Spectrum of HNF1B mRNA splicing variants in various gynecological lesions

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VFN PRAHA

Hepatocyte nuclear factor 1-beta (HNF1B)

- transcription factor
  - ontogenesis
  - carcinogenesis
- HNF1B protein - regulates expression of multiple genes involved in the cell cycle modulation, susceptibility to apoptosis, and glucose metabolism

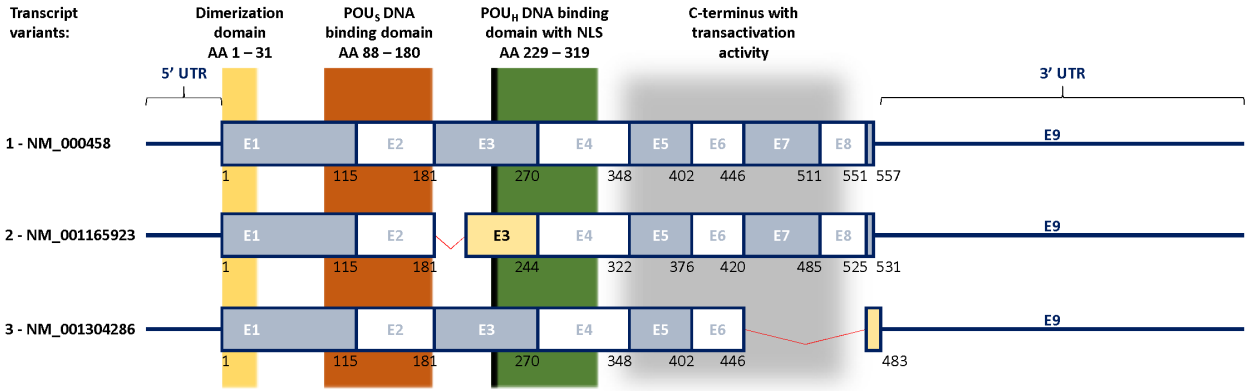


- Possible explanation - tumor-specific expression of HNF1B splicing variants (ASVs) or a dysregulation in the splicing pattern → expression of divergent protein isoforms.
- Two HNF1B ASVs have been described

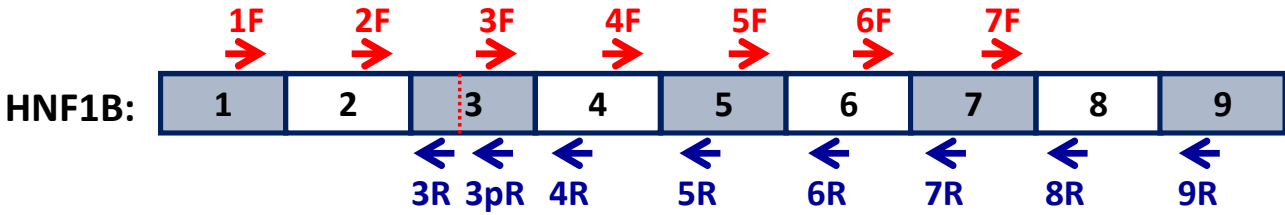
Aim: Characterize the spectrum of HNF1B mRNA splicing variants in the female genital tract with a possible functional impact.

METHODS

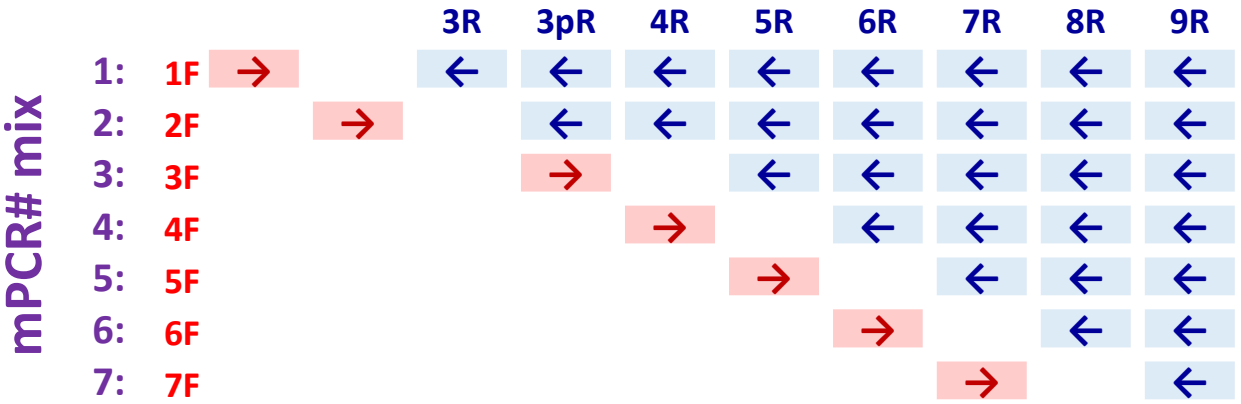
- Tissue types
  - endometroid endometrial/ovarian carcinoma-EEC/EOC, high grade serous ovarian carcinoma-HGSC
- Total RNA isolation and characterization (RQN> 8,8)
- cDNA synthesis (SSIII reverse transcriptase, random hexamers)
- cDNA sample pooling to respective tissue pools
- Multiplex PCR amplification of all present exon-exon junctions (for each tissue pool)
- NGS deep sequencing of exon-exon junctions for identification of low-expressed ASVs
- NGS data analysis in IGV



Scheme of the currently known HNF1B reference transcripts (according to the RefSeq database).



Scheme of the mPCR primer locations across the full-length HNF1B transcript



Scheme of 7 mPCR reactions with respective primer usage.

RESULTS

- Identification of 18 ASVs
  - 16 were novel
  - 4 identified in-frame predominant variants (2 novel)

- Spectrum of detected ASVs varies among tumors
  - High grade serous ovarian carcinoma (HGSC)
    - only 4 predominant variants were identified
  - Endometroid endometrial/ovarian carcinoma (EEC/EOC)
    - all 18 variants identified

3p	Δ3_8	Δ6_8
Δ2,3p	Δ4	**Δ7**
Δ2	Δ4_7,8p	Δ7,8p
Δ2_4	Δ5	**Δ7_8**
Δ2_8	Δ5_8	**Δ8**
Δ3	Δ6_7	ins91bp i4

Red – novel „predominant" variants
Blue- known „predominant" variants

- Differences in tumor specific expression levels
  - Relative expression of two predominant variants (**Δ7, Δ7_8)** is higher in HGSC in contrast with EOC/EEC: Δ7 – 10x; Δ7_8 – 4x

- Predominant variants **Δ7, Δ7_8 and Δ8** lack substantial part of coding sequence for C- terminus transactivation domain – proteins with regulatory function?? (distinct biological activity)

CONCLUSION

- We revealed a series of HNF1B ASVs which have not yet been characterized.

- Our data
  - suggests
    - the presence of several predominant ASVs with potentially different functions than canonical HNF1B
  - shows
    - the overall splicing pattern differs among tumors
    - differences in tumor specific expression levels

      → precise quantification of predominant variants (qPCR, ddPCR) in a wide spectrum of tumor and normal tissue samples

- Identification and precise characterization of HNF1B ASVs is important and can represent a first step in the assessment of their biological meaning and significance for tumorigenesis in female genital tract.

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