

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

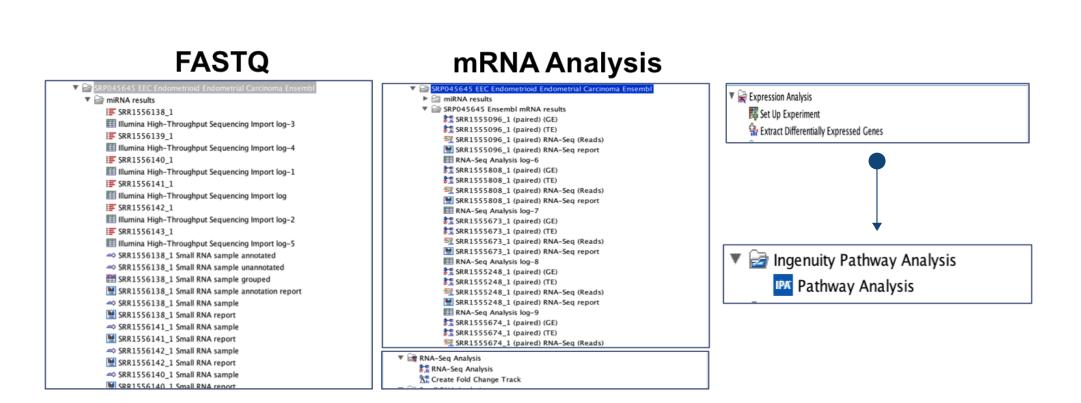
Jean-Noel Billaud, Stuart Tugendreich, Debra Toburen, QIAGEN Bioinformatics (Redwood City, CA)

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

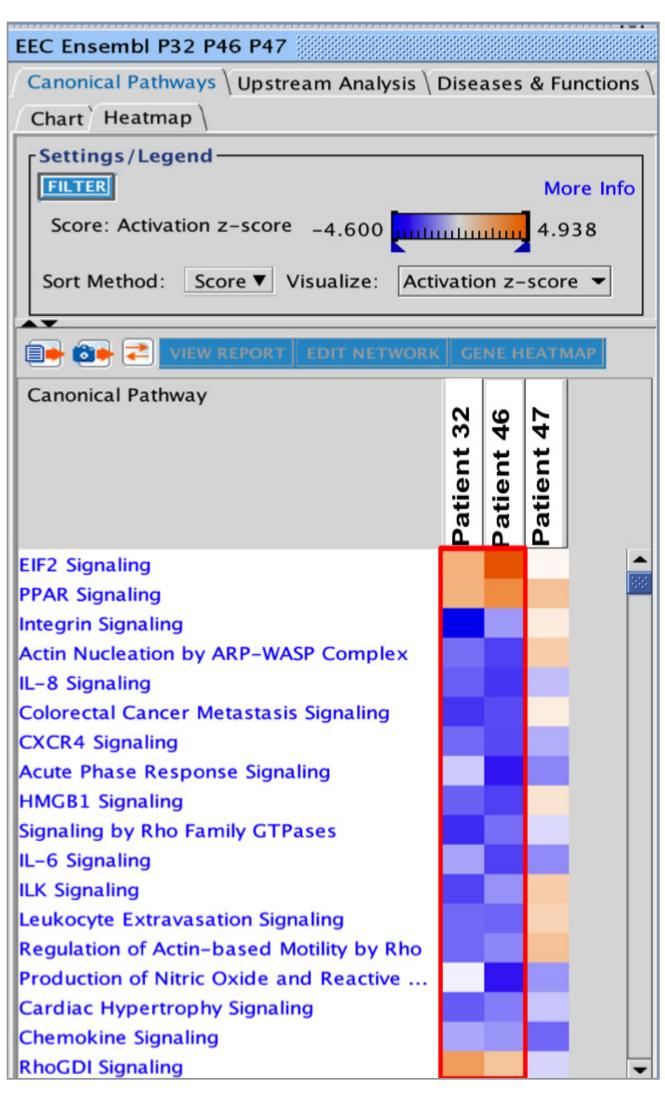
- Total RNA extracted from tissues obtained after surgical resection from three women at stage one EEC (two Stage IA and one Stage IB (all Grade 1) was subjected to RNA-sequencing.
- The publicly available dataset (SRP045645) was downloaded directly from the Sequence Read Archive and the FASTQ files 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)



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)

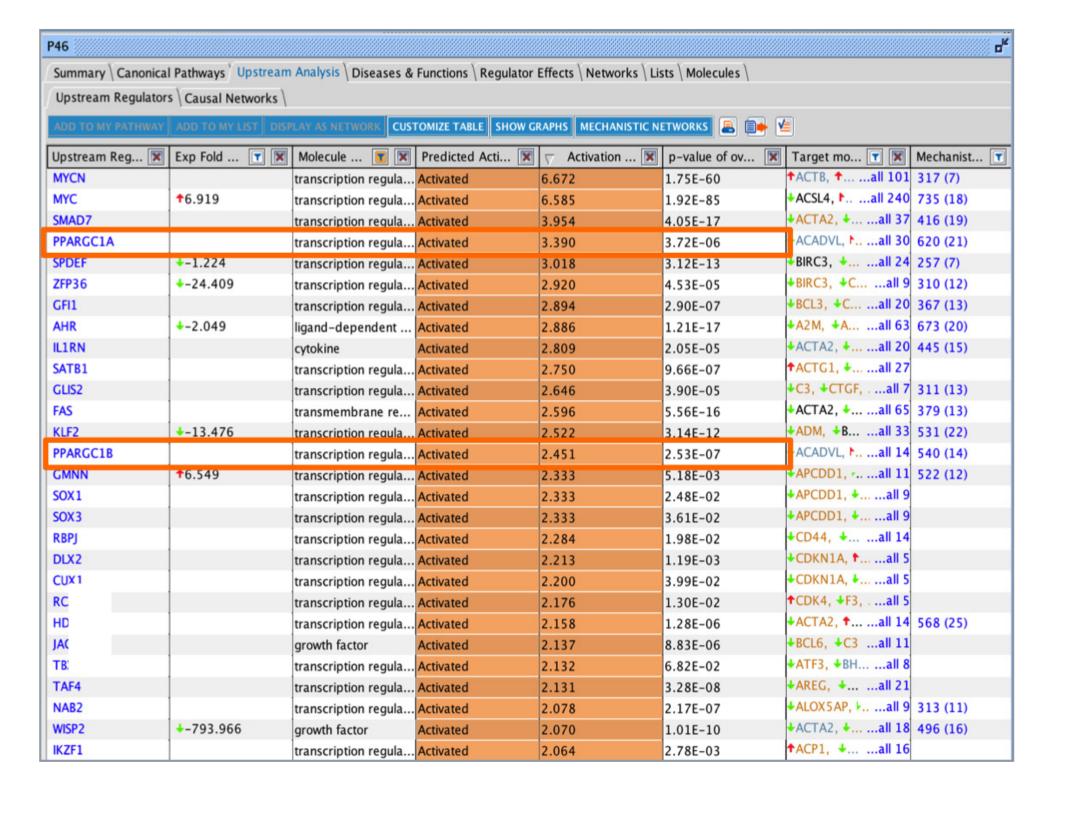
However two of the three are more alike than the other based on activity pattern:

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

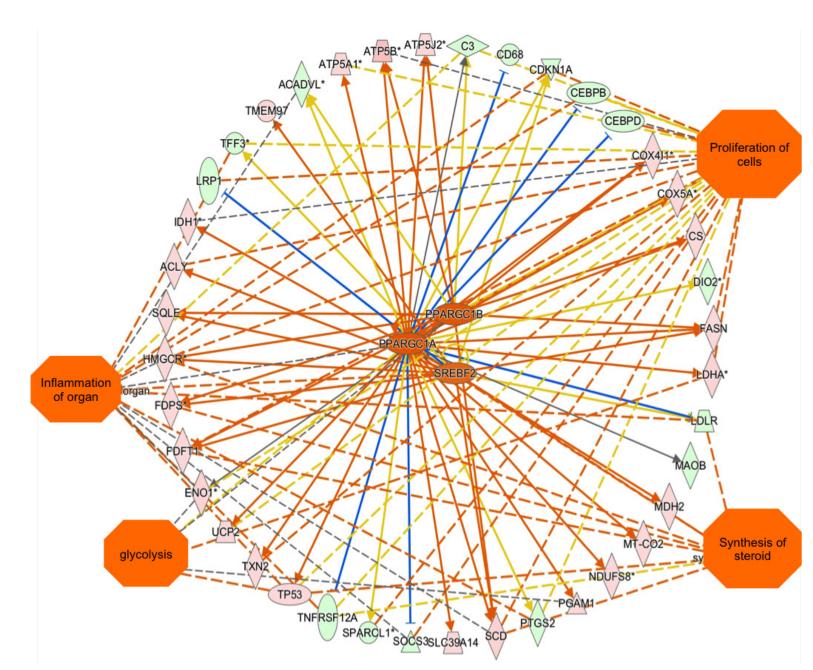
Upstream Analysis of Patient 46

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

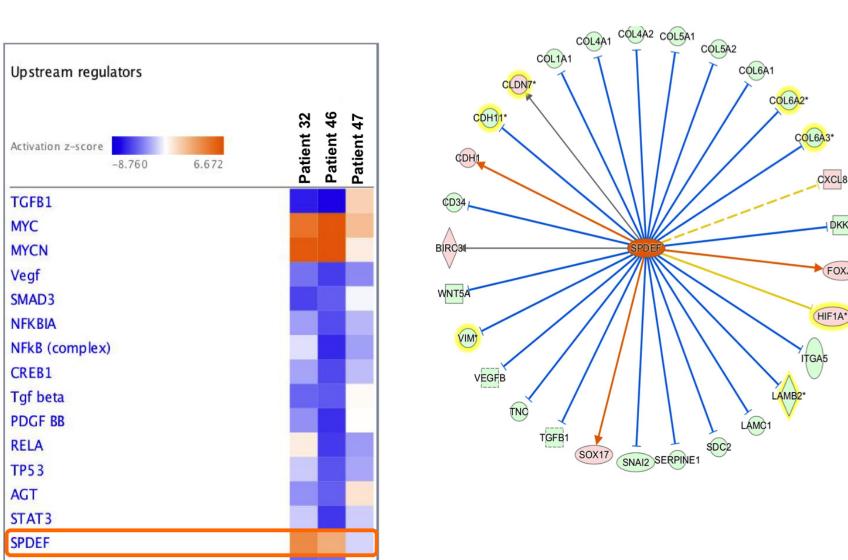


Drivers of Fatty acid and Sterol Metabolism are predicted to be activated.

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

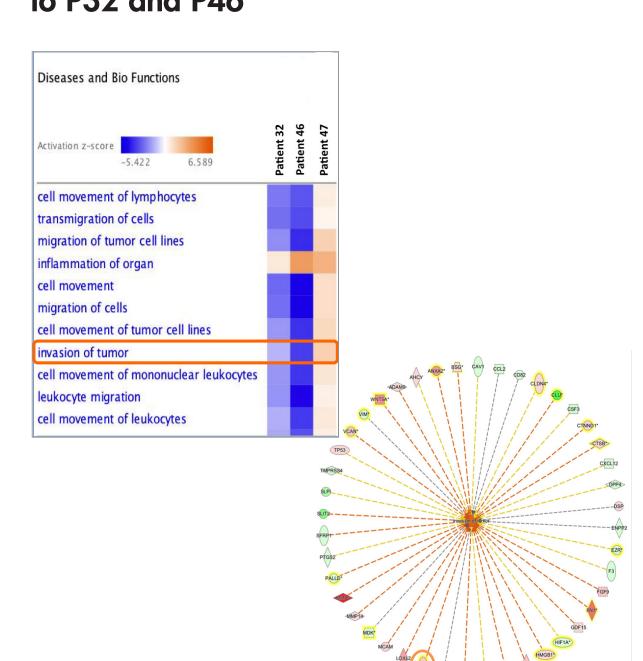


Cell migration & invasion:

• Inhibited in P32 and P46 (SPDF)

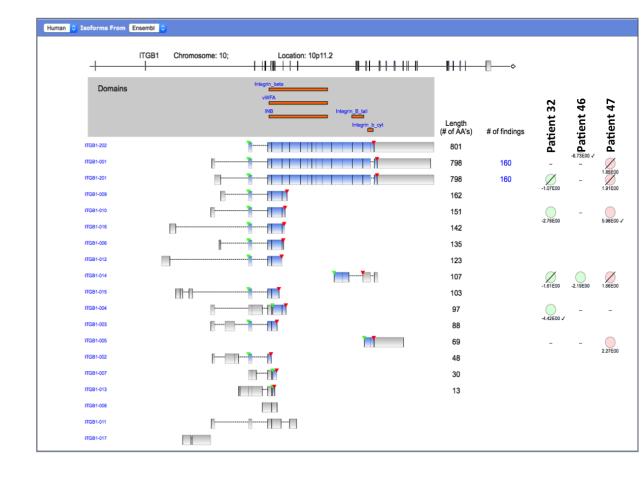
- Inhibited in P32 and P46 (SPDEF activated)
- Induced in P47 (SPDEF inhibited)

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



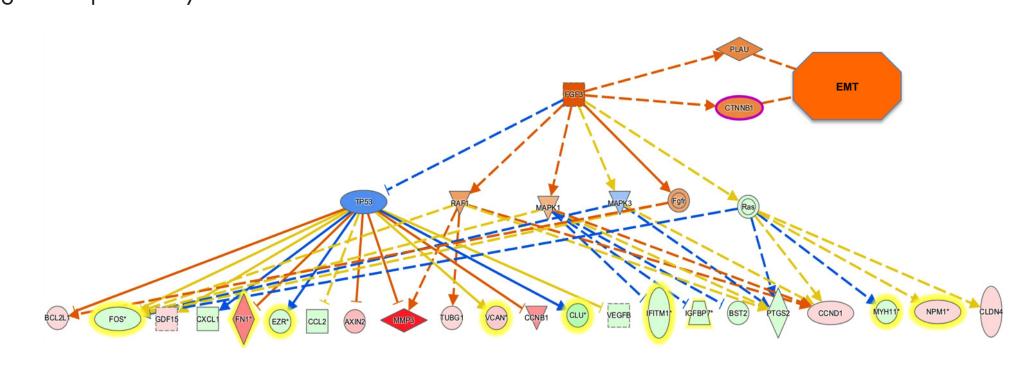
ITGB1 splicing variants: potential regulator of invasion of carcinoma cells

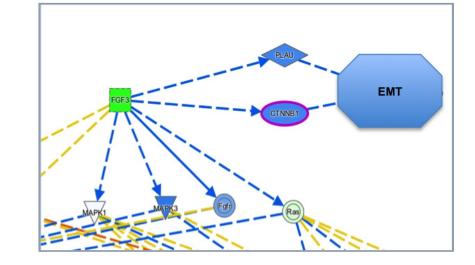
Highlight of a key gene and its isoforms: up-regulation of ITGB1-010 (isoform) may promote cell migration/invasion during metastasis to other tissues



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).

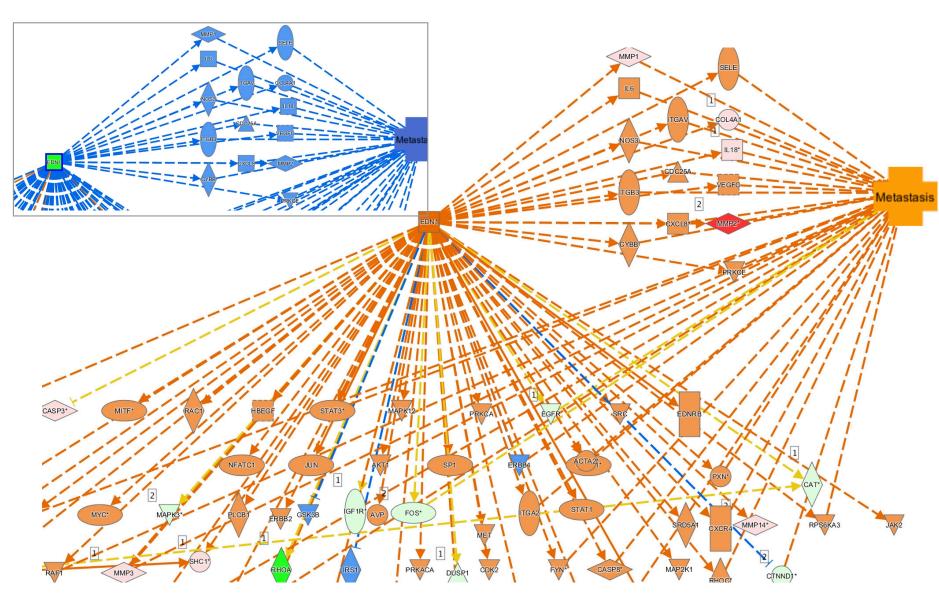
IsoProfiler to discover isoforms that may drive tumor progression

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

Molecule									Add column(s) 📳	Disease or Function Evid	lence	
Λ 🔽	Transcri 🔻 🔀		🔻 🕱					Exp Fold Ch	Expression	🔻 🕱 🔻 🕱	Disease or F	🔻 🕱
F 101 1	IGF1-002	IGF1B	protein is		ENST000	+ -58.770				increased increases	quantity of cells	not applic
	ITGB1-201	INTEGRI	protein is	transme	ENST000	+ −1.073		† 1.912		increased increases	cell movement,	not applic
	ITGB1-201	INTEGRI	protein is		ENST000	+ −1.073		† 1.912	0 0 0	increased increases	cell movement,	not applic
►ITM2B	ITM2B-001	ADAN	protein is	other	ENST000	↓ -1.057	↓ -3.202	↓ -2.049		increased affects	Dementia,	upregulat
►LAMB3	LAMB3-001	LAMB3	protein is	transporter	ENST000	† 14.333	↓ -2.559	↓ -1.422		increased increases	cell movement,	not applic
►LAPTM4B	LAPTM4B-001	LAPT	protein is	other	ENST000	† 2.314	† 1.552	† 2.559		increased affects	abdominal cancer	upregulat
►LYN	LYN-003	LYNB	protein is	kinase	ENST000	† 2.662				increased affects	B-cell chronic ly	upregulat
►MBD2	MBD2-001	MBD2 A i	protein is	transcript	ENST000	† 1.417	↓ -1.121	† 1.509		increased increases	repression of	not applic
▼MYH9	MYH9-001	MYH9	protein is	enzyme	ENST000	+ -2.983	↓ -1.240	† 1.465		unknown affects	hearing loss,	not applic
	MYH9-001	MYH9	protein is		ENST000	↓ -2.983	↓ -1.240	† 1.465	0 0 0	unknown affects	hearing loss,	not applic
▶PBX1	PBX1-001	PBX1A	protein is	transcript	ENST000	↓ -4.480		↓ -1.602		unknown increases	transactivation,	not applic
▶PGR	PGR-001	PGR-B	protein is	ligand-de	ENST000	↓ -5.392		↓ -1.480		increased affects,de	Ovarian Cancer a	upregulat
▶PPP1R1B	PPP1R1B-002,	T-DA	protein is	phosphat	ENST000	† 4.300,	† 2.966			increased increases	cell survival,	not applic
▶RTN4	RTN4-007,	NOGO B2	protein is	other	ENST000	↓ -3.185,	↓ -1.989	↓ -1.819, ↓ -1.695		decrease affects,de	arteriosclerosis,	downregu
►SEPT9	SEPT9-008	SEPT9B	protein is	enzyme	ENST000	+ -1.873	† 1.554	† 1.004		increased affects,in	M phase,	not applic
▶SET	SET-001	SET al	protein is	phosphat	ENST000	† 2.596	† 1.351	† 1.706		unknown decrease	DNA replication	not applic
▶SPP1	SPP1-001,	SPP1 iso	protein is	cytokine	ENST000	† 8.254,	↓ -5.091			increased affects,de	Breast Cancer an	not applic
▶STAT1	STAT1-001	STAT1 a	protein is	transcript	ENSTOOO	† 1.104	↓ -1.853	† 1.266		increased affects,de	G0 phase,	not applic
►STAT3	STAT3-001	STAT3 a	protein is	transcript	ENST000	† 1.233	↓ -1.558	↓ -1.655		increased affects	Thyroid Cancer a	upregulat
►SYNPO2	SYNPO2-003	SYNPO2 i	protein is	other	ENSTOOO	↓ -38.657				increased affects	development of c	not applic
▶TAX1BP1	TAX1BP1-002	TXBP15	protein is	other	ENST000	† 1.179	↓ -3.356	↓ -1.229		increased decreases	apoptosis,	not applic
▶TSC22D1	TSC22D1-001	TSC22D1	protein is	transcript	ENSTOOO	↓ -1.312				increased decreases	senescence of cell	not applic
▼VCAN	VCAN-001	VERSICA	protein is	other	ENST000	+ -12.853		† 2.127		increased affects	Breast Cancer an	upregulat
	VCAN-001	VERSICA	protein is		ENST000	+ -12.853		† 2.127	0 0 0	increased affects	breast cancer,	upregulat
►WFDC2	WFDC2-006	WFDC2	RNA isofo	other	ENST000	† 9.597	↓ -2.409	↓ -6.516		increased affects	Uterine Cancer a	upregulat
►WNT5A	WNT5A-001	WNT5A i	protein is	cytokine	ENST000	↓ -4.727		† 8.093		increased decreases	proliferation of b	not applic

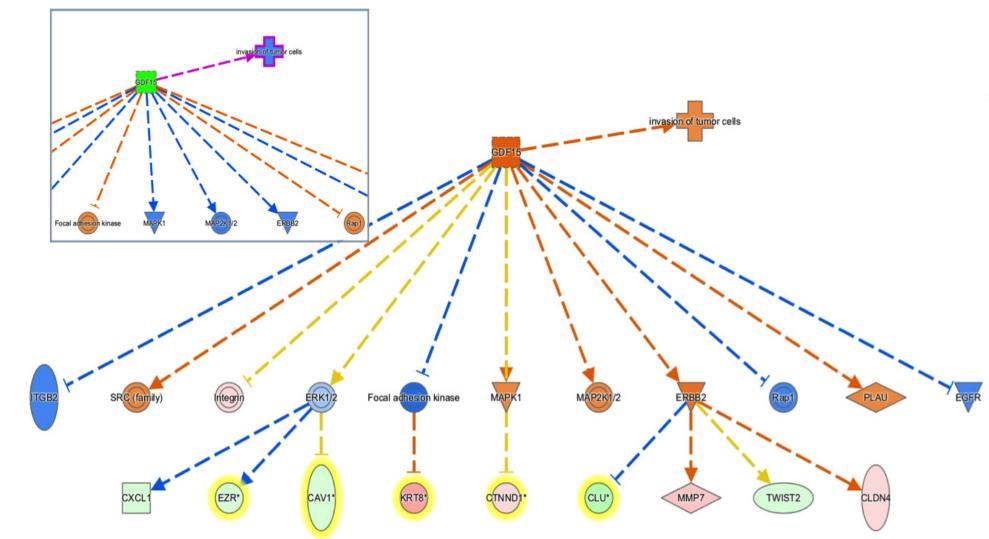
EDN1-driven CN is linked to Metastasis in EEC

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 o 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 intermedi ates (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).