Identification of potential immune targets in controlling Endometrioid Endometrial Carcinoma metastatic progression

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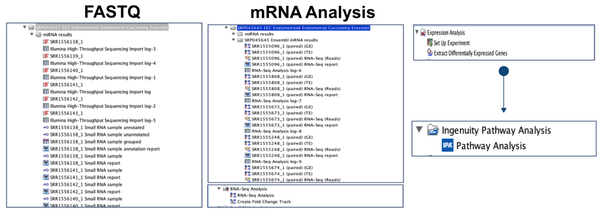
Introduction

- Endometrial adenocarcinoma is a common cause of gynecological cancer death in Europe and North America.
- The most dominant subtype, Endometrioid Endometrial Cancer (EEC) accounts for >80% of this cancer and is estrogen-dependent.
- At diagnosis, 75% of women have the disease confined to the uterus, which is considered Stage One. Five-year survival for Stage One patients is 80%, however, about 15–20% develop metastasis.
- Most EECs are low-grade tumors (G1 or G2, comprised of moderately to well-differentiated cells) that are early stage (i.e. before extra-uterine spread).
- Risk Factors: Menopause, but up to 25% of cases premenopausal, Obesity, Nulliparity, Diabetes mellitus, Prolonged, unopposed estrogen exposure in post-menopause, Tamoxifen and oral contraceptive pills.
- Patients are generally treated with surgery, radiation, chemotherapy or hormone therapy

Materials & Methods

- Total RNA extracted from tissues obtained after surgical resection from three women at stage one EEC (two Stage IA and one Stage IB (all Grade 1) was subjected to RNA-sequencing.
- The publicly available dataset (SRP045645) was downloaded directly from the Sequence Read Archive and the FASTQ files were processed with Biomedical Genomics Workbench (BX) for secondary analysis including mapping, quantification and differential expression analysis.
- Through streamlined integration the data was uploaded to Ingenuity Pathway Analysis (IPA) for biological interpretation.
- Sequencing: mRNA (100 bp paired-end reads) and small RNA (50 bp single-end reads): Illumina HiSeq 2000 of tumor (T) and adjacent non-tumorous (Adj Non-T) tissues.
- BX to IPA: Expression Profile from RNA-seq: 1. Download FASTQ from SRA (convert .sra to FASTQ). 2. Import the FASTQ files into BX. 3. Set up the RNA-seq analysis in BX: mRNA (select Reference Genome: human Ensembl V81, Hg38), select Mapping options, select Expression Level Option. 4. Set up the experiment at transcript level (TE): Tumor (T) vs. Adjacent Non-Tumor (Adj Non-T). 5. Send dataset to IPA using Plugin from BX. 6. Analyze the processed dataset in IPA (mRNAs)

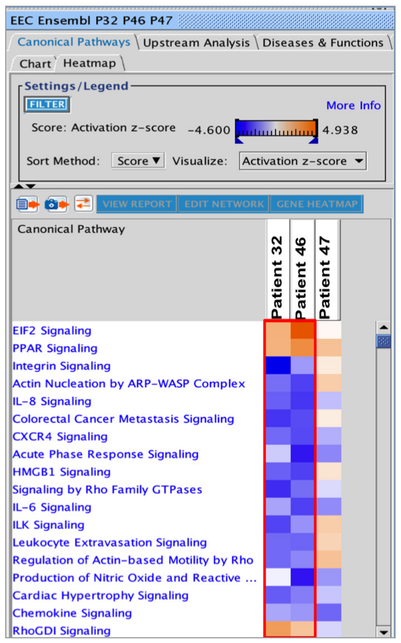
FASTQ **mRNA Analysis**



Biological Analysis with IPA

Dataset: 3291 isoforms with >20 RPKM in either T or Adj Non-T, |fold change|>1, p<0.05
Analysis: 740 mRNAs with |fold change|>2 in IPA, (130 miRNAs) in MicroRNA Target Filter

Comparison of Canonical Pathways in patients P32, P46, P47



The patients' mRNA expression data indicates activation and inhibition of many of the same CP involved in tumorigenesis:

- Proliferation (EIF2 signaling)
- Cell movement (Integrin signaling, ILK signaling, Actin nucleation by ARP-WASP Complex, Signaling by Rho family GTPases, …)
- Metabolic pathways (PPAR signaling)

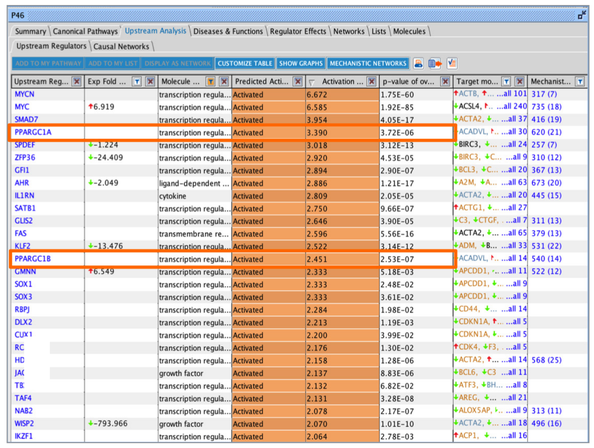
However two of the three are more alike than the other based on activity pattern:

- P32 and P46 are likely Stage IA
- P47 is likely Stage IB

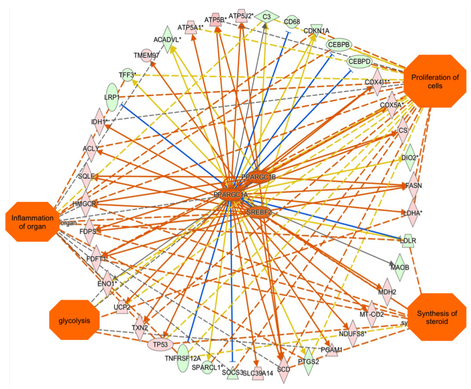
Upstream Analysis of Patient 46

Typical Transcriptional Program in tumor progression (early stage): MYC, SMAD7,…

Network of 2 selected (+ 1 not shown here) transcription regulators in Patient 46 (see below)



Biological Processes Predicted to be Activated in Patient 46, Overlay statistically significant diseases and functions

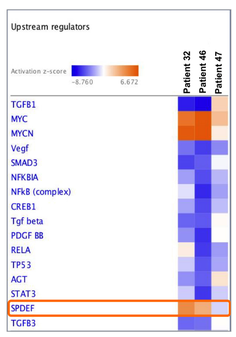


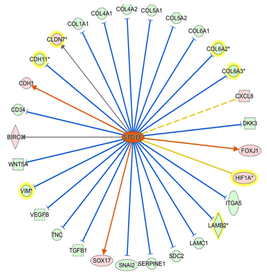
Drivers of Fatty acid and Sterol Metabolism are predicted to be activated.

Proliferation of cells and Inflammation are strongly activated, Synthesis of steroid (estrogens, progesterone, …) and glycolysis are activated as well.

Comparison of the Upstream Analysis in P32, P46, and P47

Growth Factors and Transcription Regulators also distinguish the patients from one another

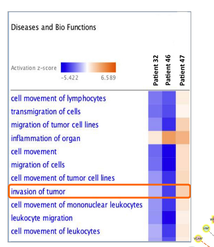


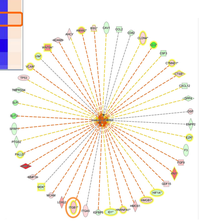


Cell migration & invasion:

- Inhibited in P32 and P46 (SPDEF activated)
- Induced in P47 (SPDEF inhibited)

Downstream Effect Analysis indicates increased "invasion of tumor" in P47 compared to P32 and P46



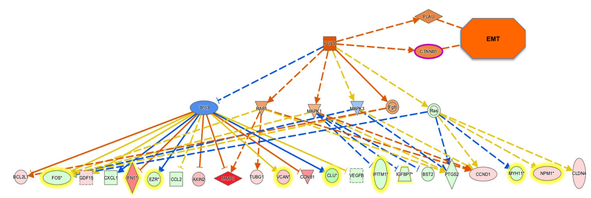


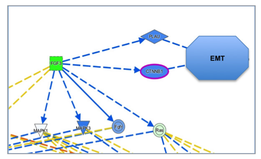
ITGB1 splicing variants: potential regulator of invasion of carcinoma cells

Highlight of a key gene and its isoforms: up-regulation of ITGB1-010 (isoform) may promote cell migration/ invasion during metastasis to other tissues

Causal Network Analysis (CNA): FGF3 is linked to Epithelial-to-Mesenchymal-Transition (EMT) in EEC

FGF3-driven CN (depth 2) is shown below (7 regulators plausibly explaining the expression pattern of 164 downstream targets (22 are shown here). Frequent amplification of this gene has been found in human tumors, which may be important for neoplastic transformation and tumor progression (BrCa). Hypothesis to be tested and validated: FGF3 is predicted to be activated and is driving a CN potentially connected to EMT via CTNNB1 and PLAU.

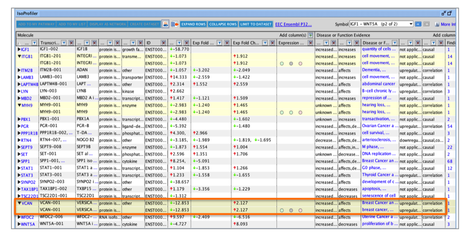




This CN allows to set a new hypothesis in conjunction with MAP (Molecule Activity Predictor). MAP simulates the inhibition of FGF3 and the impact on the EMT (epithelial-to-mesenchymal-transition). When FGF3 is inhibited or downregulated, the EMT is decreased (blue circle).

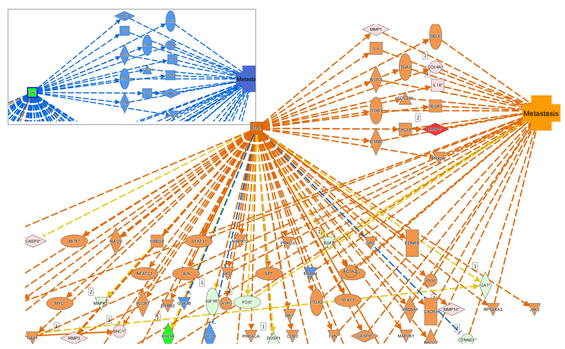
IsoProfiler to discover isoforms that may drive tumor progression

VCAN (versican): upregulation of VCAN-001 is involved in malignant solid tumor (in BrCa). This isoform is upregulated in P47



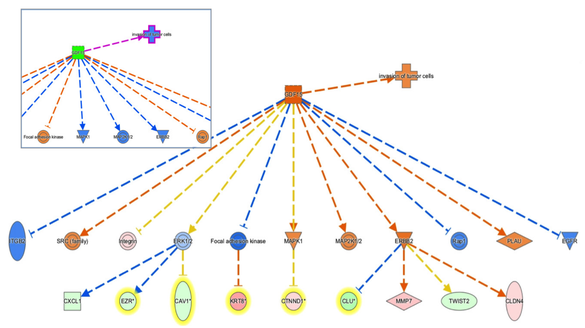
EDN1-driven CN is linked to Metastasis in EEC

EDN1-driven CN (depth 2) is shown below (57 regulators plausibly explaining the expression pattern of 378 downstream targets (none shown here). Upregulation or involvement of EDN1 has been shown in colorectal cancer, human chondrosarcoma, prostate cancer, multiple myeloma, and non-small lung cancer. Hypothesis to be tested and validated: EDN1 is predicted to be activated and is driving a CN potentially connected to metastasis via many intermediates (IL6, ITGB3, CXCL8 etc…). Inhibiting EDN1 (green) would decrease metastasis (blue).



GDF15-driven CN is linked to invasion in EEC

GDF15-driven CN (depth 2) is shown below (12 regulators plausibly explaining the expression pattern of 92 downstream targets (9 are shown here). Overexpression of GDF15 has been show to be involved in many cancers (melanoma, prostate, thyroid, pancreatic, ovarian, colon). Plasma GDF-15 is elevated in patients with endometrial cancer and is a marker for phenotype, including lymph node metastasis and disease-specific survival. Hypothesis to be tested and validated : GDF15 is predicted to be activated and is driving a CN potentially connected to invasion. Inhibiting GDF15 (green) would decrease



Conclusion

We have identified three important immune related proteins as key factors toward tumor progression (cell invasion, EMT and Metastasis) using our QIAGEN "Sample to Insight" solution that helps delivering data analysis (BX) and biological interpretation (IPA) and suggesting new hypotheses to be tested and validated.

Using Biomedical Genomics Workbench, we have been able to: Upload RNA-seq data (FASTQ files from SRA); Align to the genome of interest (human Ensembl); Quantitate and obtain differential expression between samples; Seamlessly send data directly into IPA for biological interpretation.

Using IPA, we have been able to: Understand signaling pathways involved in EEC progression; Discover potential transcriptional program(s); Visualize differentially expressed splicing variants (view of ITGB1, VCAN); Discover biological processes participating in tumor progression; Highlight new hypotheses (FGF3, EDN1 and GDF15-CN).