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WNT SIGNALING PATHWAY ANALYSIS IN BREAST AND ENDOMETRIAL CANCERS

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Introduction

In recent years, an increase in the number of new cases of diagnosed hormone-dependent neoplasms has been observed. According to the currently available data, one of the signaling pathways involved in the development and progression of these tumors is the WNT pathway, which is particularly important for the regulation of the cell cycle and embryonic development.

Aim of the study

The aim of the study was to evaluate the influence of WNT pathway effectors on patients struggling with breast and endometrial cancers in two independent studies incorporating different bioinformatical tools. The research was focused on identification of novel biomarkers and/or therapeutic targets for this group of patients. The analyses could also contribute to the better understanding of WNT regulation mechanisms and the biological processes associated with the genes of interest.

Materials & Methods

FOR BREAST CANCER ANALYSIS - completed

1. Weighted Gene Correlation Network Analysis (WGCNA) on expression data from The Cancer Genome Atlas (TCGA) regarding 2573 WNT target genes selected on the basis of data deposited in the Gene Transcription Regulation Database (GTRD)
2. Differentially Expressed Genes (DEGs) extraction using EdgeR from comparisons between tumor samples and normal samples, as well as ER positive and ER negative patients
3. Kaplan-Meier survival estimation curves for each DEG regarding patients with breast cancer, further grouped into specific signatures in each comparison
4. Diagnostic potential evaluation of the signatures with ROC curves

FOR ENDOMETRIAL CANCER ANALYSIS - ongoing

1. Seurat (Single Cell RNA-Seq Analysis) on Gene Expression Omnibus datasets regarding WNT-associated potential biomarkers (GSE173682, GSE156728, GSE154763, GSE180091, GSE165404)
2. Cell clusters distribution on UMAP (Uniform Manifold Approximation and Projection) charts and cell type classification
3. Gene expression analysis for each cluster
4. Selection of the most significant combinations regarding gene expression and cell type
5. Kaplan-Meier survival analysis evaluating prognostic potential of the chosen signature

Results

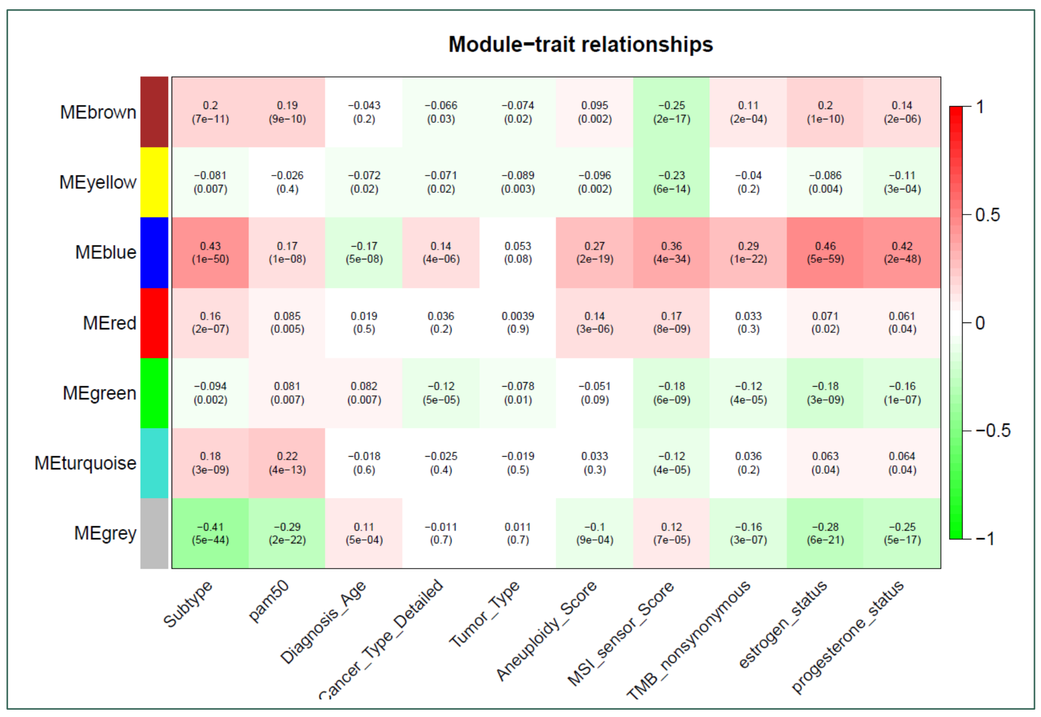

Fig. 1. The Weighted Gene Correlation Network Analysis heatmap showing correlation between genes grouped into modules of similar expression patterns and particular clinical traits of breast cancer patients. The greatest correlation was found for genes included in the blue module and estrogen receptor status. This module comprised of 183 genes, which subsequently underwent further analysis to evaluate their potential linkages with specific clinical attributes.

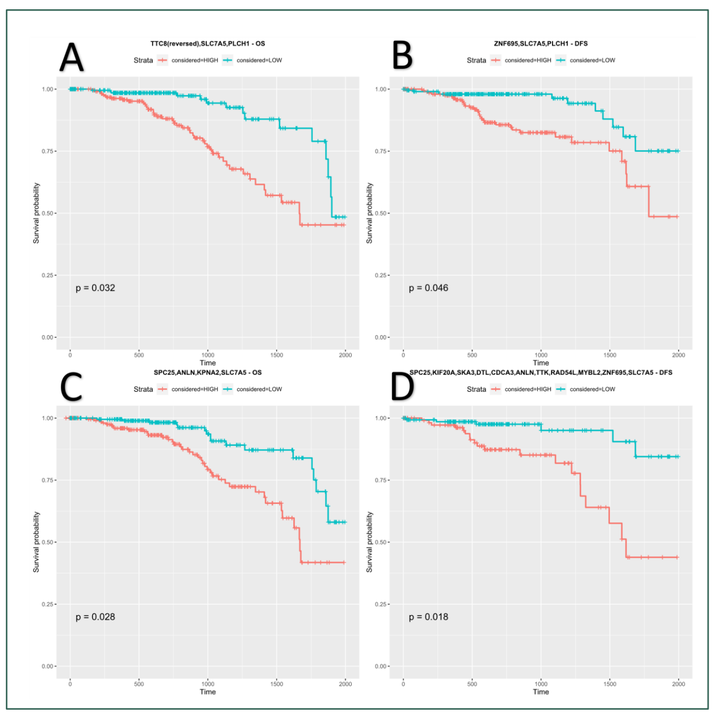

Fig. 2. Kaplan-Meier estimators for multi-gene signatures evaluating combined effect for ER comparisons (A: Overall Survival – TTC8, SLC5A7, PLCH1 with TTC8 expression being reversed as its higher expression was found favorable in contrast to other genes; B: Disease-free Survival – ZNF695, SLC7A5, PLCH1), and tumor versus normal tissue comparisons (C: Overall Survival – SPC25, ANLN, KPNA2, SLC7A5; D: Disease-free Survival – SPC25, KIF20A, SKA3, DTL, CDCA3, ANLN, TTK, RAD54L, MYBL2, ZNF695, SLC7A5)

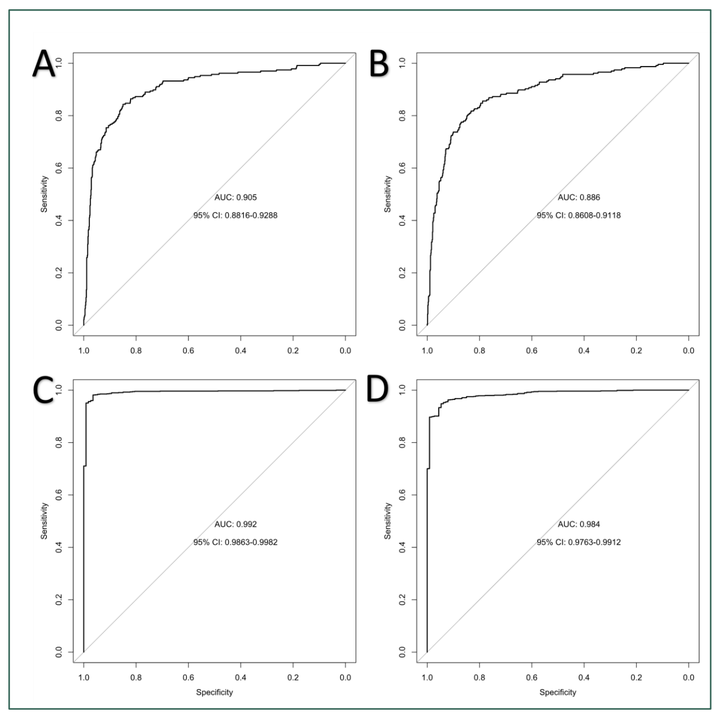

Fig. 3. ROC curves for each signature-based binary regression model for ER status comparisons (A: Overall Survival – TTC8, SLC5A7, PLCH1; B: Disease-free Survival – ZNF695, SLC7A5, PLCH1), and tumor versus normal tissue comparisons (C: Overall Survival – SPC25, ANLN, KPNA2, SLC7A5; D: Disease-free Survival – SPC25, KIF20A, SKA3, DTL, CDCA3, ANLN, TTK, RAD54L, MYBL2, ZNF695, SLC7A5)

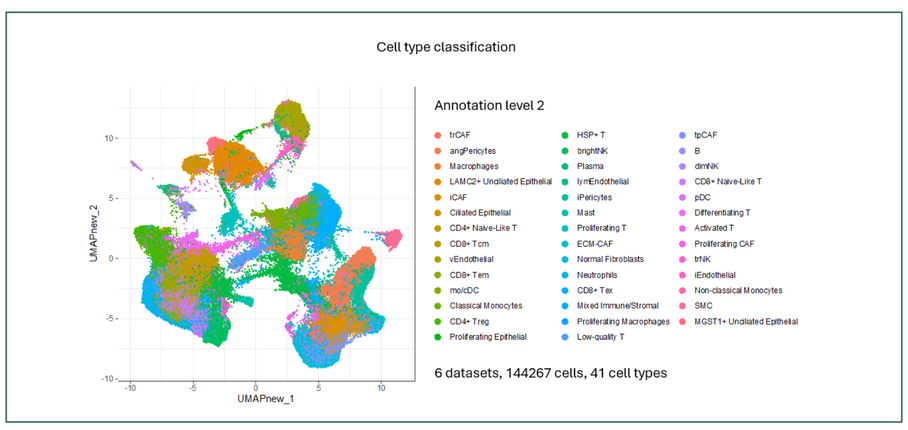

Fig. 4. Based on expression of canonical gene markers in endometrial cancer, 10 main cell types were found (B, T, NK, DC, Endothelial, Epithelial, Myeloid, Mast, SMC, and Fibroblasts)

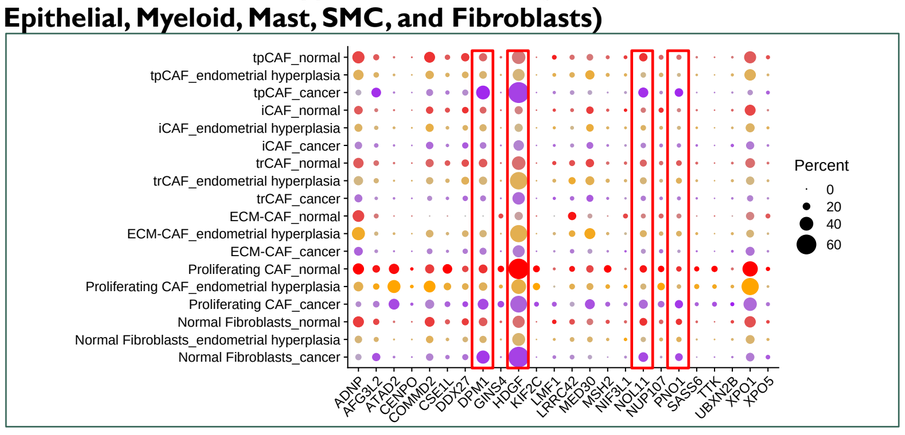

Fig. 5. Heatmap representing the expression of the chosen WNT genes in comparison of normal and endometrial cancer cells in particular subtypes. The greatest difference was found in e.g. fibroblasts

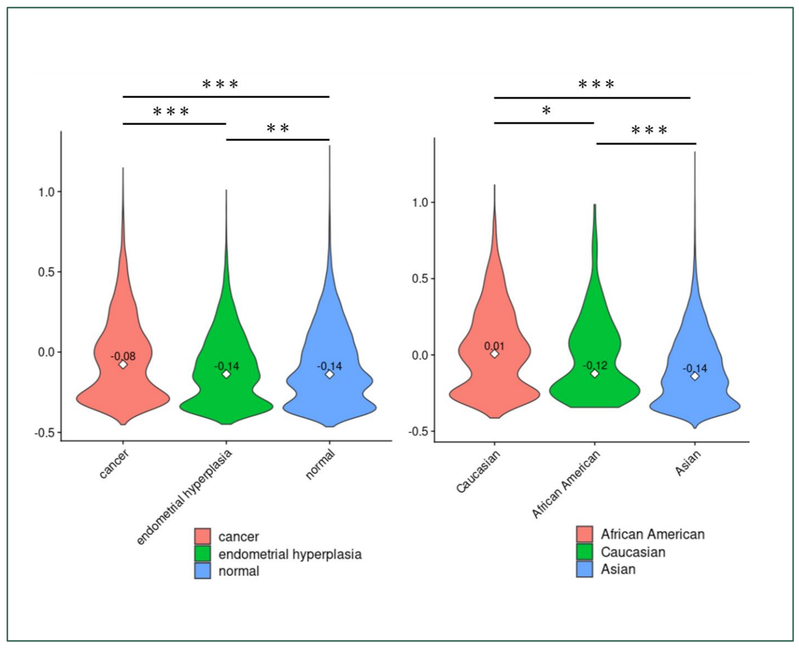

Fig. 6. Enrichment analysis of *DPM1*, *HDGF*, *NOL11*, and *PNO1* signature showed that the signature significantly distinguishes normal, hyperplasia, and endometrial cancer samples, with the addition of showing difference between races

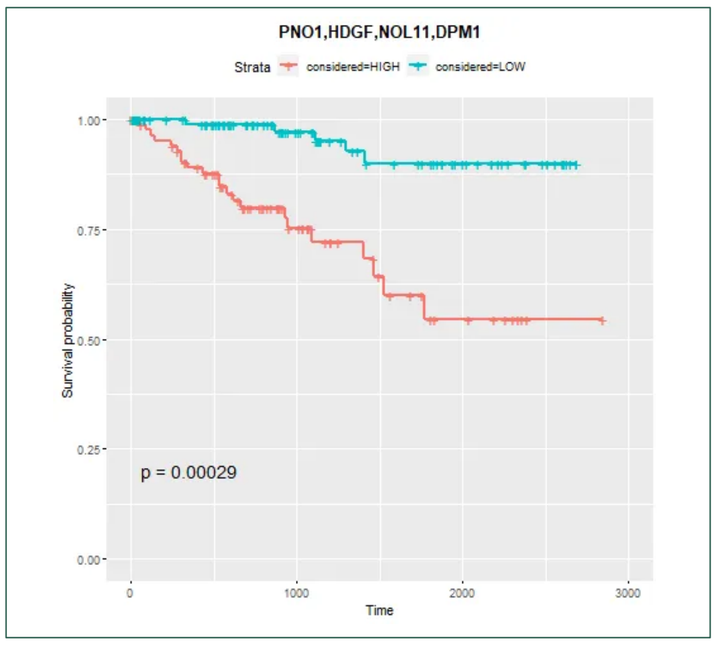

Fig. 7. Kaplan-Meier estimator for Overall Survival evaluating *DPM1*, *HDGF*, *NOL11*, and *PNO1* signature suggests that high expression of this module results in significantly worsened survival outcomes in endometrial cancer patients

Conclusion

The preliminary results obtained in the analyses suggest a high prognostic and diagnostic potential of the extracted genes in both breast cancer and endometrial cancer patients. It seems rational to expand further research to include also *in-vitro* analyzes. This approach may provide valuable insight into the biological processes regulated by genes related to the WNT signaling pathway in hormone-dependent cancers, especially in the context of endometrial cancer, on which upcoming *in-vitro* studies will be conducted.