Transcriptional regulation of *O*-GlcNAc homeostasis is disrupted in pancreatic cancer

Kevin Qian, Simeng Wang, Minnie Fu, Jinfeng Zhou, Jay Prakash Singh, Min-Dian Li, Yunfan Yang, Kaisi Zhang, Jing Wu, Yongzhan Nie, Hai-Bin Ruan, Xiaoyong Yang

1. Figure S1-S3
2. Table S1-S7

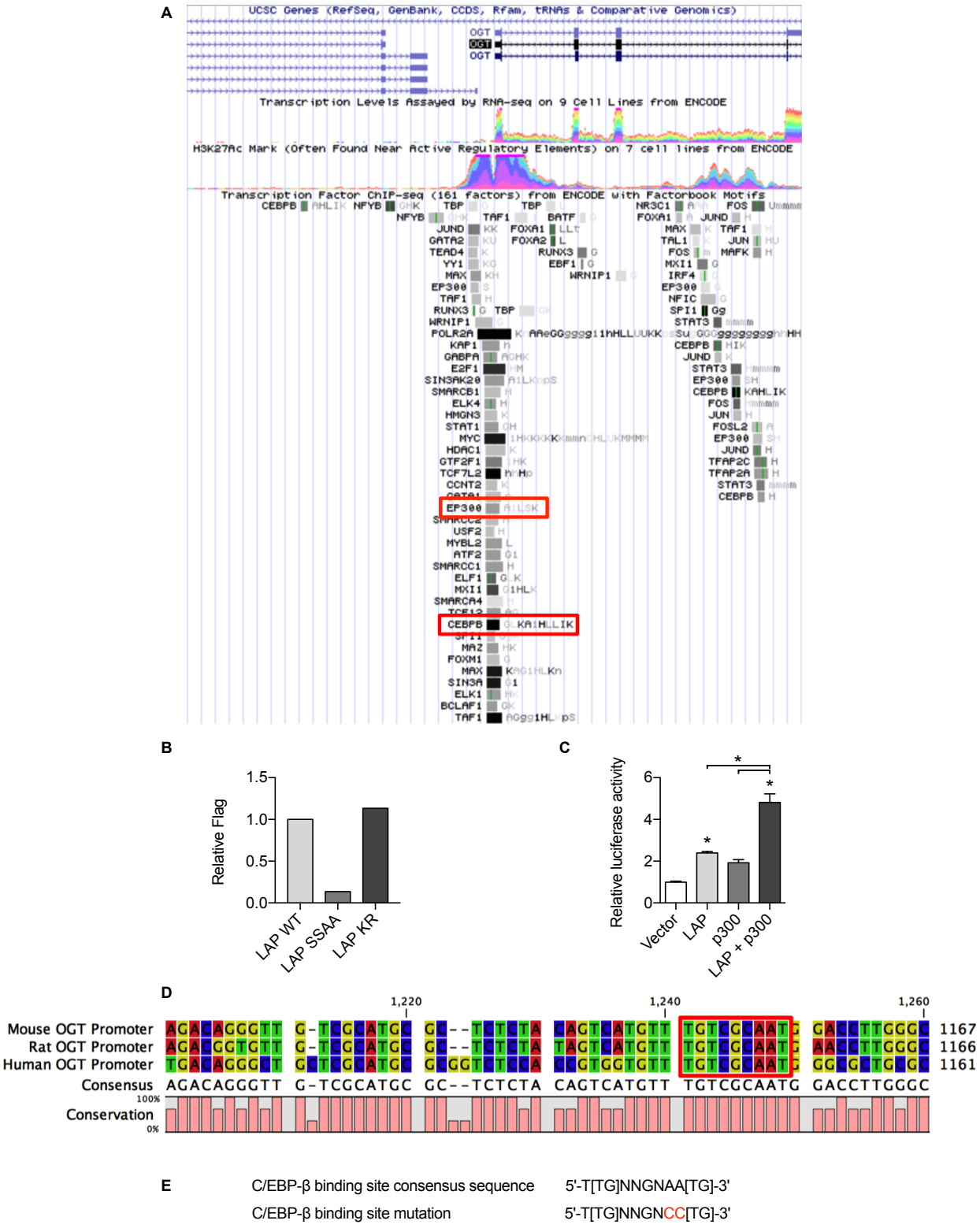
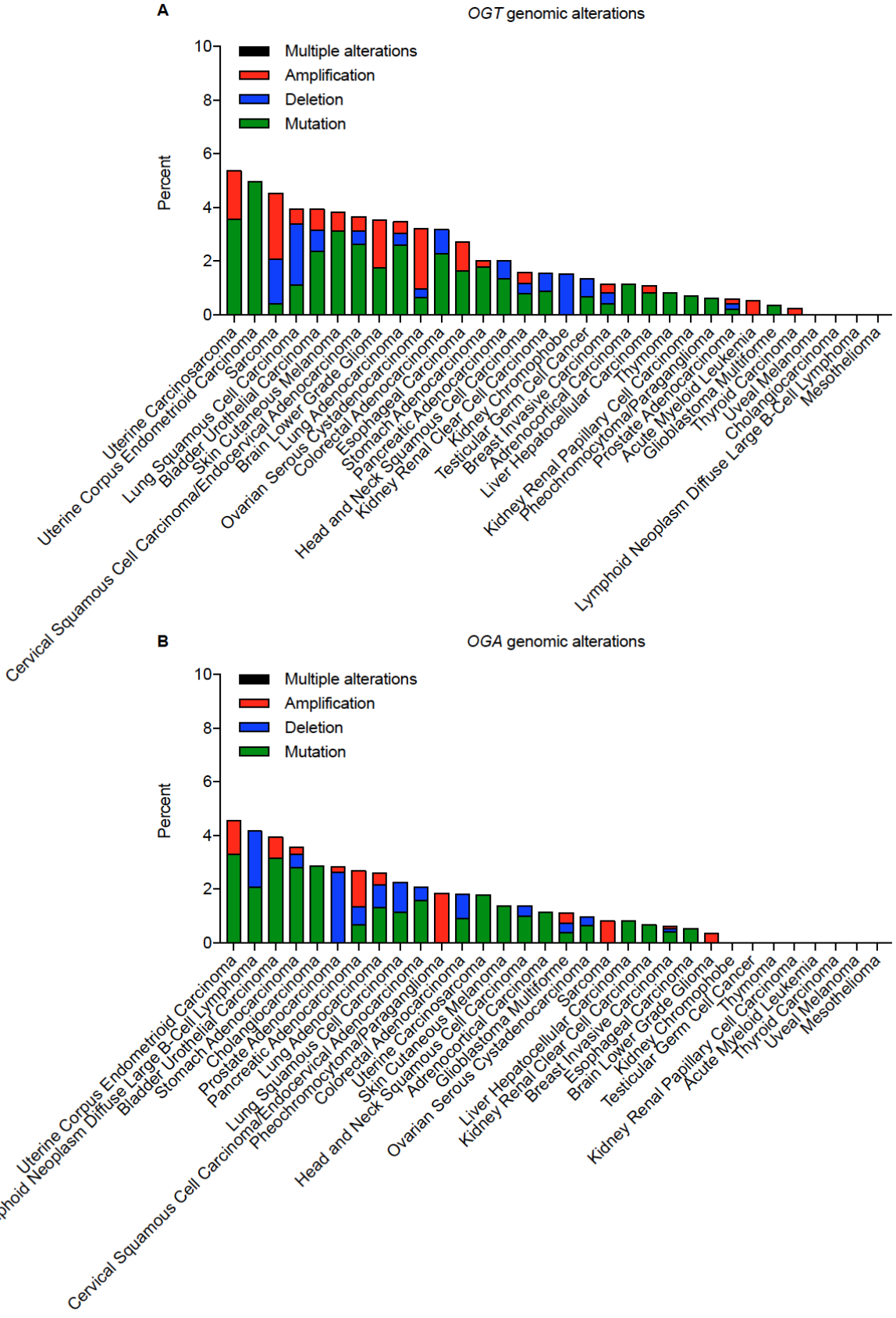

A
UCSC Genes (RefSeq, GenBank, CCDS, Rfam, tRNAs & Comparative Genomics)
OGT
Transcription Levels Assayed by RNA-seq on 9 Cell Lines from ENCODE
H3K27Ac Mark (Often Found Near Active Regulatory Elements) on 7 cell lines from ENCODE
Transcription Factor ChIP-seq (161 factors) from ENCODE with Factorbook Motifs
EP300
CEBPB
B
Relative Flag
1.5
1.0
0.5
0.0
LAP WT
LAP SSAA
LAP KR
C
Relative luciferase activity
6
4
2
0
Vector
LAP
p300
LAP + p300
D
1,220
1,240
1,260
Mouse OGT Promoter AGACAGGGTT G-TCGCATGC GC--TCTCTA CAGTCATGTT TGTCGCAATG GACCTTGGGC 1167
Rat OGT Promoter AGACGGTGTT G-TCGCATGC GC--TCTCTA TAGTCATGTT TGTCGCAATG AACCTTGGGC 1166
Human OGT Promoter TGACAGGGCT GCTCGCATGC GCGGTCTCCA CCGTGGTGTT TGTCGCAATG GGCGCTGCGC 1161
Consensus AGACAGGGTT G-TCGCATGC GC--TCTCTA CAGTCATGTT TGTCGCAATG GACCTTGGGC
100%
Conservation
0%
E
C/EBP-β binding site consensus sequence 5'-T[TG]NNGNAA[TG]-3'
C/EBP-β binding site mutation 5'-T[TG]NNGNCC[TG]-3'

Figure S1. Related to Figure 2. (A) UCSC Genome Browser view of the human *OGT* promoter with corresponding RNA-seq and H3K27ac and transcription factor ChIP-seq data from ENCODE. Notable p300 and C/EBPβ binding sites boxed in red. (B) Quantification of Flag band intensities from the Western blot in Figure 2D. (C) *Ogt* promoter luciferase assay performed in HEK 293T cells transfected with the indicated plasmids (n = 4). All values represent mean ± SEM. *$p < 0.05$ by one-way ANOVA with Tukey's multiple comparisons test. (D) Sequence alignment of the human, mouse, and rat *OGT* promoters with the conserved C/EBPβ binding motif boxed in red. (E) C/EBPβ binding site consensus sequence with the two mutated bases highlighted in red.


A
OGT genomic alterations
Multiple alterations
Amplification
Deletion
Mutation
Percent
0
2
4
6
8
10
Uterine Carcinosarcoma
Uterine Corpus Endometrioid Carcinoma
Lung Squamous Cell Carcinoma
Bladder Urothelial Carcinoma
Skin Cutaneous Melanoma
Cervical Squamous Cell Carcinoma/Endocervical Adenocarcinoma
Brain Lower Grade Glioma
Lung Adenocarcinoma
Ovarian Serous Cystadenocarcinoma
Colorectal Adenocarcinoma
Esophageal Carcinoma
Stomach Adenocarcinoma
Pancreatic Adenocarcinoma
Head and Neck Squamous Cell Carcinoma
Kidney Renal Clear Cell Carcinoma
Kidney Chromophobe
Testicular Germ Cell Cancer
Breast Invasive Carcinoma
Adrenocortical Carcinoma
Liver Hepatocellular Carcinoma
Thymoma
Kidney Renal Papillary Cell Carcinoma
Pheochromocytoma/Paraganglioma
Prostate Adenocarcinoma
Acute Myeloid Leukemia
Glioblastoma Multiforme
Thyroid Carcinoma
Uveal Melanoma
Cholangiocarcinoma
Lymphoid Neoplasm Diffuse Large B-Cell Lymphoma
Mesothelioma
B
OGA genomic alterations
Multiple alterations
Amplification
Deletion
Mutation
Percent
0
2
4
6
8
10
Uterine Corpus Endometrioid Carcinoma
Lymphoid Neoplasm Diffuse Large B-Cell Lymphoma
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Cervical Squamous Cell Carcinoma/Endocervical Adenocarcinoma
Pheochromocytoma/Paraganglioma
Colorectal Adenocarcinoma
Uterine Carcinosarcoma
Skin Cutaneous Melanoma
Head and Neck Squamous Cell Carcinoma
Ovarian Serous Cystadenocarcinoma
Adrenocortical Carcinoma
Glioblastoma Multiforme
Sarcoma
Kidney Renal Clear Cell Carcinoma
Liver Hepatocellular Carcinoma
Breast Invasive Carcinoma
Esophageal Carcinoma
Brain Lower Grade Glioma
Kidney Chromophobe
Testicular Germ Cell Cancer
Thymoma
Kidney Renal Papillary Cell Carcinoma
Acute Myeloid Leukemia
Thyroid Carcinoma
Uveal Melanoma
Mesothelioma

Figure S2. Related to Figure 3. Percentage of the indicated cancer types with the indicated genomic alterations in the (A) *OGT* and (B) *OGA* genes.

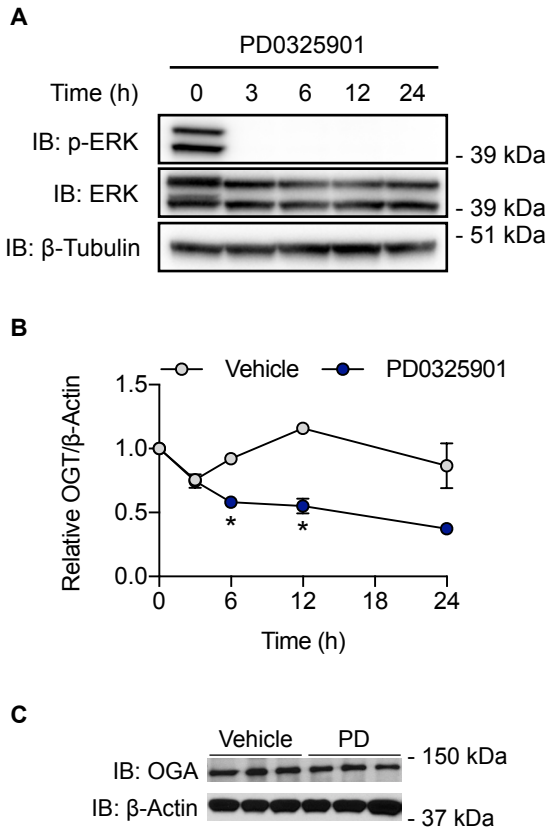

Figure S3. Related to Figure 5. (A) Primary mouse PDAC cells were treated with PD0325901 (10 μM) for 0, 3, 6, 12, or 24 hr and p-ERK levels were measured by Western blot. (B) Quantification of OGT and β-Actin band intensities from the Western blot in Figure 5F (n = 2). All values represent mean ± SEM. *$p < 0.05$ by multiple t tests with Holm-Sidak correction for multiple comparisons. (C) Western blot analysis of OGA protein levels in whole cell lysates used for the OGA activity assay in Figure 5G.

Table S1. Top genes coexpressed with *OGT* in human, mouse, and rat tissues

Rank	Human	Mouse	Rat
-	*OGT*	*Ogt*	*Ogt*
1	*DDX17*	*Pnisr*	*Ddx17*
2	*MDM4*	*Rbm5*	*Zfc3h1*
3	*ZFC3H1*	*Firre*	*Zc3h11a*
4	*RBM5*	*Thoc2*	*Kdelr3*
5	*DMTF1*	*Clk1*	*Zranb2*
6	*TTC17*	*Zfp871*	*Pnisr*
7	*NKTR*	*Abcc5*	*Morc3*
8	*ALG13*	*Atrx*	*Epm2aip1*
9	*MGEA5*	*Malat1*	*Cspp1*
10	*PNISR*	*Ddx17*	*Son*
…	…	…	…
90	*RAPGEF6*	*2610008E11Rik*	*Mgea5*
…	…	…	…
107	*ATG2B*	*Mgea5*	*Casp2*

Table S2. Top genes coexpressed with *OGA* in human, mouse, and rat tissues

Rank	Human	Mouse	Rat
-	*MGEA5*	*Mgea5*	*Mgea5*
1	*BTAF1*	*Phf3*	*Fyttd1*
2	*YTHDC1*	*Kdm3a*	*Ctnnb1*
3	*CHD2*	*Ubxn7*	*Adnp*
4	*MDM4*	*Zmym2*	*Pot1*
5	*OGT*	*Csnk1g1*	*Zc3h11a*
6	*DMTF1*	*Sf3b1*	*Ddx3x*
7	*NKTR*	*Ctr9*	*Rab6a*
8	*ZFC3H1*	*Sbno1*	*Gdi2*
9	*RBM5*	*Nbr1*	*Crk*
10	*CLK1*	*Rlf*	*Slc25a36*
…	…	…	…
40	*HERC4*	*Kctd18*	*Ogt*
…	…	…	…
61	*STK4*	*Ogt*	*Atp6ap1*

Table S3. Correlation between *OGT* and *OGA* mRNA expression in human cancers

Cancer Type	Pearson	Spearman	n
Uveal Melanoma	0.72	0.76	80
Pancreatic Adenocarcinoma	0.70	0.61	179
Thyroid Carcinoma	0.68	0.62	509
Acute Myeloid Leukemia	0.61	0.69	173
Adrenocortical Carcinoma	0.61	0.50	79
Sarcoma	0.57	0.54	263
Bladder Urothelial Carcinoma	0.53	0.46	408
Skin Cutaneous Melanoma	0.53	0.56	472
Thymoma	0.53	0.61	120
Stomach Adenocarcinoma	0.51	0.56	415
Testicular Germ Cell Cancer	0.49	0.55	156
Kidney Renal Clear Cell Carcinoma	0.47	0.52	534
Cholangiocarcinoma	0.46	0.39	36
Lymphoid Neoplasm Diffuse Large B-Cell Lymphoma	0.45	0.64	48
Uterine Carcinosarcoma	0.45	0.34	57
Kidney Renal Papillary Cell Carcinoma	0.44	0.59	291
Prostate Adenocarcinoma	0.43	0.47	498
Breast Invasive Carcinoma	0.43	0.46	1100
Brain Lower Grade Glioma	0.42	0.42	530
Pheochromocytoma and Paraganglioma	0.42	0.43	184
Esophageal Carcinoma	0.42	0.42	185
Lung Adenocarcinoma	0.41	0.49	517
Glioblastoma Multiforme	0.41	0.41	166
Head and Neck Squamous Cell Carcinoma	0.41	0.42	522
Uterine Corpus Endometrioid Carcinoma	0.40	0.41	333
Colorectal Adenocarcinoma	0.38	0.42	382
Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma	0.36	0.44	306
Ovarian Serous Cystadenocarcinoma	0.35	0.44	307
Liver Hepatocellular Carcinoma	0.35	0.35	373
Lung Squamous Cell Carcinoma	0.33	0.54	501
Mesothelioma	0.31	0.20	87
Kidney Chromophobe	-0.05	0.09	66

Table S4. Comparison of OGT, OGA, and RL2 levels in normal pancreas and PDAC

	OGT			OGA			RL2		
Tissue	Low (-, +)	High (++)	p*	Low (-, +)	High (++)	p*	Low (-, +)	High (++)	p*
Normal	73	8	<0.0001	60	21	<0.0001	62	19	<0.0001
PDAC	23	58		15	66		20	61	
*Fisher's exact test									

Table S5. Correlation between OGT and OGA levels in PDAC

	OGA	
OGT	Low (-, +)	High (++)
Low (-, +)	10	13
High (++)	5	53
Spearman r = 0.405, p < 0.001		

Table S6. Correlation between OGT and RL2 levels in PDAC

	RL2	
OGT	Low (-, +)	High (++)
Low (-, +)	15	8
High (++)	5	53
Spearman r = 0.592, p < 0.001		

Table S7. Correlation between OGA and RL2 levels in PDAC

	RL2	
OGA	Low (-, +)	High (++)
Low (-, +)	8	7
High (++)	12	54
Spearman r = 0.317, p < 0.001		