

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 nontumorous (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



Canonical Pathways (CP) of Patient P32 involved in tumor progression (both signaling and metabolic)



Pathway Activity Analysis: Proliferation pathway (EIF2 signaling) is activated (orange), Cell movement/ motility (ILK signaling, Integrin Signaling) are inhibited (blue)

Comparison of Canonical Pathways in patients P32, P46, P47

The patients' mRNA expression date cates activation and inhibition of m the same CP involved in tumoriger

- Proliferation (EIF2 signaling)
- Cell movement (Integrin signali) ILK signaling, Actin nucleation ARP-WASP Complex, Signaling Rho family GTPases, ...)
- Metabolic pathways (PPAR sig ling)

However two of the three are more than the other based on activity pa

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

Sample to Insight

Canonical Pathways \ Upstream Analysis \ Diseases & Functions	Regulator Effects Networks	Lists My Pathways	Molecules	
			Export : 🍅	0 + (0
ysis Settings				
Canonical Pathways				
Name	p-	value	Ove	rlap
EIF2 Signaling		1.53E-33	28.6 %	53/185
Regulation of eIF4 and p70S6K Signaling		1.91E-13	19.2 %	28/146
Antigen Presentation Pathway	•	6.13E-13	40.5 %	15/37
mTOR Signaling	· · · · ·	3.85E-12	16.0 %	30/188
Agranulocyte Adhesion and Diapedesis		4.42E-12	15.9 %	30/189
	法委托委会委任任 2			
Upstream Regulators				
ostream Regulators				
Name	p-value	of overlap	Predicted	Activation
MYCN		3.25E-60		
TGFB1	· · · · · ·	9.72E-60		
MYC	•	3.22E-57	Activ	ated
beta-estradiol		1.57E-51		
dexamethasone	•	1.93E-51		
	121466666			
usal Networks				
usal Networks Name	p-value	of overlap	Predicted	Activation
usal Networks Name GRP	p-value	of overlap 1.87E-78	Predicted	Activation
usal Networks Name GRP miR-145-5p (and other miRNAs w/seed UCCAGUU)	p-value	of overlap 1.87E-78 4.60E-77	Predicted	Activation
usal Networks Name GRP miR-145-5p (and other miRNAs w/seed UCCAGUU) KLK14	p-value	of overlap 1.87E-78 4.60E-77 2.06E-76	Predicted	Activation

ta indi-	EEC Ensembl P32 P46 P47
nany of	Canonical Pathways \ Upstream Analysis \ Diseases & Functions \
nesis:	Chart Heatmap
	Score: Activation z-score -4,600 4,938
ling, 1 by	Sort Method: Score ▼ Visualize: Activation z-score ▼
ig by	E VIEW REPORT EDIT NETWORK GENE HEATMAP
	Canonical Pathway
gna-	Patient 32 Patient 46 Patient 47
e alike	EIF2 Signaling
attorn	PPAR Signaling
	Integrin Signaling
	Actin Nucleation by ARP-WASP Complex
١٨	IL-8 Signaling
IA	Colorectal Cancer Metastasis Signaling
	CXCR4 Signaling
	Acute Phase Response Signaling
	HMGB1 Signaling
	Signaling by Rho Family GTPases
	IL-6 Signaling
	ILK Signaling
	Regulation of Actin_based Motility by Rho
	Production of Nitric Oxide and Reactive
	Cardiac Hypertrophy Signaling
	Chemokine Signaling
	RhoGDI Signaling

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)

Summary \ Canonica	Pathways Upstream	Analysis \ Diseases &	Functions \ Regulator	Effects \ Networks \ Lis	sts \ Molecules \		
Upstream Regulator	s \ Causal Networks \						
ADD TO MY PATHWAY	ADD TO MY UST DIS	PLAY AS NETWORK CUST	TOMIZE TABLE SHOW G	RAPHS MECHANISTIC N	etworks 🖪 📑		
Jpstream Reg 🕱	Exp Fold 🝸 🕱	Molecule 📧 🕱	Predicted Acti 💌	V Activation 🕱	p-value of ov 🕱	Target mo 🝸 🕱	Mechanist
AYCN		transcription regula	Activated	6.672	1.75E-60	↑ACTB, ↑all 101	317 (7)
IYC	† 6.919	transcription regula	Activated	6.585	1.92E-85	ACSL4, Mall 240	735 (18)
MAD7		transcription regula	Activated	3.954	4.05E-17	+ACTA2, +all 37	416 (19)
PARGC1A		transcription regula	Activated	3.390	3.72E-06	ACADVL, Mall 30	620 (21)
DEF	↓ -1.224	transcription regula	Activated	3.018	3.12E-13	→BIRC3, ↓all 24	257 (7)
FP36	↓-24.409	transcription regula	Activated	2.920	4.53E-05	◆BIRC3, ◆Call 9	310 (12)
FI1		transcription regula	Activated	2.894	2.90E-07	◆BCL3, ◆Call 20	367 (13)
HR	+-2.049	ligand-dependent	Activated	2.886	1.21E-17	◆A2M, ◆Aall 63	673 (20)
1RN		cytokine	Activated	2.809	2.05E-05	◆ACTA2, ◆all 20	445 (15)
TB1		transcription regula	Activated	2.750	9.66E-07	↑ACTG1, ↓all 27	
LIS2		transcription regula	Activated	2.646	3.90E-05	+C3, +CTGF,all 7	311 (13)
s		transmembrane re	Activated	2.596	5.56E-16	◆ACTA2, ◆all 65	379 (13)
LF2	+-13.476	transcription regula	Activated	2.522	3.14E-12	+ADM, +Ball 33	531 (22)
PARGC1B		transcription regula	Activated	2.451	2.53E-07	ACADVL, Mall 14	540 (14)
MNN	† 6.549	transcription regula	Activated	2.333	5.18E-03	APCDD1,all 11	522 (12)
DX1		transcription regula	Activated	2.333	2.48E-02	◆APCDD1, ◆all 9	
X3		transcription regula	Activated	2.333	3.61E-02	◆APCDD1, ◆all 9	
BPJ		transcription regula	Activated	2.284	1.98E-02	+CD44, +all 14	
LX2		transcription regula	Activated	2.213	1.19E-03	+CDKN1A, + all 5	
JX1		transcription regula	Activated	2.200	3.99E-02	♦CDKN1A, ♦all 5	
CAN1		transcription regula	Activated	2.176	1.30E-02	↑CDK4, +F3,all 5	
DAC5		transcription regula	Activated	2.158	1.28E-06	+ACTA2, + all 14	568 (25)
G2		growth factor	Activated	2.137	8.83E-06	+BCL6, +C3all 11	
BX2		transcription regula	Activated	2.132	6.82E-02	◆ATF3, ◆BHall 8	
AF4		transcription regula	Activated	2.131	3.28E-08	◆AREG, ◆all 21	
AB2		transcription regula	Activated	2.078	2.17E-07	ALOX5AP, Fall 9	313 (11)
ISP2	+-793.966	growth factor	Activated	2.070	1.01E-10	◆ACTA2, ◆all 18	496 (16)
(ZF1		transcription regula	Activated	2.064	2.78E-03	↑ACP1, ↓all 16	

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

Jpstream regulators				
Activation z-score -8.760 6.672	Patient 32 Patient 46	Patient 47	COLI	
GFB1			Cont	~
IYC			<u> </u>	
IYCN			CDH1	
egf				-
MAD3			\frown	
IFKBIA			CD34	
IFkB (complex)				
REB1				
gf beta			BIRC3	
DGF BB			V	
ELA			MANTE A	-
P53			VVITJA	
GT				/
ТАТЗ			VIM	
PDEF				
rgfb3				<
			VEGF	В
ell migration & invasion:				TN

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

CXCL14-driven CN is linked to Metastasis in EEC

CXCL14-driven CN (depth 3) is shown below (4 regulators plausibly explaining the expression pattern of 51 downstream targets (none shown here). Upregulation of CXCL14 has been show to be involved in breast cancer, papillary thyroid carcinoma, prostate cancer, pancreatic cancer. Hypothesis to be tested and validated : CXCL14 is predicted to be activated and is driving a CN potentially connected to metastasis via RAP1. Inhibiting CXCL14 (green) would decrease metastasis (blue).





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 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, CXCL14 and GDF15-CN)