is being investigated (Miranti, 2009).

C-MET IN TUMOR CELLS (+/- CD82)

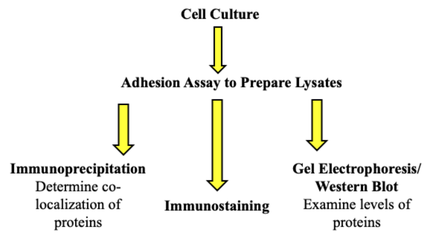


Normal
CD82(+++)
Met (+)

Metastatic
CD82(-)
Met (+++)

Figure 2. In tumorigenesis, expression of CD82 and c-Met changes. In a normal cell, CD82 activity is high while c-Met is active but low. In metastatic prostate tumor cells, CD82 is lost and c-Met is over expressed.

EXPERIMENTAL APPROACH



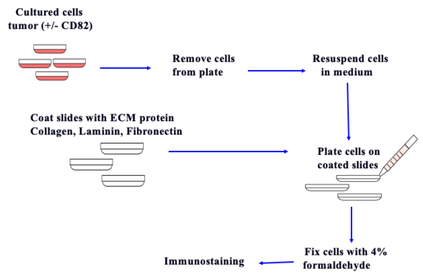
METHODS

Cell Culture

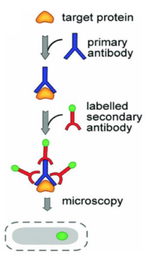
Cell Lines

- PC3 (bone)
  - 5V- empty vector
  - #29- CD82 re-expressed
  - #11- CD82 re-expressed

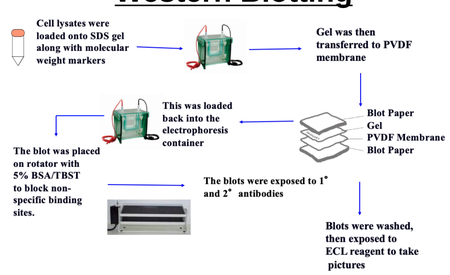
Adhesion Assay



Immunostaining



Gel Electrophoresis/Blot Transfer & Western Blotting



RESULTS

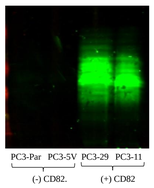
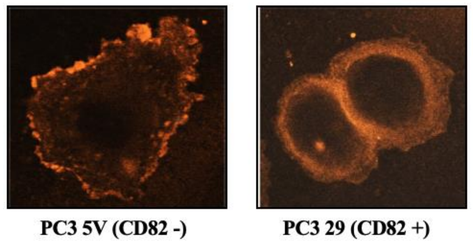
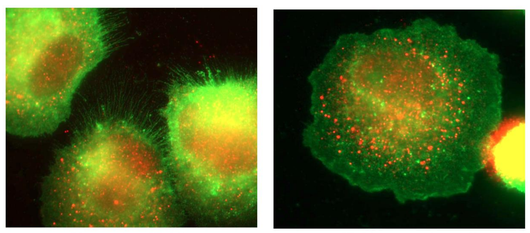


Figure 3. Shows CD82 expression on a western blot. Tumor (metastatic) cells (PC3-Par) and vector transfected (5V) have very little CD82, while cells with CD82 reintroduced (PC3-29 & PC3-11) have high expression. The blots were treated with TS82B abs.



PC3 5V (CD82 -) **PC3 29 (CD82 +)**

Figure 4. Confocal microscopy of c-Met surface immunostaining of PC3 cells (+/-) CD82. PC3 Cells(+/-) CD82, adhered to matrix stained with biotinylated Met antibodies followed by staining with streptavidin conjugated Q-dot antibodies.



Vector (no CD82) **CD82 expressing cell**

Figure 5. Fluorescent microscope pictures of merged images of PC3 cells. Vector (A) and CD82 re-expressing cells (B) CD151 is stained green and c-Met stained red.

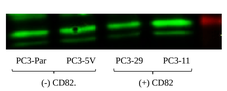


Figure 6. Shows total CD151 expression on a western blot in the PC3 cell lysates. Tumor (metastatic) cells (PC3 –parental & vector transfected PC3 5V) as well as clones of PC3 cells with CD82 reintroduced (PC3 -11 & PC3-29) all show same levels of CD151 expression.

RESULTS

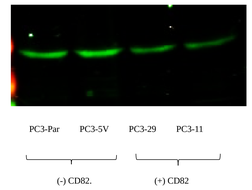


Figure 7. Shows TGF-β II receptor expression on a western blot. Tumor (metastatic) cells (PC3 –parental & 5V, vector transfected have higher expression of the receptor while tumor cells with CD82 reintroduced (PC3 -11 & PC3-29) have lower expression comparatively.

CONCLUSION

- CD82 regulates c-Met activation.
- CD82 does not co-localize with c-Met. c-Met appears to be redistributed along the cell surface in cells re-expressing CD82. By redistributing c-Met, CD82 may be preventing c-Met activation.
- CD151 associates with c-Met in other tumors and activates it.
- Total CD151 expression does not change with CD82 expression in prostate metastatic cells. However CD151 expression at the cell surface changes with CD82 re-expression i.e., CD82 re-expression seems to move CD151 inside the cell.
- By removing CD151 from the cell surface, CD82 may be inhibiting c-Met association with CD151 and thus preventing c-Met activation.
- CD151 promotes cell metastasis via TGF-β/SMAD signaling activation, whereas CD82 downregulates cell migration and invasion through TGF-β/SMAD signaling inhibition in renal cell carcinoma.
- CD82 expression seems to reduce the level of TGF-β receptor expression, thus suggesting it may be regulating TGF-β signaling in prostate cells.

FUTURE STUDIES

- Determine if CD82 directly affects TGF-β receptor expression or indirectly affects its expression via relocating CD151 and c-MET.
- Evaluate if TGF- β signaling is regulated by CD151, and if this is independent of CD82's regulation.
- Determine how potential signaling cross talk between c-MET and TGF-β/SMAD may be involved in or affect CD82's and CD151's role in metastasis.
- We will continue our efforts in finding the mechanism through which CD82 regulates c-Met and explore the role of TGF-β involvement in this regulation.

REFERENCES

- Miranti, C. K. (2009). Controlling cell surface dynamics and signaling: How cd82/kai1 suppresses metastasis. *Elsevier: Cell Signaling,2*, 196-211. doi:10.1016/j.cellsig.2008.08.023