The role of PBRM1 BAH domains in gene regulation and cell biology

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Introduction

- Genes can be regulated by the activities of transcription factors and by nucleosomes (1).
- Post-translational modifications (PTMs) of histones have been shown to affect gene expression, in part, through the recruitment of effector proteins (or complexes) bearing specialized domains that read or interpret the histone code (**Figure 1**) (2).
- PBRM1 is a reader domain that has two bromo-adjacent BAH domains (BAH 1 and BAH2) which is involved with transcriptional regulation
- Many families of reader domains have been extensively investigated; however, new 'orphan' reader domains' histone PTM binding preferences unclear (3).
- **Various mutant forms of the BAH domains are present in cancer and our objective is to answer the question how these mutant forms affect transcriptional regulation**

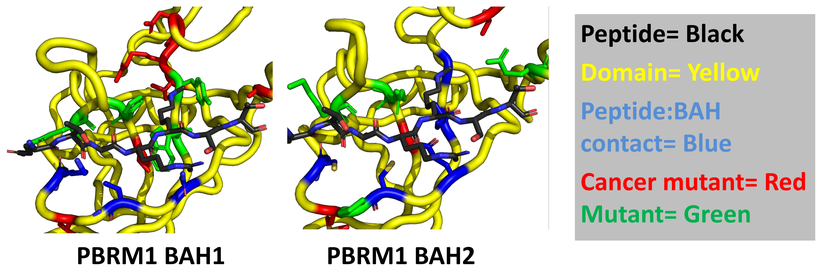

Figure 1: Mutants of PBRM1 BAH1 and BAH2 affect the structure of the domain (adapted from Rothbart et al, 2012)

Research Goals

- After maintaining a cell line with our mutant constructs, we want to analyze the gene expression of an array of genes marked for cancer importance in Clear Cell Renal Cell Carcinoma (ccRCC) usting RT-qPCR
- We hope this will provide insight on the functional importance of BAH domains on PBRM1's biochemical function

References

1. Allis CD, Jenuwein T. The molecular hallmarks of epigenetic control. Nat Rev Genet. 2016;17(8):487-500.
2. Rothbart SB, Strahl BD. Interpreting the language of histone and DNA modifications. Biochimica et biophysica acta. 2014;1839(8):627-43.
3. Andrews FH, Strahl BD, Kutateladze TG. Insights into newly discovered marks and readers of epigenetic information. Nat Chem Biol. 2016;12(9):662-8.
4. https://2bind.com/
5. Rothbart SB, Krajewski K, Strahl BD, Fuchs SM. Peptide microarrays to interrogate the "histone code". Methods Enzymol. 2012;512:107-35.

Methods

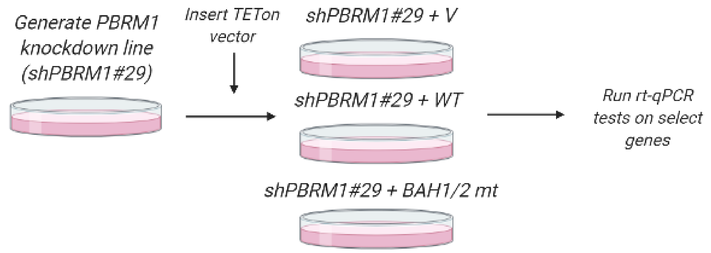

Figure 2: Development of PBRM1 knockdown and rescue cell lines

HEK39T cell lines were developed with a PBRM1 knockdown generated by transfection of a SMART inducible shRNA vector against PBRM1 (shPBRM1#29). Rescue lines were generated by transfecting compatible TETon plasmids with an empty vector, wild type, or BAH1/2 mutant. rt-qPCR was used to assay knockdown in rescue lines on select genes marked for cancer importance.

Results

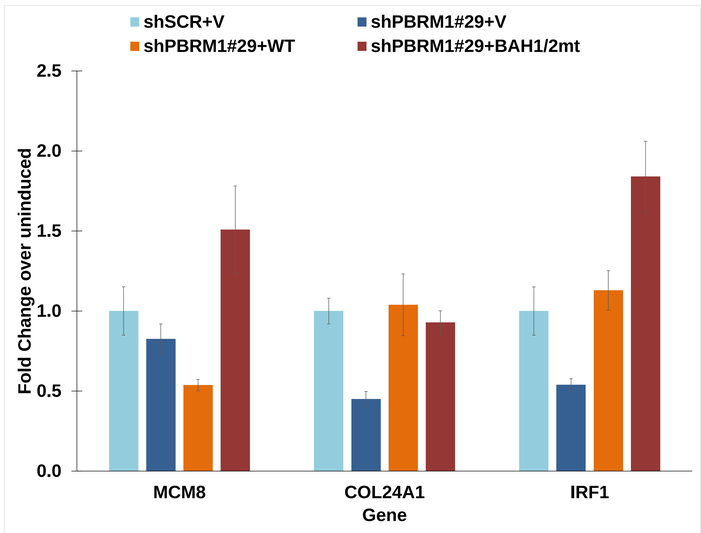

Figure 3: Examined representatives of cohorts of genes to determine whether data obtained matches previously published data through the shPRBRM1#29 knockdown with wild type rescue

The graph showcases the fold change over uninduced samples, determined by normalizing variable targets against GAPDH and finding the difference. Based on the graph, we can see downregulation for MCM8 expression compared to upregulation of COL24A1 and IRF1 when looking at the shPBRM1#29 + WT expression

Conclusions

- Based on knockdown cell lines, BAH mutants display different functionality for different cohorts of genes
- MCM8 is a gene involved in the cell cycle where compared to normal expression (shSCR + V), the knockdown of PBRM1 (shPBRM1#29 + WT) is decreased showing down regulation
- COL24A1 is a gene involved in adhesion where the knockdown of PBRM1 has around the same level of gene expression as normal expression
- IRF1 is a gene involved in immune response, where interestingly, PBRM1 knockdown results in increased expression indicating potential importance for regulating tumor response
- Interestingly when PBRM1 is rescued with mutated BAH1/2 domains, MCM8 and IRF1 have increased expression suggesting an inhibitory function of the BAH domains

Future Directions

- Next we are looking at the chromatin binding capabilities of each line in order to understand the strength of the PBRM1 BAH interactions with the chromatin
- Currently we are doing this by conducting chromatin association assays and attempting to detect the strength of the exogenous PBRM1 inserted through the TETon vector

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