Investigation of Transcriptional Regulators of ATP7B and Their Role in Cisplatin Resistance in Cancer

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Introduction

ATP7B is a Cu-pump, which gets translocated from the golgi to lysosomes upon increased Cu+ levels, sequestering and eventually removing Cu+ via exocytosis. Not surprisingly, this important function requires tight regulation and changes in ATP7B activity is linked to several diseases. While lack of ATP7B activity leads to Wilson's disease, its overexpression results in drug resistance in cancers. Therefore, understanding how ATP7B is regulated is crucial to shed light on the physiology of these conditions. To date, there is very little information on the transcriptional regulation of ATP7B. MTF1 is the only known transcription factor, which controls its expression, yet MTF1 levels do not always correlate with ATP7B levels suggesting additional factors involved in its regulation.

Results and Conclusion

To find these additional factors, we initially performed an i*n silico* analysis using TRANSFAC/PROMO software and have found that several transcription factors may bind to ATP7B promoter (Figure 1 and 2). VISTA analysis showed that the conserved regions were mostly focused around 3000 to +1, indicating that the regulatory sequences are most likely located in this region. To investigate which of these factors bind to the ATP7B promoter, Genomic Locus Proteomics methodology will be used (Figure 3). In this technique, deadCas9 protein is fused to APEX2 enzyme and guided to the ATP7B promoter via specific gRNAs (Figure 5). The proteins which are close to the dCas9APEX2 will be biotinylated and marked proteins will be identified by mass spectrometry. Depending on their enrichment scores, selected proteins will be investigated and confirmed with ChIP. Eventually, CRISPR knockout of the candidate proteins will be tested for their effects on cancer cell lines for cisplatin resistance.

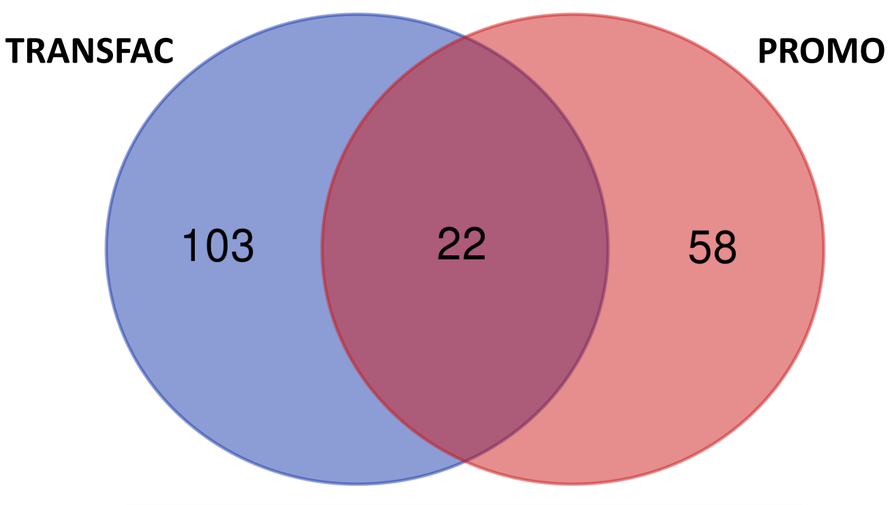

Figure 1: Transcription Factor Analysis of ATP7B. In –3000 to +1 region, binding sites were found for 125 different transcription factors in the TRANSFAC database and for 80 different factors in the PROMO database. It was seen that 22 factors intersect in both analyzes.

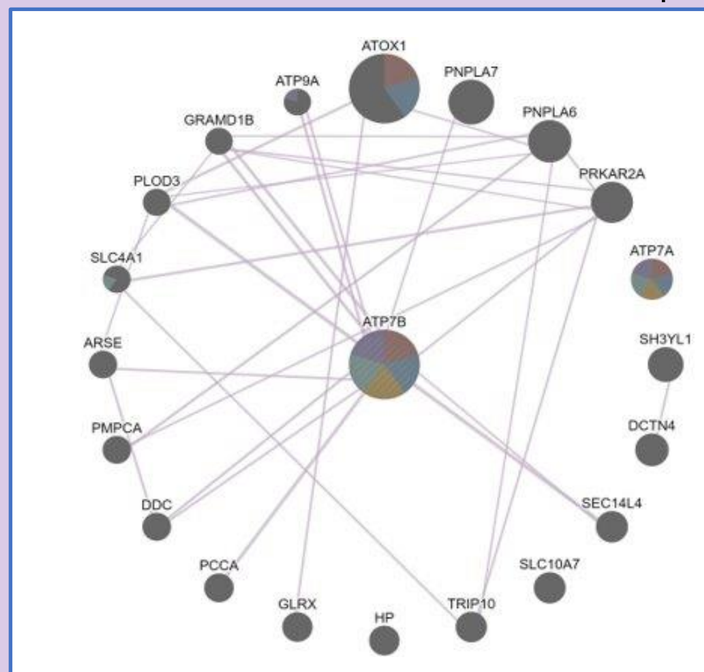

Figure 2A: Demonstration of genes that co-expressed with ATP7B. Transcription factor binding site analysis done by CiiDER software, a dataset was prepared from genes known to be co-expressed with ATP7B

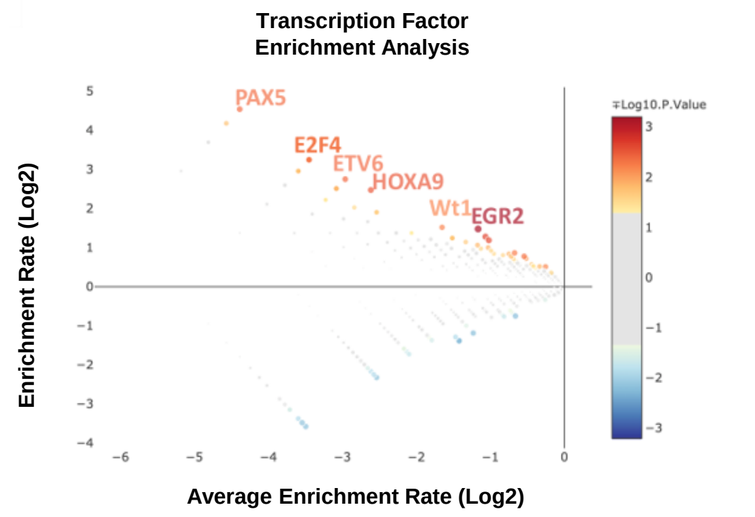

Figure 2B: Transcription Factor Enrichment analysis of ATP7B and Co-expressed genes. Potential binding sites of transcription factors from -3000 to +1 from transcription start site of ATP7B.

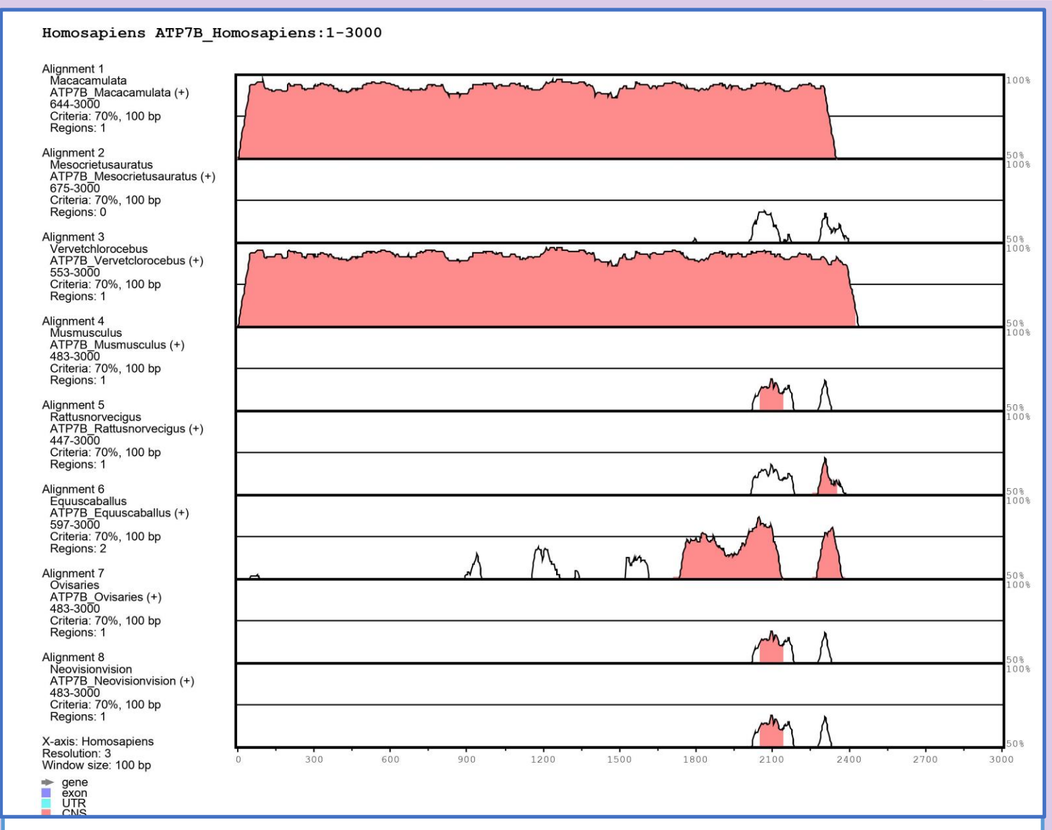

Figure 4: Alignment of ATP7B promoter between species. Evolutionary conservation of +1 to -3000 bp region of ATP7B between 9 species

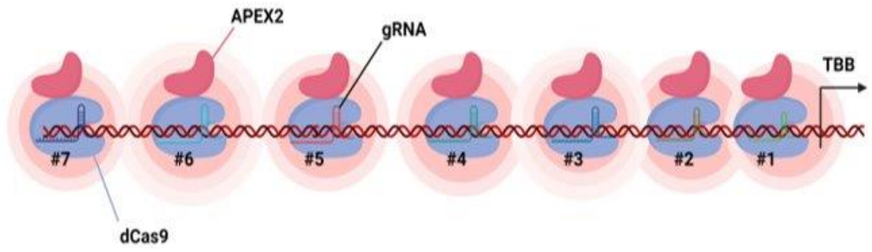

Figure 5: Schematic Representation of dCas9-APEX2 Complex and gRNAs to Target -3000 to +1 on Promoter of ATP7B
gRNAs are designed to target -3000 to +1 region of ATP7B promoter.