

Mechanistic insights of O-GlcNAcylation that promote progression of cholangiocarcinoma cells via nuclear translocation of NF- κ B

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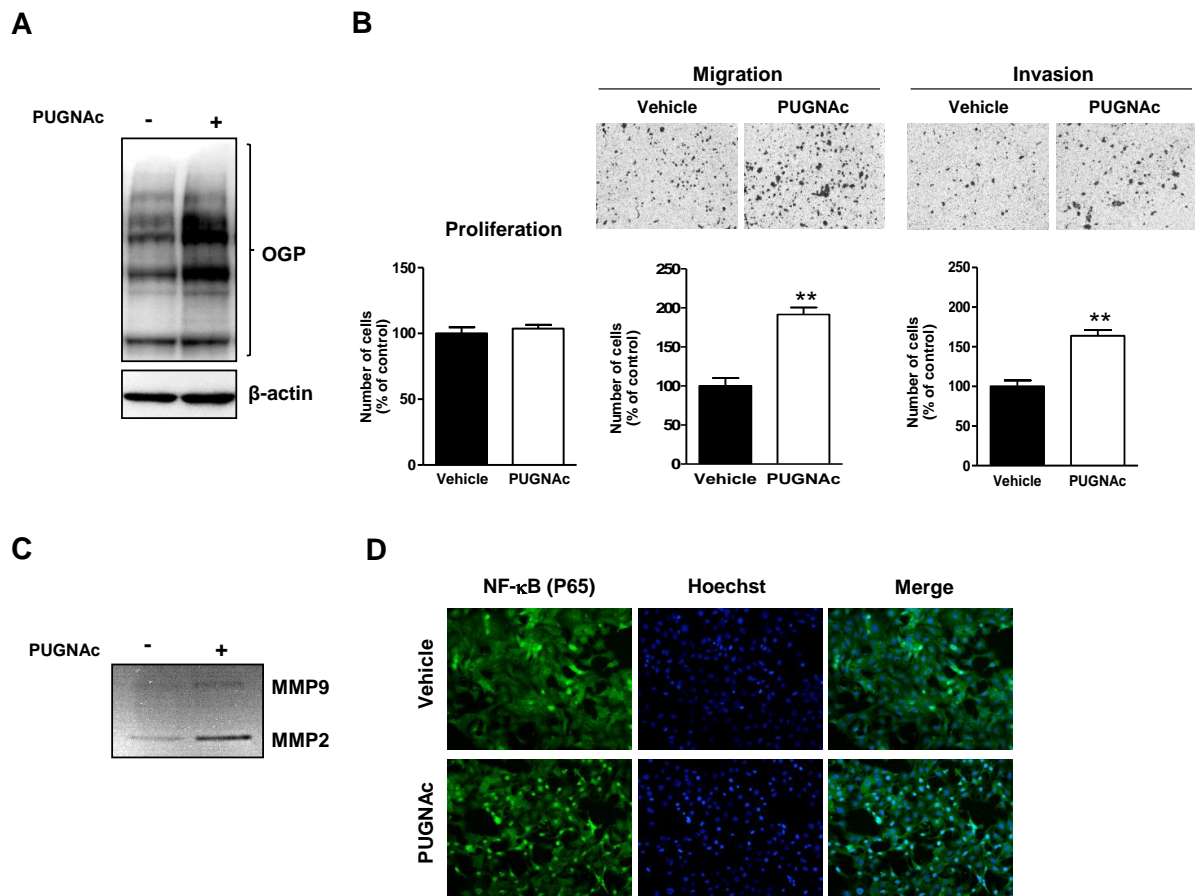
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Supplementary Figure S1. Increasing O-GlcNAcylation in KKKU-100 using PUGNac yielded similar effects as siOGA treated cells. The CCA cell line, KKKU-100, was treated with PUGNac, a well-known OGA inhibitor, for 24 h. The effects of O-GlcNAcylation of PUGNac treated and the control cells were compared on (A) Western blotting of O-GlcNAcylated products (OGP), (B) cell growth, migration and invasion observed at 28 h, (C) MMP2 and MMP9 zymogram and (D) nuclear translocation of NF-κB.

Supplementary Table S1: Primers for determination of genes using real-time PCR

Gene	Ref. No.	Forward	Reverse
<i>CDH1</i>	NM_004360	5'-CTTAGAGGTCAGCGTGTG-3'	5'-AGCAAGAGCAGCAGAATC-3'
<i>CDH2</i>	NM_007664.4	5'-CCTGAGGGATCAAAGCCTGG-3'	5'-ACATGTTGGGTGAAGGGGTG-3'
<i>FN1</i>	NM_002026.2	5'-TGGTGTACACAGAGGCTACTAT-3'	5'-GAAGGGTTACCAGTTGGGGA-3'
<i>ITGA5</i>	NM_002205.2	5'-GATGCCCTACCGAATCCTGC-3'	5'-TGATGATCCACAGTGGGACG-3'
<i>ITGB1</i>	NM_002211.3	5'-GAGTCGCGAACAGCAGG-3'	5'-AGCAAACACACAGCAAACCTGA-3'
<i>MMP2</i>	NM_001127891.1	5'-TGTTAGGCAAGTGACTTCTCAGT-3'	5'-AACAGGTTGCAGCTCTCCTT-3'
<i>MMP3</i>	NM_002422.3	5'-AGACTTTCAGGGATTGACTC-3'	5'-ACAATTAAGCCAGCTGTTACTCT-3'
<i>MMP7</i>	NM_002423.3	5'-GATAAGCACTGTTCTCCAC-3'	5'-CACAGTCACACCATAAAGGA-3'
<i>MMP9</i>	NM_004994.2	5'-ACGCACGACGTCTTCCAGTA-3'	5'-CTTGGTCCACCTGGTTCAACT-3'
<i>PAI1</i>	NM_000602.4	5'-CAGGACGAACCGCCAATCG-3'	5'-CAGGTGGGCCACGTAGGATG-3'
<i>SNAI2</i>	NM_003068.4	5'-ACAGCGAACTGGACACACAT-3'	5'-GCGGTAGTCCACACAGTGAT-3'
<i>OPN</i>	NM_000582.2	5'-GCGAGGAGTTGAATGGTG-3'	5'-ATAATCTGGACTGCTTGTGG-3'
<i>TIMP1</i>	NM_003254.2	5'-GCTTCTGGCATCCTGTTGTTG-3'	5'-GGTCTGGTTGACTTCTGGTGT-3'
<i>TIMP2</i>	NM_003255.4	5'-ACCCTCTGTGACTTCATCGTG-3'	5'-CGTCCGGGAGGAGATGTAG-3'
<i>TWIST1</i>	NM_000474.3	5'-GGAGTCCGAGTCTTACGAG-3'	5'-TCTGGAGGACCTGGTAGAGG-3'
<i>VCAN</i>	NM_004385	5'-GGCACCTGTTATCCTACTG-3'	5'-GTCTCGGTATCTTGCTCAC-3'
<i>VIM</i>	NM_003380.3	5'-AACTTTTCTCCCTGAACCTGAG-3'	5'-TCAAGGTCATCGTGATGCTGAG-3'
<i>B2M</i>	AK022379	5'-AAGATGAGTATGCCTGCCG-3'	5'-CGGCATCTTCAAACCTCC-3'

Supplementary Table S2: Relative expression of metastasis associated genes in KKU-213 treated with siOGT or siOGA

Genes	siOGT	<i>P</i>	siOGA	<i>P</i>
E- cadherin (CDH1)	0.59	0.007	0.72	<0.001
Fibronectin (FN1)	0.97	0.530	1.14	0.315
Integrin- α 5 (ITGA5)	0.57	<0.001	1.18	0.014
Integrin- β 1 (ITGB1)	0.65	<0.001	1.21	0.006
MMP2	0.69	0.005	1.60	<0.001
MMP3	0.81	<0.001	0.89	0.023
MMP7	0.58	<0.001	1.43	0.015
MMP9	1.50	<0.001	2.81	<0.001
N-cadherin (CDH2)	0.88	0.003	1.07	0.182
Osteopontin (OPN)	1.56	0.002	1.32	0.049
Serpine 1 (PAI-1)	0.72	<0.001	1.67	0.008
Slug (SNAI2)	0.88	0.003	1.15	0.072
TIMP1	0.84	<0.001	0.89	0.249
TIMP2	0.82	<0.001	1.10	0.022
Twist	0.94	0.198	1.06	0.680
Versican (VCAN)	0.95	0.291	0.90	0.049
Vimentin (VIM)	1.11	<0.001	1.28	0.006