MOL231: Alternative Splicing of Fibroblast Growth Factor Receptors 1 and 2 in Endometrial Cancer Cell Lines:

Any correlation with epithelial mesenchymal transition?

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Background and aims

Fibroblast Growth Factor Receptors (FGFRs) are transmembrane proteins that regulate cell signaling. FGFRs have previously been shown to be implicated in various cancers. (3)

Alternative splicing (AS) of FGFRs affects receptor function and might be linked to cancer progression.

Recent research suggests that the splicing factor **polypyrimidine tract-binding protein 1 (PTBP1)** regulates inclusion or exclusion of the **1α-exon (exon 3) in FGFR1** (*Figure 1A*).(1, 2) In addition, both **FGFR1 and FGFR2 undergo alternative splicing involving exon 8 or exon 9**, producing the **3b and 3c isoforms**, respectively (*Figure 1B*).(3) The 3b isoform is typically expressed in **epithelial** cells, whereas the 3c isoform predominates in **mesenchymal** cells, suggesting that a shift from 3b to 3c may be associated with tumor progression.

Vimentin is an intermediate filament protein that plays an important role in maintaining cell structure. Elevated vimentin expression is associated with tumor progression in various cancers including **endometrial cancer (EC).**

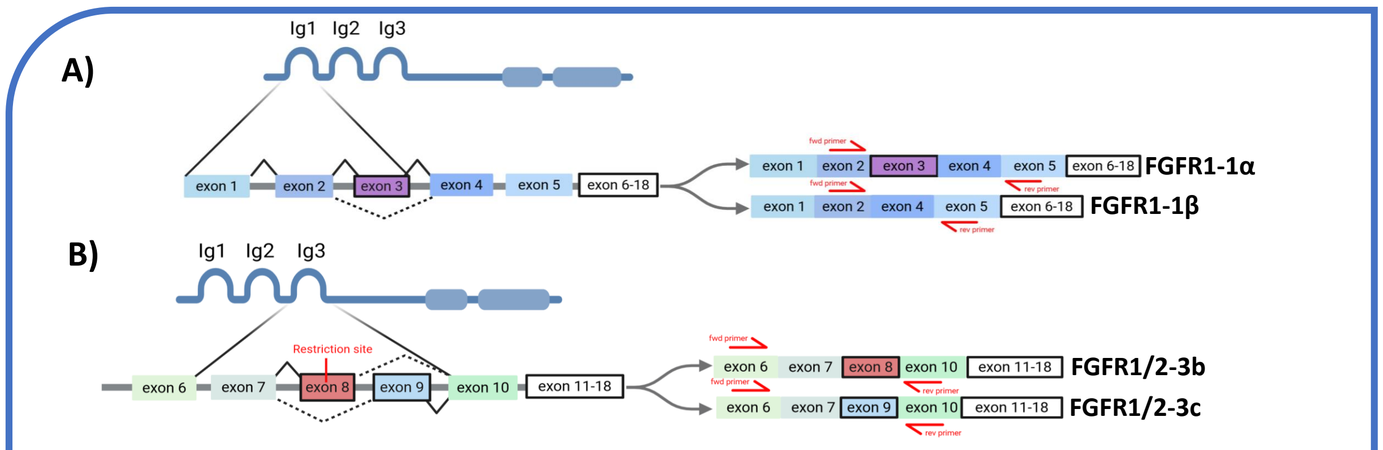

Figure 1: Schematic of FGFR1 and FGFR2 isoform generation by alternative splicing.
A) FGFR1-1α is produced by inclusion of exon 3 (purple), while FGFR1-1ß results from exclusion of exon 3 and has increased ligand affinity. AS of exon 3 causes changes in the first immunoglobulin-like (Ig) domain of FGFR1.
B) Mutually exclusive exons 8 and 9 are located within the third immunoglobulin-like (Ig) domain. Alternative splicing generates the 3b and 3c isoforms, which differ in ligand-binding properties.
Primers used in this project are indicated by red arrows. Restriction sites indicated by a red line. Figure created with BioRender.

The focus of this research was to:

1. Compare alternative splicing levels of the FGFR1-1α exon, as well as the relative expression of FGFR1 and FGFR2 3b and 3c isoforms, in EC cell lines.
2. Compare the protein expression levels of PTBP1 and vimentin in the same cell lines.

Materials and methods

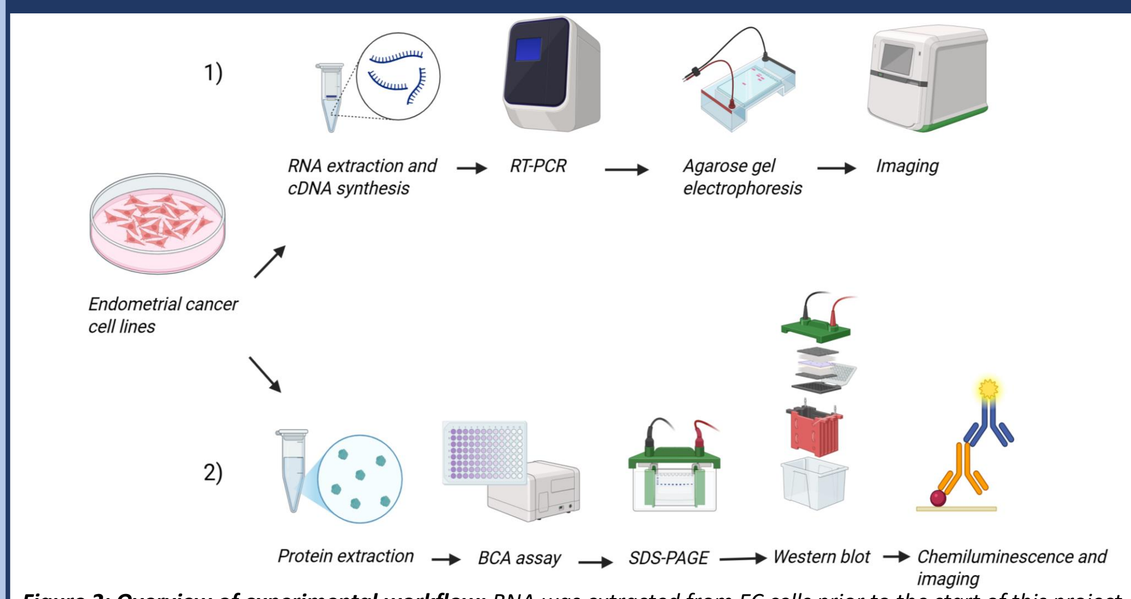

Figure 2: Overview of experimental workflow: *RNA was extracted from EC cells prior to the start of this project, and cDNA was synthesized from the extracted RNA.*
1) Experimental procedures used to investigate the levels of FGFR1 and FGFR2 splicing isoforms in the EC cell lines.
2) Experimental procedures used to examine the protein levels of the markers Vimentin and PTBP1 in the EC cell lines.
Figure created with BioRender.

Results

1) Alternative splicing of exon 3 observed across the EC cell lines

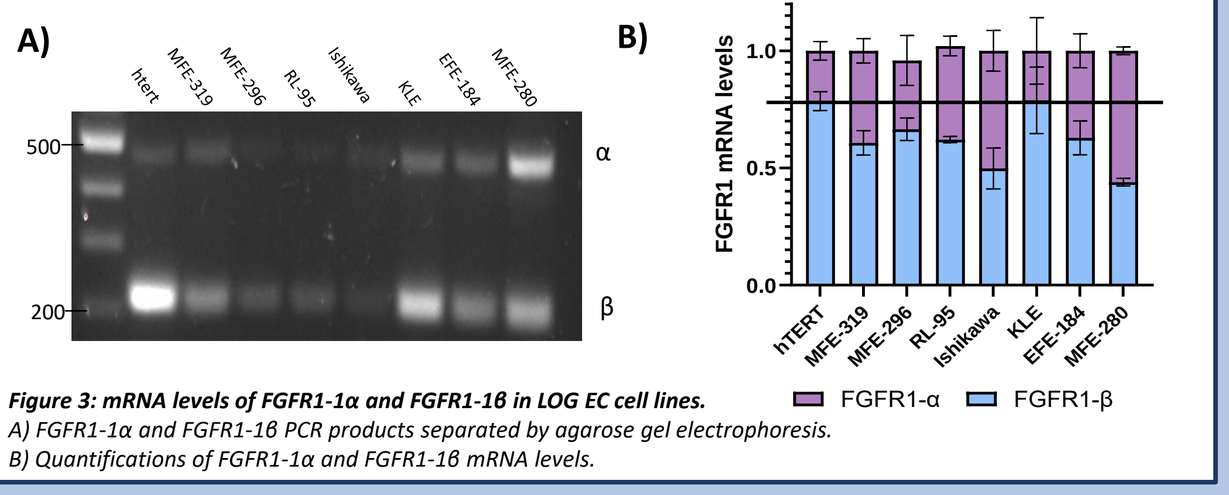

Figure 3: mRNA levels of FGFR1-1α and FGFR1-1ß in LOG EC cell lines.
A) FGFR1-1α and FGFR1-1ß PCR products separated by agarose gel electrophoresis.
B) Quantifications of FGFR1-1α and FGFR1-1ß mRNA levels.

2) FGFR1 and FGFR2 alternative splicing in endometrial cancer cells

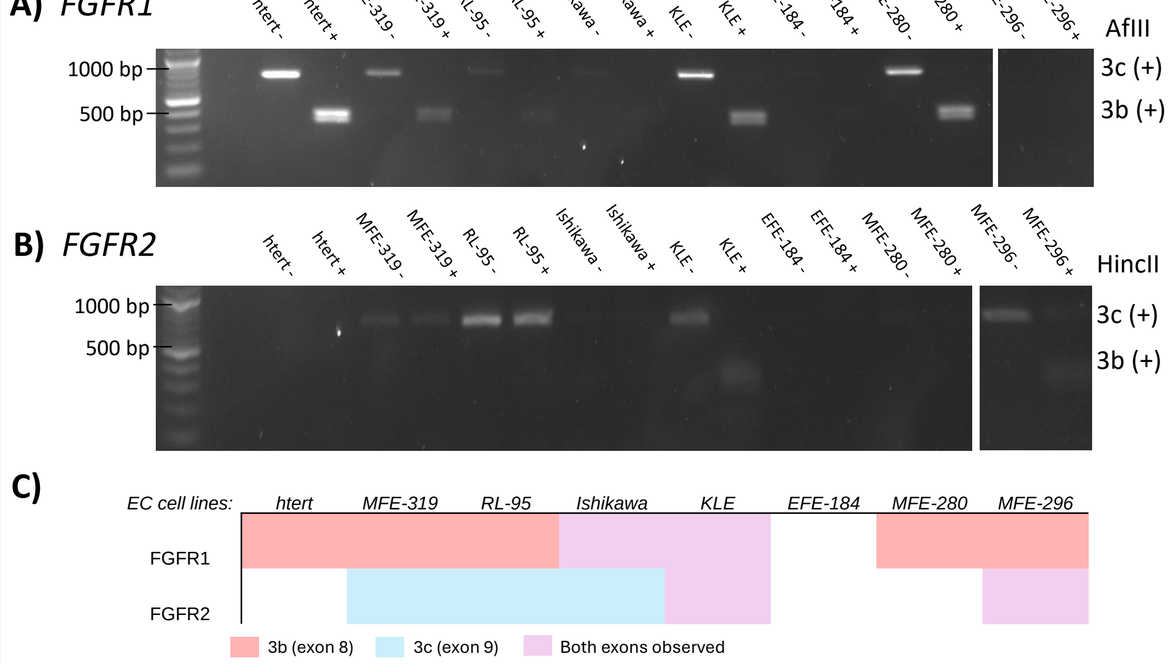

Figure 4: FGFR1 and FGFR2 alternative splice variants in LOG EC cell lines.
A) FGFR1 RT-PCR products were treated with the restriction enzyme AflII, producing cleaved FGFR1-3b and leaving FGFR1-3c uncleaved. Samples were analyzed by agarose gel electrophoresis.
B) FGFR2 RT-PCR products were treated with the restriction enzyme HincII, producing cleaved and uncleaved products that were separated by agarose gel electrophoresis as in (A).
C) Summary diagram of FGFR1 and FGFR2 3b and 3c isoform expression in the EC cell lines. Red indicates 3b expression, blue indicates 3c expression and purple means that both isoforms are present.
Undigested control samples are marked with a: -
No color means there was too little data to observe.

3) Universal PTBP1 expression and variable Vimentin expression was observed across the cell lines

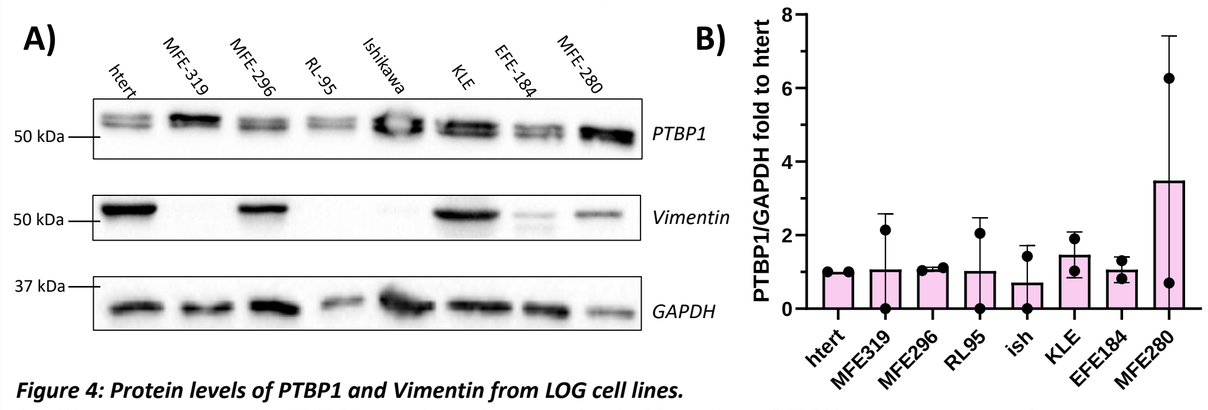

Figure 4: Protein levels of PTBP1 and Vimentin from LOG cell lines.
A) Western blot analysis of PTBP1 and vimentin expression in EC cell lines. GAPDH was used as a loading control.
B) Quantification of PTBP1 protein levels normalized to GAPDH and compared to hTERT. The values represent ± SD from two independent experiments.

Conclusions and future work

- All the cell lines expressed both the 1α-exon and PTBP1 (at varying degrees), consistent with the theory that PTBP1 might be involved in the upregulation of 1α-exon inclusion in cancers.
- The EC cell lines exhibited a low level of the FGFR1-3c isoform but a higher level of the FGFR2-3b isoform, which might indicate that FGFR2 is more relevant to EC progression. However, further research is needed to clarify the mechanisms.
- Vimentin levels did not appear to correlate with the mesenchymal variants 1α or 3c, except for KLE and MFE-296 cells, suggesting a partial link to epithelial mesenchymal transition.
- Future research should focus on understanding how FGFR splicing is regulated in endometrial cancer and how it affects tumor growth and treatment response.

References

1. Jin, W. et.al (2003). Polypyrimidine Tract-Binding Protein Down-Regulates Fibroblast Growth Factor Receptor 1 α-Exon Inclusion. *Cancer Research 63,* 6154-6157.
2. Yu, Q. et.al (2023). PTBP1 as a potential regulator of disease. *Molecular and Cellular Biochemistry, 478*(1-2), 1–14.
3. Chioni, A. et.al (2021) Biological Significance and targeting of the FGFR Axis in Cancer. *Cancers, 13, 5681.*

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