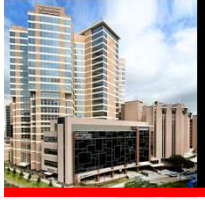

IRF2BP1-mediated regulation of MHC-I in breast cancer cells

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INTRODUCTION

Brain metastasis is one of the dangerous consequences of breast cancer and is responsible for most deaths from this disease. Aberrant function of protein kinases permits the uncontrolled outgrowth of cancer cells. We previously observed that cyclin-dependent kinase 5 (CDK5) promotes metastasis growth in experimental mouse models of brain metastasis through affecting the antitumor immunity. Another protein of interest is Interferon Regulatory Factor 2 Binding Protein 1 (IRF2BP1). While there is not much known about this yet, a similar protein, Interferon Regulatory Factor 2 Binding Protein 1 (IRF2BP1) has been known to be an important factor in the immune system. From these findings we are interested in investigating the relationship between CDK5 and IRF2P1. Previous studies have linked a positive relationship between IRF2 and the class I major histocompatibility complex (MHC-I). [1] Since IRF2BP1 regulated IRF2, we are also interested in investigating the relationship between IRF2BP1 and MHC-I and its associated proteins.

METHODS

Cell Culture: 4T1.Br3 and T11.Br1 wells were transfected independently with a shRNA-control and a shRNA mediated knockdown of *Cdk5* and *Irf2bp1*. These cells were then cultured in DMEM/F12 (1:1) supplemented with 10% FBS and 1% Penicillin and Streptomycin.
RNA Extraction: RNA was extracted from all cells using TRI reagent (MRC) and following the TRIZOL RNA Isolation Protocol from the W.M. Keck Foundation Biotechnology Microarray Resource Laboratory at Yale University.
cDNA Synthesis: RNA from each cell line was converted into cDNA using the using iScript™ gDNA Clear cDNA Synthesis Kit (Bio-Rad)
qPCR: Synthesized cDNA was used to perform real time quantitative PCR using SYBR Green as the florescent dye on the Cdk5 cell group in order to confirm whether the *shCdk5* knockdown was performed correctly.
Protein Extraction: Membrane, Cytoplasmic, and Nuclear protein fractions were extracted from cells using the Cell Fractionation Kit from Cell Signaling Technology.
Immunoblot: Immunoblots were run on the 4T1.Br3 and T11.Br1 sh*Cdk5* and shCtl cell group on 12% SuperSep™ Phos-tag™ gels in order to determine the phosphorylation of IRF2BP1 and total IRF2BP1. Control antibodies for this gel were β-actin and CDK5. These samples were also run in parallel using 12% Bis-Tris Gel, followed by probing with IRF2BP1, β-actin, and CDK5 antibodies.

REFERENCES

1. Kriegsman, B. A., Vangala, P., Chen, B. J., Meraner, P., Brass, A. L., Garber, M., & Rock, K. L. (2019). Frequent Loss of IRF2 in Cancers Leads to Immune Evasion through Decreased MHC Class I Antigen Presentation and Increased PD-L1 Expression. *Journal of immunology (Baltimore, Md. : 1950)*, *203*(7), 1999–2010. https://doi.org/10.4049/jimmunol.1900475

RESULTS

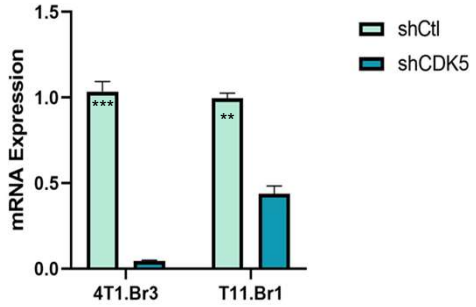

Figure 1: CDK5vrelative expression in *Cdk5* gene knockdown cells. 4T1.Br3 and T11.Br1 breast cancer cells were stably transfected with control shRNA or shRNA targeting *Cdk5*. Total RNA was extracted followed by cDNA synthesis and RT-qPCR analysis to profile the relative expression of *Cdk5*. Unpaired T test.

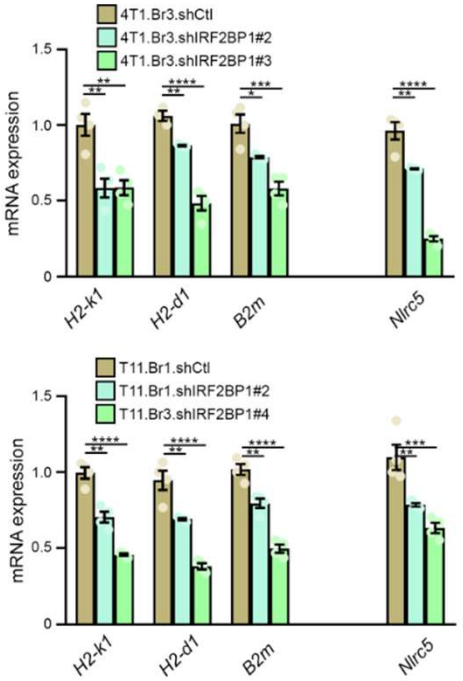

Figure 3: mRNA expression of key MHC-I genes in cells with *Irf2bp1* knockdown. 4T1.Br3 and T11.Br1 breast cancer cell lines stably transfected with control shRNA or shRNA targeting *Irf2bp1* (2 clones). Total RNA was extracted followed by cDNA synthesis and RT-qPCR analysis to profile the relative expression of key MHC-I genes (*H2-k1*, *H2-d1*, *B2m*, and *Nlrc5*). One-way ANOVA with Dunnett's post hoc test.

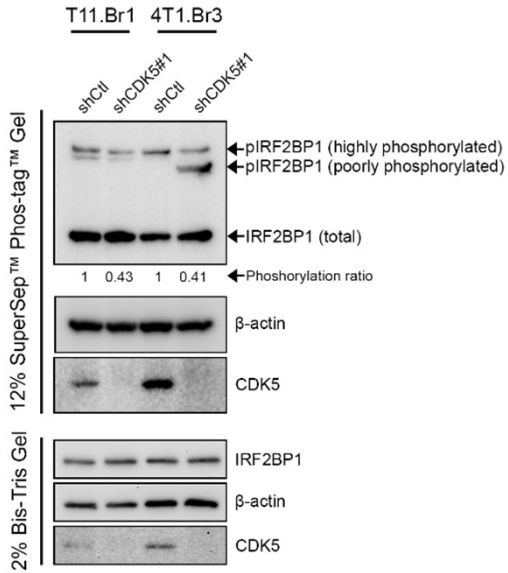

Figure 2: Phosphorylation of IRF2BP1 in CDK5 knockdown cells. 4T1.Br3 and T11.Br1 breast cancer cell lines stably transfected with control shRNA or shRNA targeting CDK5 were immunoblotted for the indicated proteins. Cells were tested for phosphorylation status of IRF2BP1 using 12% SuperSep™ Phos-tag™. The samples were also run in parallel using the 12% Bis-Tris Gel gel.

CONCLUSION

CDK5 may phosphorylate IRF2BP1
In immunoblots conducted on 4T1.Br3 and T11.Br1 cells, shCDK5 cells displayed less prominent bands corresponding to phosphorylated IRF2BP1. In addition, in T11.Br1 cells we also observed a shift of pIRF2BP1 bands indicative of reduced IRF2BP1 phosphorylation (Figure 2). This suggests that CDK5 may lead to phosphorylation of IRF2BP1.
IRF2BP1 knockdown reduces mRNA expression of MHC-I genes
In qPCR conducted on 4T1.Br3 and T11.Br1 cells, shIRF2BP1 clones showed a significant decrease in mRNA expression of genes *H2-k1*, *H2-d1*, *B2m*, and *Nlrc5* (Figure 3). This indicates that IRF2BP1 controls MHC-I production in breast cancer cell lines.
Conclusion
Based on these results, shRNA-mediated knockdown of *Irf2bp1* in breast cancer cells led to a significant reduction of key immune-related genes, such as those associated with the class I major histocompatibility complex (MHC-I): *H2-k1*, *H2-d1*, *B2m*, and *Nlrc5*. From this we believe that CDK5 may alter the expression of MHC-I through the phosphorylation of IRF2BP1.