Characterization of PDLIM7 expression and localization in breast cancer cells

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Abstract

The PDZ and LIM domain (PDLIM) protein family is associated with organ development (Krcmery et al., 2010), cytoskeletal architecture and oncogenesis (Liu et al., 2015). Little is known about the expression profile, cellular localization and function of PDLIM7 in cancer cell lines. I hypothesize that just like the other PDLIM proteins, PDLIM7 plays a role in cell migration and adhesion. In this research, I found that PDLIM7 colocalizes with actin fiber and partially colocalizes with vinculin on the focal adhesion of MDAMB468 cells. PDLIM7 is also differentially expressed in MDAMB468, MCF-7 and HCT-116 cancer cell lines. I generated PDLIM7 knockout (KO) cells using CRISPR-Cas9 plasmid in MDAMB468, MCF-7 and HCT-116 cancer cell lines by incorporating a modified enhanced green fluorescent protein plasmid (pEGFP) into the target PDLIM7 sequence as a reporter of selection for successful KO cells. Upon successful knockout, I aim to investigate the effect of PDLIM7 depletion on cell migration, adhesion and expression profiles of other adhesion molecules, such as integrin, talin and kindlin. The significance of this research is that it could help us better understand the underlying function of PDLIM7 in different types of cancer cells.

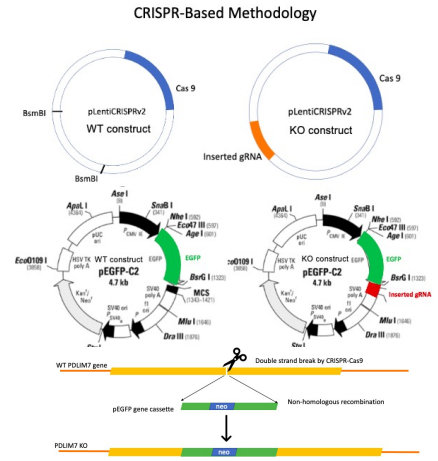
Introduction

PDLIM protein is made of PDZ domain and LIM domain. It has been reported that some PDLIM proteins were implicated in regulating cell adhesion and migration in cancer metastasiss. PDLIM7 is also a protein member of the same family. It is located on chromosome 5, and its pre-mRNA is subjected to alternative splicing giving rise to three different isoforms (The Human Protein Atlas, 2020). Meta-analysis from UALCAN shows that PDLIM7 mRNA is significantly upregulated in metaplastic breast cancer compared to other tumor histologic subtypes. However, the protein expression of PDLIM7 in the breast cancer cell line and its subcellular localization remain unknown. I hypothesize that just like other PDLIM family proteins, PDLIM7 also plays a pivotal role in regulating cell adhesion and migration, which potentially contributes to cancer metastasis. In this project, characterized the expression profile of PDLIM7 protein in breast cancer cells study its subcellular localization in MDAMB468 breast cancer cell lines establish a CRISPR-based method to knock-out (KO) PDLIM7 gene expression in breast cancer cell lines MDAMB468 as well as MCF-7 to deplete the expression of PDLIM7 protein and assess whether the loss of PDLIM7 alters cell adhesion, cell migration and the expression of proteins involved in cell adhesion and migration such as integrins, talin, kindlins.

Acknowledgement

I would like to thank my principal investigator Professor Bouaouina who supervised me through the entire project, offered unconditional support and guidance, and pushed this project forward. I would also like to thank Professor Bachu for his advice and expertise in helping us design the gRNA and troubleshooting generating knockout constructs, my thesis panel advisors Professor Younis and Professor Affara for their expertise and advice, Bernadette and Maya who provided us with lots of technical support.

Methods



Conclusion

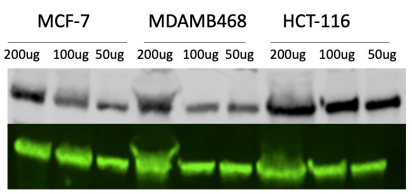
PDLIM7 isoform 1 is endogenously expressed in MDAMB468, MCF-7 and HCT-116 cancer cell lines. PDLIM7 expression in HCT-116 is substantially higher than that in breast cancer cell line MDAMB468 and MCF-7. PDLIM7 colocalizes with actin fibers in MDAMB468 breast cancer cells and partially colocalizes with vinculin in focal adhesions in MDAMB468 cells, which implies its role in cell migration and adhesion. We also generated PDLIM7 KO cells in HCT-116 and MCF-7 cell lines, which are to be confirmed by western blot and immunofluorescence staining.

References

Krcmery, J., Camarata, T., Kulisz, A., & Simon, H. G. (2010). Nucleocytoplasmic functions of the PDZ-LIM protein family: new insights into organ development. BioEssays : news and reviews in molecular, cellular and developmental biology, 32(2), 100–108.

Liu, Z., Zhan, Y., Tu, Y. et al (2015). PDZ and LIM domain protein 1(PDLIM1)/CLP36 promotes breast cancer cell migration, invasion and metastasis through interaction with α-actinin. Oncogene 34, 1300–1311.

Results



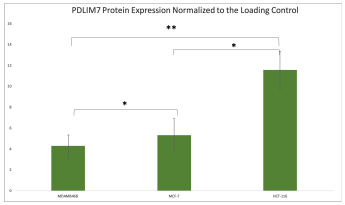


Figure 1: Expression of Endogenous PDLIM7 in MCF7, HCT-116 and MDAMB468 cancer cell lines
Panel A: Detection and Quantification of PDLIM7 in MDAMB468, MCF-7 and HCT-116 cancer cell lines. They were incubated with primary anti-PDLIM7 antibody and secondary donkey anti-rabbit antibody. The membrane was imaged at 600nm. Pan beta-tubulin serves as a loading control in western blot.
Panel B: Comparison of PDLIM7 Expression among three cell lines. Statistically analysis was done using student T-test in excel. (* means significance p values 0.1, ** means significance p value 0.05)

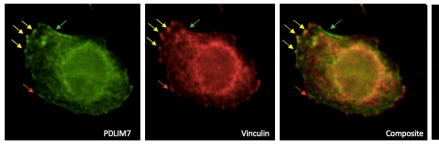


Figure 2:PDLIM7 partially colocalizes with Vinculin in MDAMB468 cells. Green arrow: PDLIM7 localized outside of focal adhesions (most probably stress fiber). Yellow arrows: partial co-localization of PDLIM7 and vinculin at focal adhesions Red arrow: focal adhesion without PDLIM7.

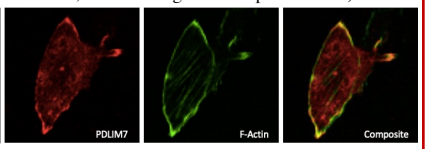
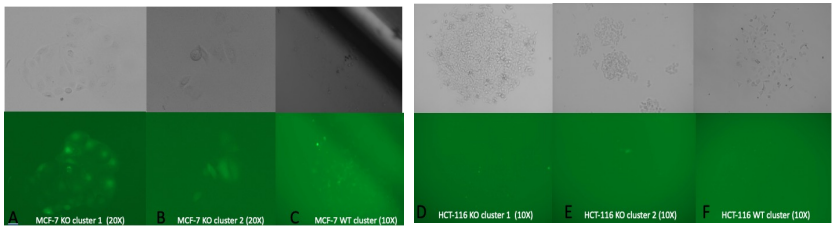


Figure 3: Colocalization of PDLIM7 and F-actin stress fiber in Breast Cancer Cells. MDAMB468 cells were transfected with PDLIM7-ds red and then stained with Phalloidin-488 (1/10 dilution) to probe for localization of F-actins.



Fiugre 4: Generation PDLIM7 KO cells using CRISPR-cas 9 and Geneticin Selection.
Panel A-C: Selection of MCF-7 cells after 14 days shows clusters of resistant cells. 1mg of Geneticin was applied to electroporated MCF-7 cells the day after electroporation. Cells were followed up daily.
Panel D-F: Selection of HCT-116 cells after 14 days shows clusters of resistant cells. 1mg of Geneticin was applied to electroporated HCT-116-7 cells the day after electroporation. Cells were followed up daily.

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