MOL231: Investigating PTBP1 Regulation of Splicing and mRNA Stability of Selected Targets in HeLa Cells

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1 Background and Aims

Polypyrimidine tract-binding protein 1 (PTBP1) is a nuclear RNA-binding protein involved in several steps of mRNA processing including splicing regulation, mRNA transport and translation. PTBP1 is frequently upregulated in cancer, where it alters splicing patterns to produce cancer-prone variants (1). Known PTBP1-regulated targets include CD44, CTTN, and PKM. For PKM, PTBP1 promotes alternative splicing to favour expression of the PKM2 isoform, commonly found in proliferating cells. PTBP1 was also shown to interact directly with nuclear phosphoinositides (PIPs) through K439–K440 in the RRM3-RRM4 linker (2).

This study aims to investigate whether the mRNA levels of CD44, CTTN and PKM alternative splicing are PTBP1-dependent, and whether PIP-PTBP1 interaction influences their regulation.

2 Experimental approach

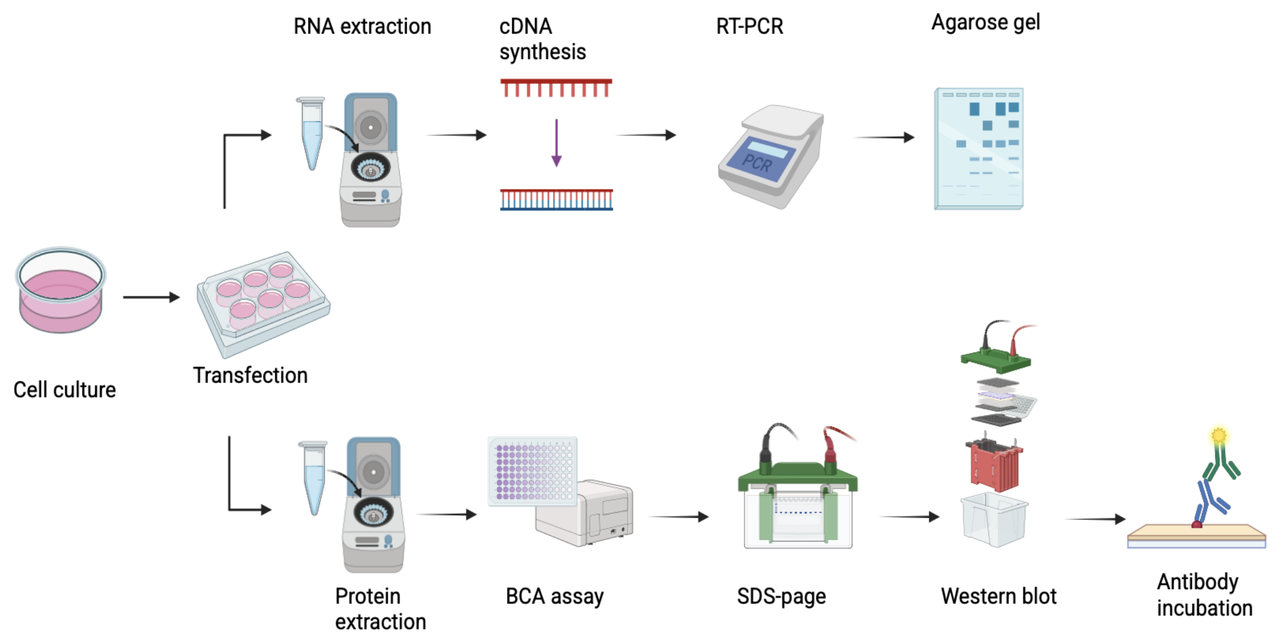

Figure 1: **Overview of the experimental approach.**
HeLa PTBP1 WT and KO cells were transfected with EGFP, EGFP-PTBP1 WT or EGFP-PTBP1 K439A-K440L constructs. The top workflow illustrates the analysis of PTBP1 mRNA targets CD44, CTTN and PKM by RT-PCR and agarose gel. The bottom workflow shows protein extraction and Western blotting to evaluate EGFP and EGFP-PTBP1 expression. Figure created with BioRender.

3 Results

Validation of PTBP1 KO and transfection efficiency

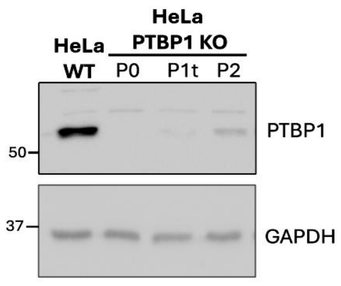

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Figure 2: **Validation of PTBP1 KO in HeLa cells.** Western blot confirms loss of PTBP1 in KO cells. GAPDH serves as loading control.

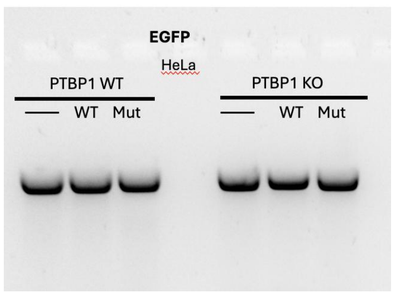

Figure 3: **Agarose gel of EGFP PCR products.**
PCR was used to confirm successful transfection of EGFP constructs. EGFP levels were later used to normalize CD44 and CTTN expression.

PTBP1 regulates CTTN expression in HeLa cells

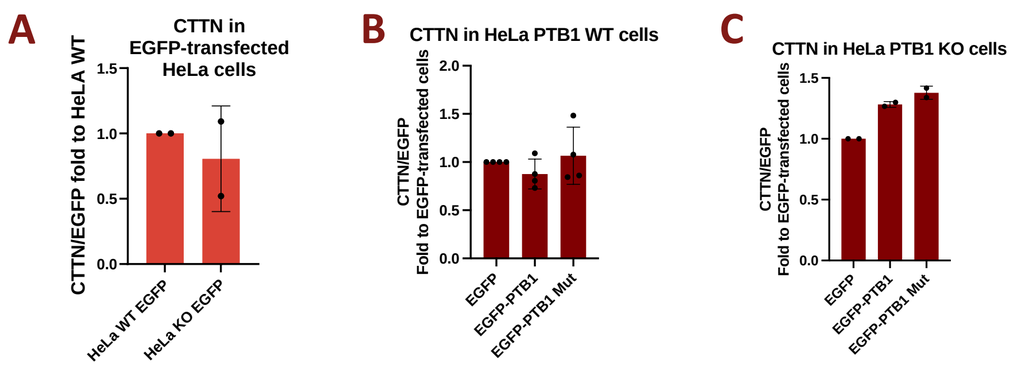

Figure 4: **CTTN/EGFP ratio in HeLa PTBP1 WT and Hela PTBP1 KO cells.**
RT-PCR of CTTN in (A) HeLa WT versus HeLa PTBP1 KO cells (from EGFP-transfected samples) and (B-C) in WT (B) and PTBP1 KO (C) HeLa cells, transfected with EGFP, EGFP-PTBP1 WT or K439A-K440L mutant (Mut). CTTN was normalised to EGFP.

PTBP1 increases CD44 mRNA levels in HeLa cells

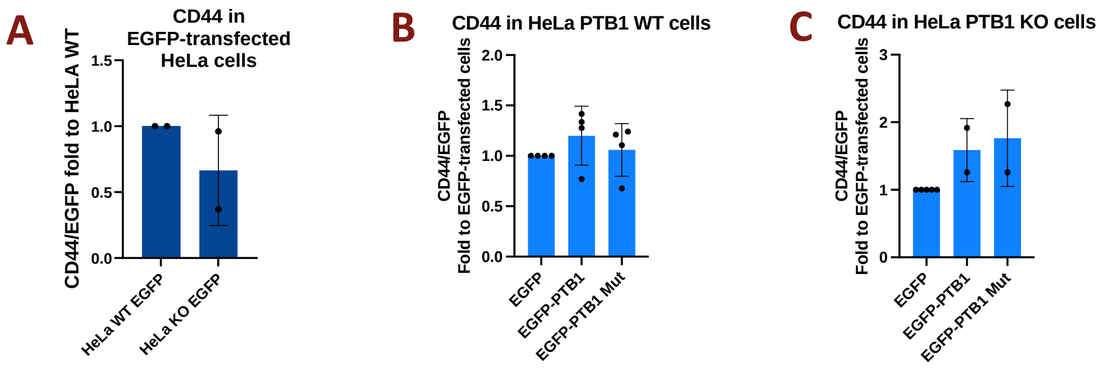

Figure 5: **CD44 mRNA levels in HeLa PTBP1 WT and KO cells.**
RT-PCR of CD44 in (A) HeLa WT versus HeLa PTBP1 KO cells (from EGFP-transfected samples) and (B-C) in WT (B) and PTBP1 KO (C) HeLa cells, transfected with EGFP, EGFP-PTBP1 WT or K439A-K440L mutant (Mut). CD44 was normalised to EGFP.

PKM1 to PKM2 switch is dependent upon PTBP1

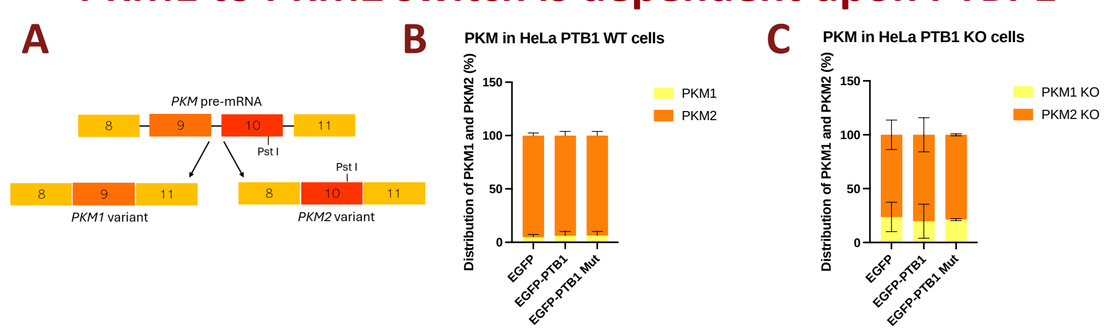

Figure 6: **PKM alternative splicing in HeLa PTBP1 WT and KO cells.**
(A) Pyruvate kinase muscle isoform 1/2 (PKM1/PKM2) alternative splicing. (B-C) Distribution of PKM1 and PKM2 (%) in HeLa WT (B) and PTBP1 KO cells (C) transfected with EGFP, EGFP-PTBP1 WT or K439A-K440L mutant (Mut).

4 Conclusion and Future Work

- CD44 mRNA levels may be PTBP1-dependent, but may not be affected by PTBP1-PIP interaction
- CTTN mRNA levels do not appear to be PTBP1-dependent
- PTBP1 promotes PKM2 over PKM1 via alternative splicing, which does not appear to be affected by PTBP1-PIP interaction.
- **Future work:** Investigate the effects of CD44 on migration properties in HeLa WT and KO cells, perform transfection with extended incubation time to assess the effects of prolonged PTBP1 WT and mutant expression in HeLa KO cells, compare protein levels for PKM1 and PKM2 and analyse impacts on glycolysis metabolism.

References

1. Yu, Q., Wu, T., Xu, W., Wei, J., Zhao, A., Wang, M., Li, M., & Chi, G. 479, 2875–2894 (2024). *PTBP1 as a potential regulator of disease*. *Molecular and Cellular Biochemistry*.
2. Andreas Midlang (master thesis, UiB, 2021).

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