MOL231: **Potential Regulation of PTBP1 Specific mRNA levels by PIPK in Endometrial Cancer Cells**

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Background and Aims

mRNA processing leads to the generation of functional mature mRNAs. Errors in mRNA processing can lead to tumour promoting mRNA variants and drug resistance[1]. The molecular machinery contributing to mRNA processing is well understood; however, how these processes are regulated is unclear.

Polypyrimidine tract-binding protein 1 (PTBP1) is a nuclear RNA-binding protein that regulates mRNA-processing[2], which was identified to interact with the signaling phosphoinositide lipids phosphatidylinositol(4,5)bisphosphate (PtdIns(4,5)P_2) and PtdIns(3,4,5)P_3 [3,4,5].

Hypothesis: This interaction may influence how PTBP1 regulates mRNA processing, which can be tested by treating cells with different PIPK inhibitors (Figure 1). mRNA targets possibly affected by this PTBP1-lipid interaction are CD44 and CTTN, which express proteins associated with metastasis in cancers[5,6,7].

Research objective: 1) to map the levels of CD44 and CTTN in cancer cells and **2)** to investigate whether mRNA processing of CD44 and CTTN is altered when blocking specific kinases of phosphoinositide metabolism.

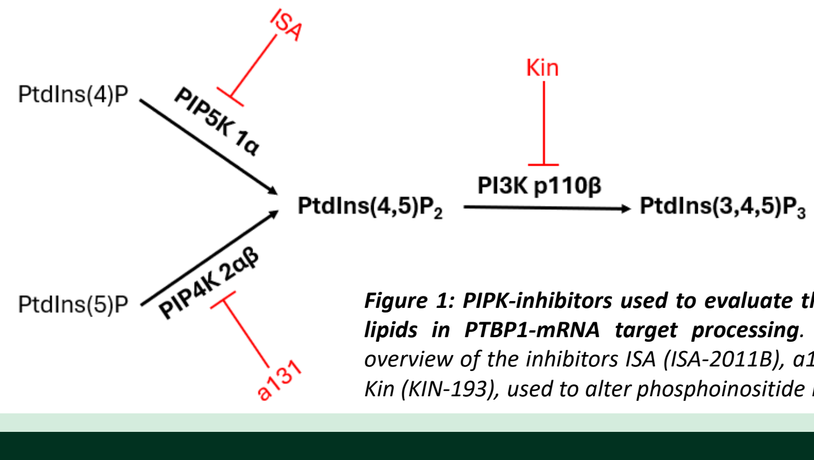

***Figure 1: PIPK-inhibitors used to evaluate the role of the signaling lipids in PTBP1-mRNA target processing**. The figure depicts an overview of the inhibitors ISA (ISA-2011B), a131 (PIP4K-IN-a131) and Kin (KIN-193), used to alter phosphoinositide metabolism.*

Methods

Overview of experimental procedures

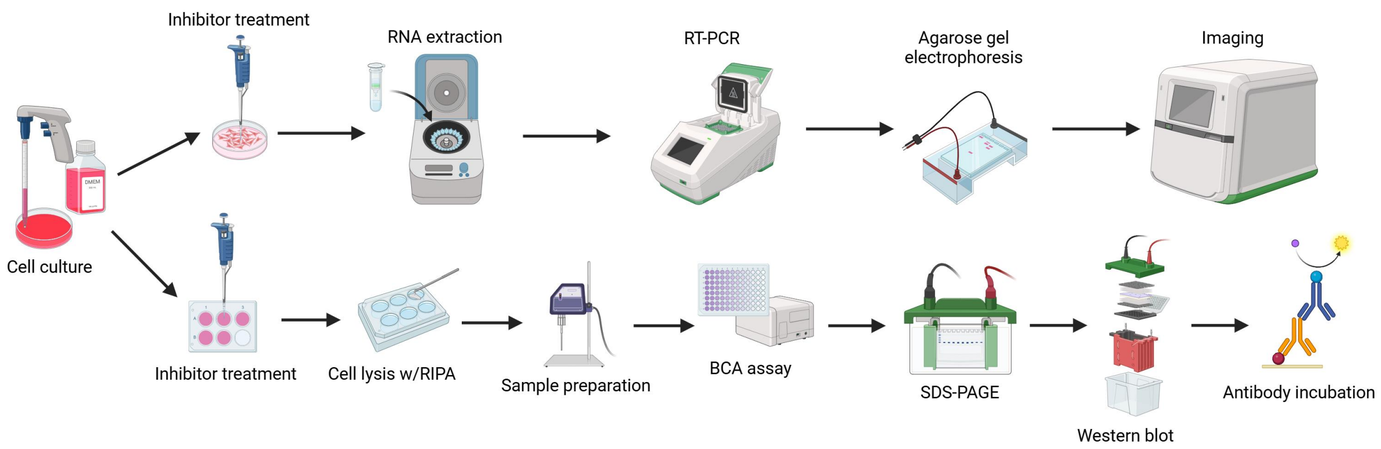

Figure 2: Flow of experimental procedures.
The top method pathway was used to investigate the PTBP1 mRNA targets CD44 and CTTN by RT-PCR in cancer cells treated with PIPK inhibitors, while the bottom pathway explains the methods used to evaluate the effect of PIPK inhibitors on PTBP1 protein levels by western immunoblotting. Figure created with BioRender.

Choice of PTBP1 splicing targets and PCR strategy

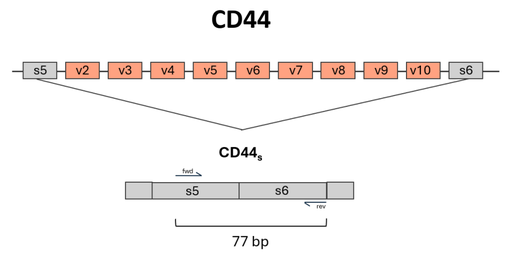

***Figure 3: Structure of the transmembrane protein CD44 with variable exons.** PCR strategy for CD44 cDNA amplification, s: short variant. v: variable exons.*

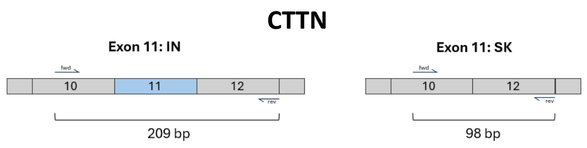

***Figure 4: Cortactin (CTTN) isoform-a showing the inclusion and skipping of exon 11.** Expected sizes of CTTN PCR product when exon 11 is included (IN) or skipped (SK).*

CD44 is a transmembrane protein involved in interactions with the ECM as a cell adhesion molecule[4]. The mRNA is proven to be a target of PTBP1-mediated alternative splicing[2] and is chosen as a study object in cancerous tissues due to its involvement in proliferation and migration[6].

Cortactin (CTTN) isoform-a is a canonical isoform that includes exon 11[8]. CTTN is an actin-binding protein associated with cell motility, migration and invasion. Retention of exon 11 is PTBP1-dependent, shown by PTBP1-knockdown experiments [2].

Results

PTBP1-mRNA targets are differently expressed in endometrial cancer cells

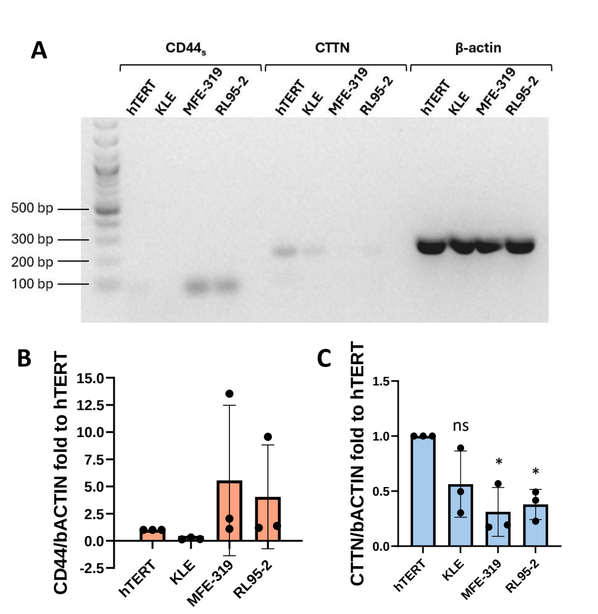

***Figure 5: mRNA-levels of CD44 and CTTN in hTERT, KLE, MFE-319 and RL95-2 LOG cancer cells. A)** CD44 and CTTN PCR products separated by agarose gel electrophoresis. **B-C)** Quantification of $CD44_s$ (B) and CTTN (C) PCR products normalized to beta-ACTIN and compared to the reference cell line hTERT.*
*Three experiments ± SDs. ANOVA non-parametric, Kruskal-Wallis post test. ns: non-significant. *P < 0.05.*

PIPK-inhibition gave variable results

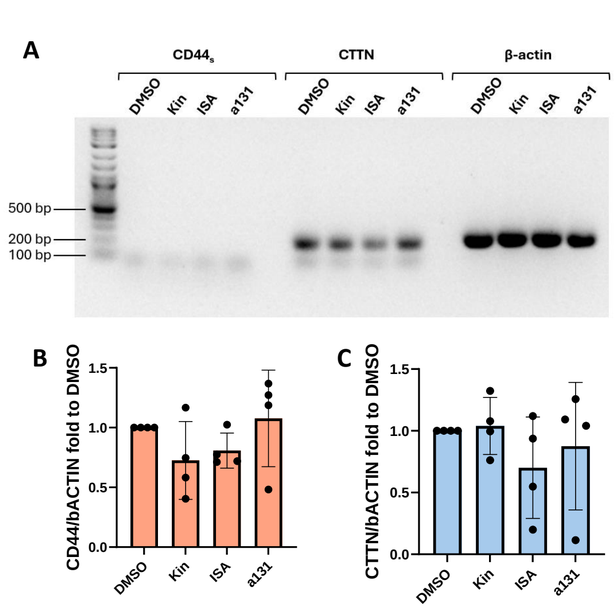

***Figure 6: mRNA-levels of CD44 and CTTN upon PIPK inhibitor-treated RL95-2 cells. A)** RL95-2 cells treated with DMSO, Kin, ISA and a131. CD44 and CTTN PCR products separated by agarose gel electrophoresis. **B-C)** Quantification of amplified CD44 (B) and CTTN (C) normalized to beta-ACTIN, in relation to DMSO.*
Four experiments ± SDs. ANOVA non-parametric, Kruskal-Wallis post test.

Lowering PtdIns(4,5)P_2 leads to decreased PTBP1 protein levels and apoptosis

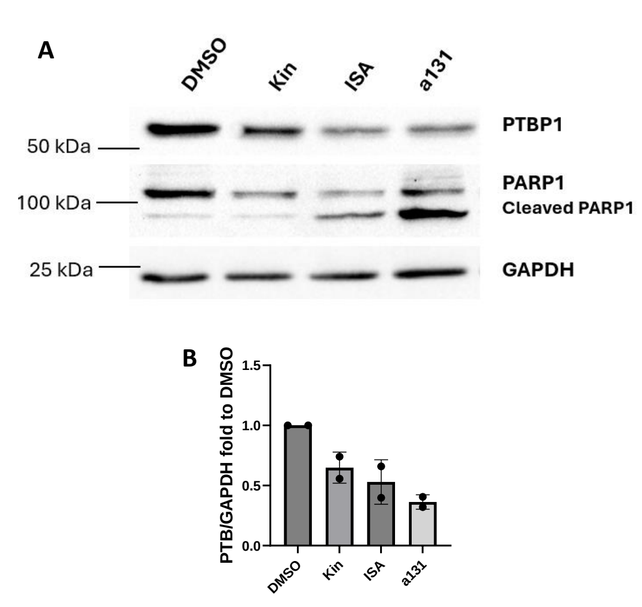

Figure 7: PTBP1 and PARP1 levels in PIPK inhibitor-treated RL95-2 cells.
A) Western immunoblots of PTBP1, PARP1 and GAPDH in RL95-2 cells treated with DMSO, Kin, ISA and a131 for 24 h. **B)** Quantification of PTBP1 protein levels normalized to GAPDH, compared to DMSO.
Two experiments ± SDs. ANOVA non-parametric, Kruskal-Wallis post test.

Conclusion and Future work

- All PIPK inhibitors induced a reduction in PTBP1 protein levels. PARP1 cleavage was observed upon PIP4K and PIP5K inhibition, indicating an increase in apoptosis.
- Cancer cells with high PI3K activity (MFE-319 and RL95-2) strongly expressed the $CD44_s$ canonical isoform, but not the splicing variant including any of the variable exons, while they expressed weakly CTTN. No significant effect on CD44 or CTTN levels were found upon PIPK inhibition in RL95-2 cells.
- **Further experiments**: knocking out PTBP1 to investigate changes in CD44 and CTTN mRNA expression, testing additional endometrial cancer cells for alternative splicing variants of CD44, testing other PTBP1 specific alternative splicing targets.

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References

1. Desterro, J. et.al (2020). Targeting mRNA processing as an anticancer strategy. *Nature Reviews Drug Discovery*, *19*(2), 112-129.
2. Takahashi, H. et.al. (2015). Significance of Polypyrimidine Tract–Binding Protein 1 Expression in Colorectal Cancer. *Molecular Cancer Therapeutics*, *14*(7), 1705-1716.
3. Lewis, AE et al, (2011) Identification of nuclear phosphatidylinositol 4,5-bisphosphate-interacting proteins by neomycin extraction. Mol Cell Proteomics. 10:M110.003376
4. Mazloumi Gavgani F, et al. (2021) Nuclear Phosphatidylinositol 3,4,5-Trisphosphate Interactome Uncovers an Enrichment in Nucleolar Proteins. Mol Cell Proteomics. 20:100102.
5. Austbø, M. L. et.al. (2024). MOL231: Interaction mapping of the phosphoinositide pathway with the alternative splicing factor PTBP1. *Biopitch.uib.no.*
6. Hassn Mesrati et.al. (2021). CD44: A Multifunctional Mediator of Cancer Progression. *Biomolecules*, *11*(12), 1850.
7. Wang, Z.N. et.al (2017). High expression of PTBP1 promote invasion of colorectal cancer by alternative splicing of cortactin. *Oncotarget*, *8*(22), 36185–36202.
8. Schnoor, M. et.al. (2018). Cortactin: Cell Functions of A Multifaceted Actin-Binding Protein. *Trends in cell biology*, *28*(2), 79–98.