Identifying transcriptomic mechanisms of gemcitabine resistance pathways in pancreatic cancer

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Background

- After decades of major research efforts, the pancreatic cancer survival rate remains low, at an **estimated 5-year survival rate of 5-6%.** [1]
- The projection that by 2030 pancreatic cancer will be the **second leading cause of cancer related death** [2] combined with by the **alarmingly low oncology clinical trial success rate (13.4%)**[1] highlights the critical need to accelerate development of new therapies.
- Gemcitabine is commonly used, and mechanisms of sensitivity and resistance are poorly understood.

Methods

- Eight gene expression datasets were leveraged:

Dataset	Assay	Tissue
Collisson et al [3]	Microarray	Cell lines
Klijn et al [4]	RNAseq	Cell lines
CCLE	Microarray	Cell lines
ICGC	RNAseq	Tumor tissue
TCGA	RNAseq	Tumor tissue
GSE28735	Microarray	Tumor tissue
GSE21501	Microarray	Tumor tissue
RIKEN FANTOM5	RNAseq	Healthy tissue

- Where available, raw data was downloaded and processed. Batch effect was checked for, and outliers were removed. Low expression genes were filtered out.
- Cell lines were defined to be in a “high” or “low” gemcitabine response group based on IC50 values from Collisson et al. Differential expression between these two groups was assessed in Collisson et al, Klijn et al, and CCLE.
- Enrichr was used to query overlapping genes, and GSEAP was used to query each study individually.
- Target genes of interest were assessed for relative tumor expression in ICGC, TCGA, GSE28735, and GSE21501. Baseline tissue expression was assessed in RIKEN FANTOM5.

Results

Dataset	# pval < 0.01	Total features tested
Collisson et al	1562 (1013 Genes)	33067 Probesets
Klijn et al	579 (550 Genes)	19302 Entrez ids
CCLE	414 Genes	14755 Genes
Overlap	131 Genes	

References

1. Siegel, Rebecca L., Kimberly D. Miller, and Ahmedin Jemal. "Cancer statistics, 2015." *CA: a cancer journal for clinicians* 65.1 (2015): 5-29.
2. Rahib, Lola, et al. "Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States." *Cancer research* 74.11 (2014): 2913-2921.
3. Collisson, E.A. *et al*. Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy. *Nat. Med.* **17**, 500–503 (2011)
4. Klijn, C. *et al*. A comprehensive transcriptional portrait of human cancer cell lines. *Nat. Biotechnol.* **33**, 306–312 (2015).

Results (continued)

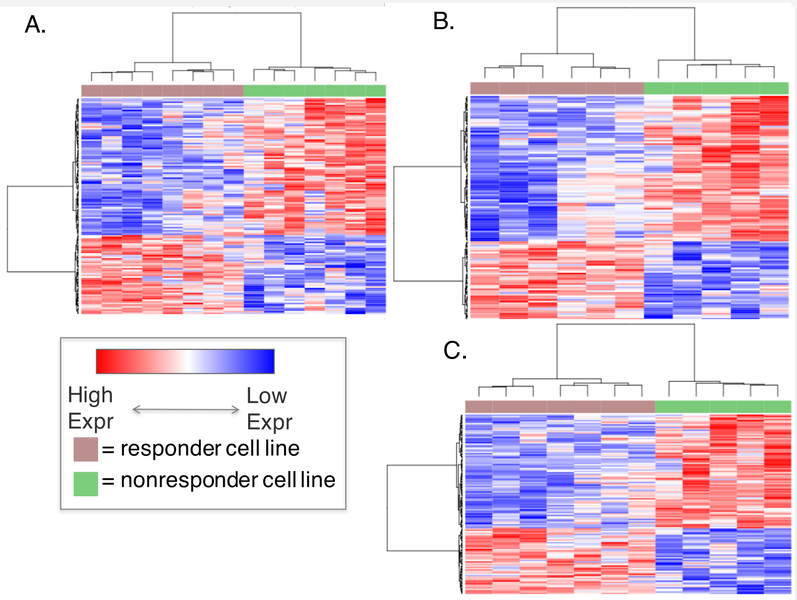


Fig. 1: Heatmaps showing 131 genes diff’ expressed between gemcitabine responder and nonresponder cell lines. Gene expression data from A. Collisson et al, B. Klijn et al, C. CCLE. Genes show a consistent robust differential expression pattern across studies

A.

Pathways up in resistant cell lines	pval	Adj pval
ErbB signaling pathway(Mus musculus) (WikiPathways)	8.82E-03	2.28E-01
G1 to S cell cycle control(Mus musculus) (WikiPathays)	1.50E-02	2.28E-01
G1 Phase (Reactome)	9.31E-03	3.52E-01
ERBB3 (GEO Kinase perturb)	1.02E-09	3.28E-08
cyclin-dependent protein serine/threonine kinase regulator activity (GO:0016538)	1.84E-04	3.78E-02

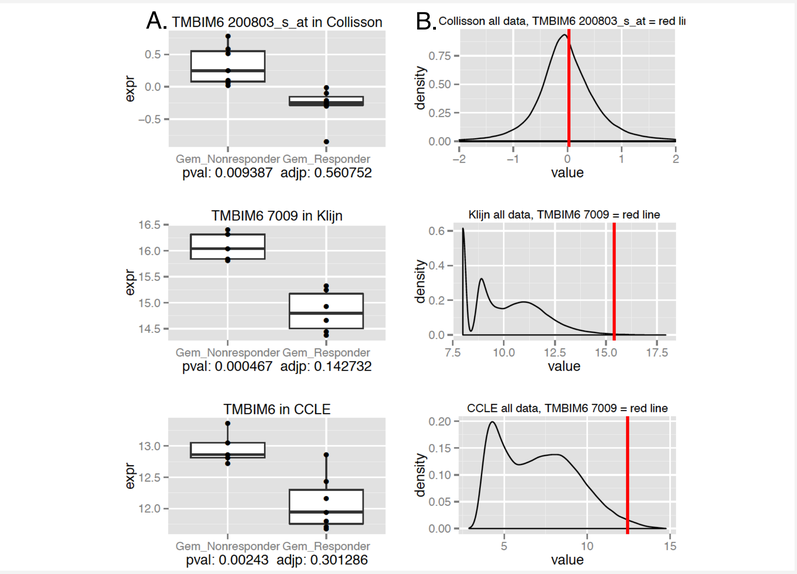
B.

Pathways up in sensitive cell lines	pval	Adj pval
IRAK4 (GEO kinase perturb)	1.65E-07	4.94E-06
ALK (GEO kinase perturb)	7.77E-06	1.17E-04
PRKACA (PPI Hub protein)	1.32E-04	8.25E-03
SLC2A4 (PPI Hub protein)	1.25E-04	8.25E-03
Vitamin B12 Metabolism(Homo sapiens) (wikipathways)	1.66E-02	3.09E-01
Folate Metabolism(Homo sapiens) (wikipathways)	2.67E-02	3.09E-01

C.

Pathways upregulated in gemcitabine sensitive cell lines in all studies	Klijn adj.p	Col Adj.p	CCLE Adj.p
KEGG_ARACHIDONIC_ACID_METABOLISM	4.72E-02	6.70E-03	7.50E-03
KEGG_DRUG_METABOLISM_CYTOCHROME_P450	< 1E-4	< 1E-4	3.00E-04
KEGG_LINOLEIC_ACID_METABOLISM	5.20E-03	1.00E-04	2.60E-03
KEGG_RETINOL_METABOLISM	1.00E-04	< 1E-4	4.00E-04

Table 2: Pathway analysis of overlapping genes using Enrichr (A, B), and overlapping GSEA results between all three studies (C).



Results (continued)

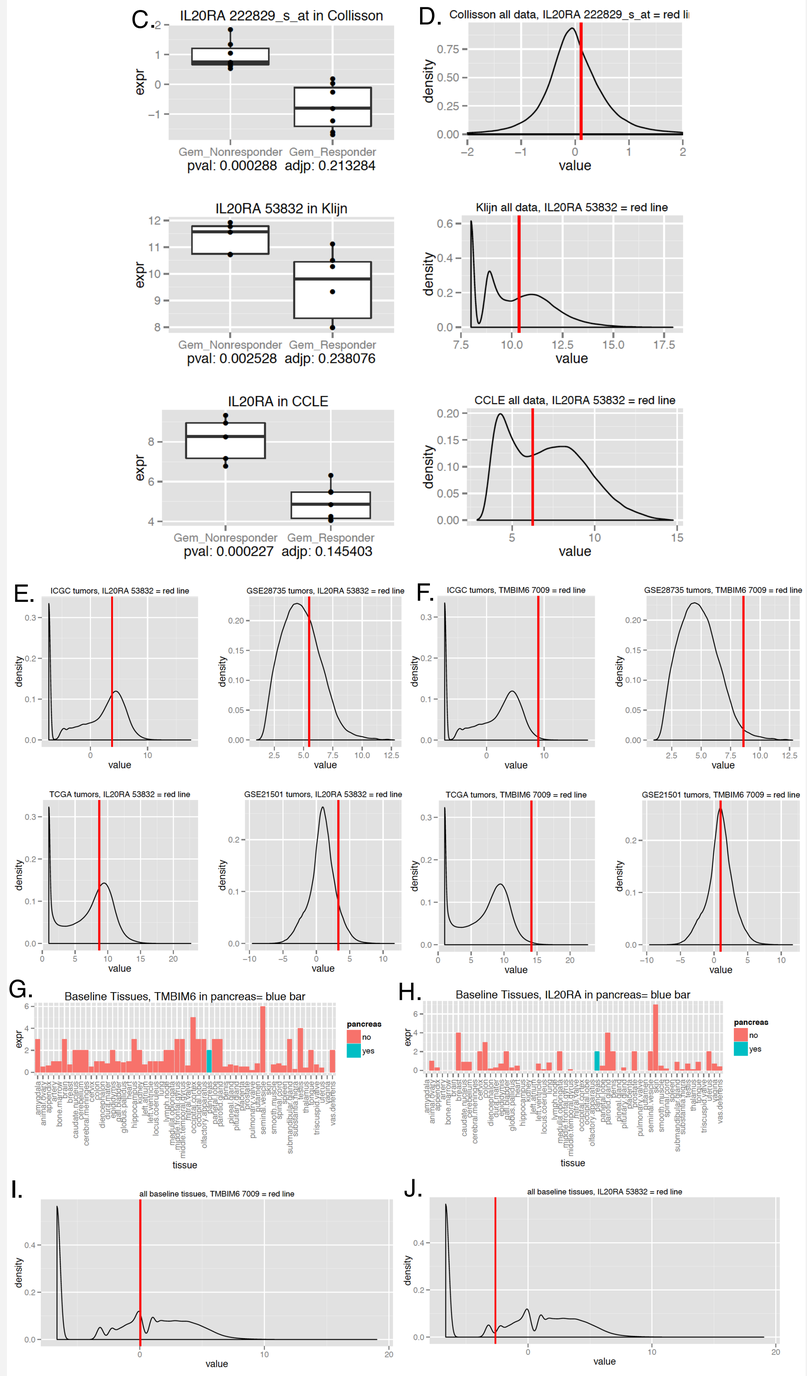


Fig. 2: Evaluation of TMBIM6 and IL20RA as targets to enhance gemcitabine therapy. A. TMBIM6 is expressed more highly in gemcitabine nonresponder cell lines across three studies B. TMIMB6 expression relative to all genes in pancreatic cancer cell lines C. IL20RA is expressed more highly in gemcitabine nonresponder cell lines across three studies. E. IL20RA and F. TMBIM6 expression relative to all genes in pancreatic cancer cell lines shows moderate to high relative expression. Comparison of G. TMBIM6 and H. IL20RA expression across healthy tissues shows low relative expression in the target tissue (pancreas). Comparison of relative expression of I. TMBIM6 and J. IL20RA against expression of all genes in all baseline tissues shows low expression

Conclusions

- Gene expression differences between gemcitabine resistant and sensitive cell lines are consistent and robust across cell line studies.
- Drugs which enhance pathways upregulated in sensitive cell lines or inhibit pathways upregulated in resistant cell lines may make ideal gemcitabine candidate therapies
- Our analysis pinpoints upregulation of apoptosis suppressor TMBIM6 ($p<0.003$) and pro-survival gene IL20RA ($p<0.003$), which appear to be ideal target genes due to their relatively high tumor expression and relatively low baseline tissue expression

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