Research Article

Cross-talk among *MEN1, p53* and Notch regulates the proliferation of pancreatic neuroendocrine tumor cells by modulating INSM1 expression and subcellular localization

Ylenia Capodanno [1], Yu Chen [1], Joerg Schrader [2], Mitsuhiro Tomosugi [3], Shoiciro Sumi [3], Akihiko Yokoyama [4], Nobuyoshi Hiraoka [5], Rieko Ohki [1,*].

[1]Laboratory of Fundamental Oncology, National Cancer Research Institute, Chuo-ku, 103-0045, Tokyo, Japan

[2] I. Medical Department, University Medical Center Hamburg-Eppendorf, Martinstrasse 52, 20246, Hamburg, Germany

[3] Laboratory of Organ and Tissue Reconstruction, Institute for Frontier Life and Medical Sciences, Kyoto University, Sakyo-ku, 606-8507, Kyoto, Japan

[4] Tsuruoka Metabolomics Laboratory, National Cancer Center, Yamagata, 997-0052, Japan

[5] Division of Molecular pathology, National Cancer Research Institute, Chuo-ku, 103-0045, Tokyo, Japan

[*]corresponding author

Laboratory of Fundamental Oncology, National Cancer Research Institute, 5-1-1 Tsukiji, Chuo-ku, 103-0045, Tokyo, Japan. Tel +81335475201 EXT.3813

rohki@ncc.go.jp

Supplementary figures

Figure S1 INSM1 expression in PNET human tissues with different tumor grades and genomic abnormalities.

Representative images of immunohistochemical staining of INSM1 in grade 1 and 2 surgically resected PNET tumor tissues having no LOH, *PHLDA3* LOH or a *MEN1* LOH genomic profile.

Figure S2 INSM1 localization and expression in multiple NET cell lines.

Representative images of immunofluorescence analysis showing INSM1 expression and localization in multiple NET cell lines having either *p53* null (H1299), *p53* mutated (Bon1, QGP1) or *p53* wild-type (NT3) genotype.

Figure S3 Notch receptors (*NOTCH1, 2, 3 and JAG1, 2*), Notch target genes (*HES1, HES5, HES6, HEY1 and HEY2*) and Notch ligands (*DLL1, DLL3*) gene expression in *p53* wildtype (NT3) compared to *p53* null (H1299) NET cells.

Gene markers underlined in orange were selected for further analysis. Expression values were calculated using cDNA from H1299 (*p53* null) cells as the calibrator sample. Values are mean of triplicates +/- SD.

Figure S4 Effect of GSI treatment on Notch target expression depends on *p53* status in PNET cells.

NT3 (*p53* wild-type) and Bon1 (*p53* mutated cells) were treated with 10 μg/ml GSI for 48 hours to force accumulation of cell surface Notch, then washed to re-initiate Notch signaling. Control samples were treated with DMSO. After 4 hours, RNA was isolated and cDNA was prepared for real-time PCR analysis of Notch receptors (*NOTCH1, 2, 3 and JAG1, 2*), Notch target genes (*HES1, HES5, HES6, HEY1 and HEY2*) and Notch ligands (*DLL1, DLL3*). Expression values were calculated using cDNA from DMSO-treated cells as the calibrator sample. Values are mean of triplicates +/- SD.

TableS 1 Clinical and pathological features of PanNET samples used in this study.

GENOMIC STATUS	SEX/AGE	TUMOR TYPE	SPECIMENT SITE	WHO GRADE (NET)
No LOH	M/55	NF PanNET	Primary	1
	M/59	NF PanNET	Primary	2
	F/55	NF PanNET	Primary	2
	F/60	NF PanNET	Primary	3
	M/35	NF PanNET	Primary	3
PHLDA3 LOH	M/51	NF PanNET	Primary	1
	F/56	NF PanNET	Primary	2
	F/56	NF PanNET	Primary	2
	M/44	NF PanNET	Primary	3
	F/37	NF PanNET	Primary	3
MEN1 LOH	M/61	NF PanNET	Primary	1
	M/71	NF PanNET	Primary	2
	M/62	NF PanNET	Primary	2
	M/48	NF PanNET	Primary	3
	F/60	NF PanNET	Primary	3

NF PanNET= Non-functional pancreatic neuroendocrine tumors; M= Male; F= Female.

Table S2 Genomic status of NET cell lines.

STATUS	H1299 (LUNG NET)	BON1 (PNET)	QGP1 (PNET)	NT3 (PNET)
p53	Null	Mutated (LOF, Homozygous stop in exon 10)	Mutated (LOF, Homozygous frameshift deletion in exon 4)	Wild-type
MEN1	Protein detected	LOH and missense mutation of the remaining allele	LOH and missense mutation of the remaining allele	LOF (missense homozygous mutation)
PHLDA3	Not expressed	LOH and potential inactivation of the remaining allele	No LOH	No LOH

Table S3 Primer list

GENE SYMBOL	FORWARD PRIMERS	REVERSE PRIMERS
MEN1	CAGGGGCCAGACAGTCAATG	GGTGGGCTCCAGCTCCTCTA
P57	CGGCGATCAAGAAGCTGTCC	CGGGCTCTTTGGGCTCT
P27	CAGCTTGCCCGAGTTCTACT	GCGTGTCCTCAGAGTTAGCC
P38	GGAAGACCATGTGGACCTGT	GGCGTTTGGAGTGGTAGAAA
CD133	TGGATGCAGAACTTGACAACGT	ATACCTGCTACGACAGTCGTGGT
NOTCH1	CCGTCATCTCCGACTTCATCT	GTGTCTCCTCCCTGTTGTTCTG
NOTCH2	GCTGATGCTGCCAAGCGT	CCGGGGAAGACGATCCAT
NOTCH3	CTGGCGAGACTGCTTTGC	CGACTGTGCCGCTTTGAG
NOTCH4	TGCAGGCATATGGGATGTAA	CATCCCCACAGTGGAGTTCT
HEY1	GCCGAGATCCTGCAGATGA	GCTGGGAAGCGTAGTTGTTG
HEY2	AGGCTACTTTGACGCACACG	CAAGTGCTGAGATGAGACACAAG
JAG1	CGGCCTCTGAAGAACAGAAC	TCACCAAGCAACAGATCCAA
JAG2	AGGTGGAGACGGTTGTTACG	ATTGATCTGGGTCATGCAGTTG
HES1	ACGTGCGAGGGCGTTAATAC	ATTGATCTGGGTCATGCAGTTG
HES5	CTACCTGAAGCACAGCAAAGC	GAAGTGGTACAGCAGCTTCATCT
HES6	AGCTGGAGAACGCCGAAGT	ATGGACTCGAGCAGAGATGGTT
DLL1	ATGTGATGAGCAGCATGGATT	GGTGTGTGCAGTAGTTCAGGTC
DLL2	TGAGCATGGCTTCTGTGAAC	AAAGGACCTGGGTGTCTCACTAC
CCND1	GTCCATGCGGAAGATCGTC	GCGGTCCAGGTAGTTCATG
GADPH	CAAGATCATCAGCAATGCCT	CAGGGATGATGTTCTGGAGAG
P53	GGAACTCAAGGATGCCCAGG	ATGGCGGGAGGTAGACTGAC
PHLDA3	GTCCATGCCTTCCACCTT	ACCATCTTTCCTTCATGCTACC
MDM2	AGGGAGATATGTTGTGAAGAAGCA	GATCCAACCAATCACCTGAATGTTC
INSM1	AACTGTCCTTCGCTTGGA	ACGAGACAAACGCGTACAGCT
FOXA2	GGAGCAGCTACTACTATGCAGAGC	CGTGTTCATGCCGTTCATCC

Figure S1

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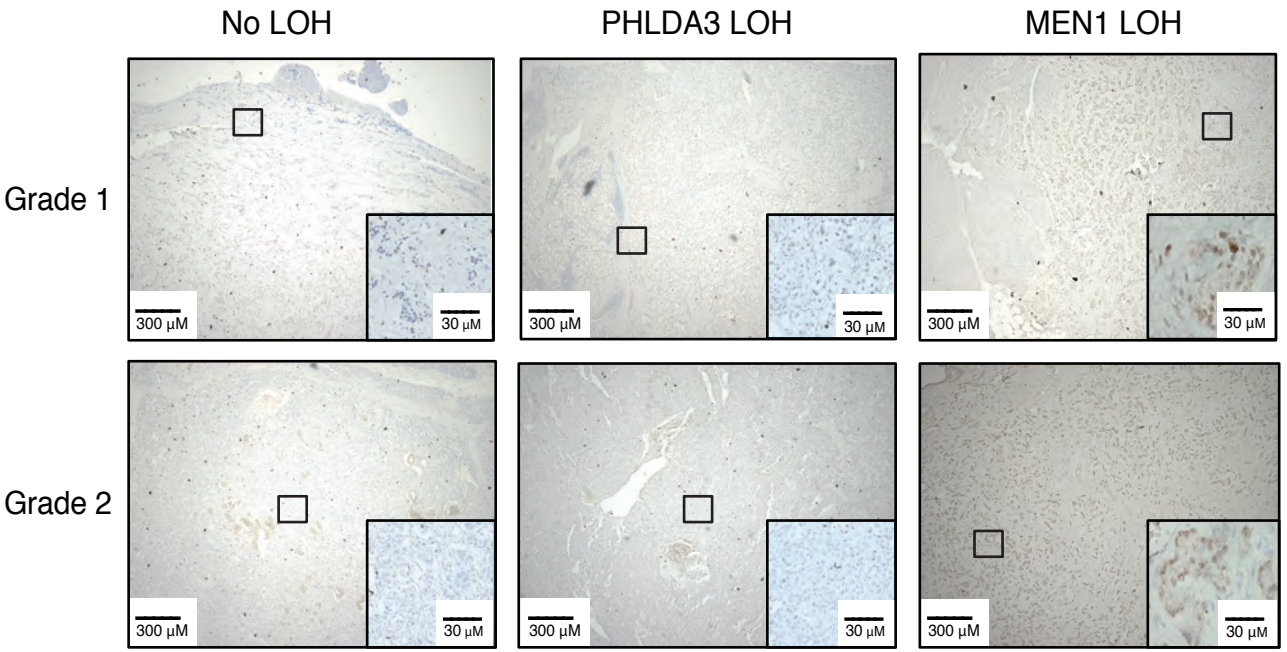

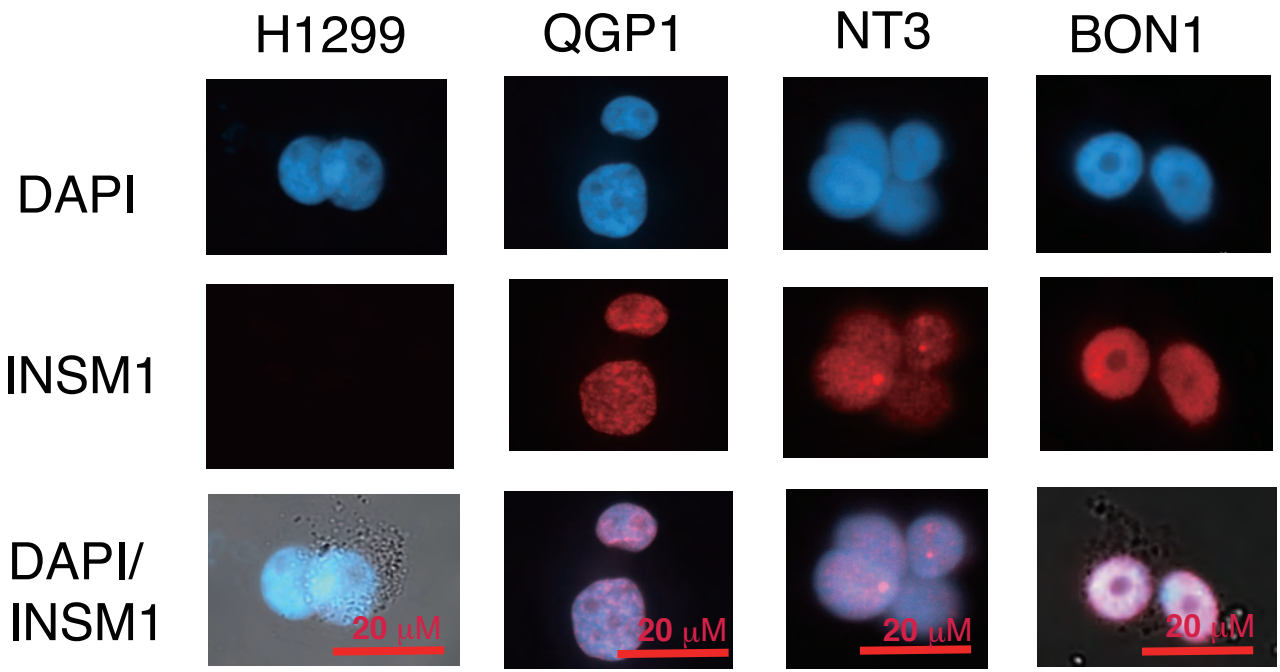
H1299
QGP1
NT3
BON1
DAPI
INSM1
DAPI/
INSM1
20 μM
20 μM
20 μM
20 μM

Figure S3

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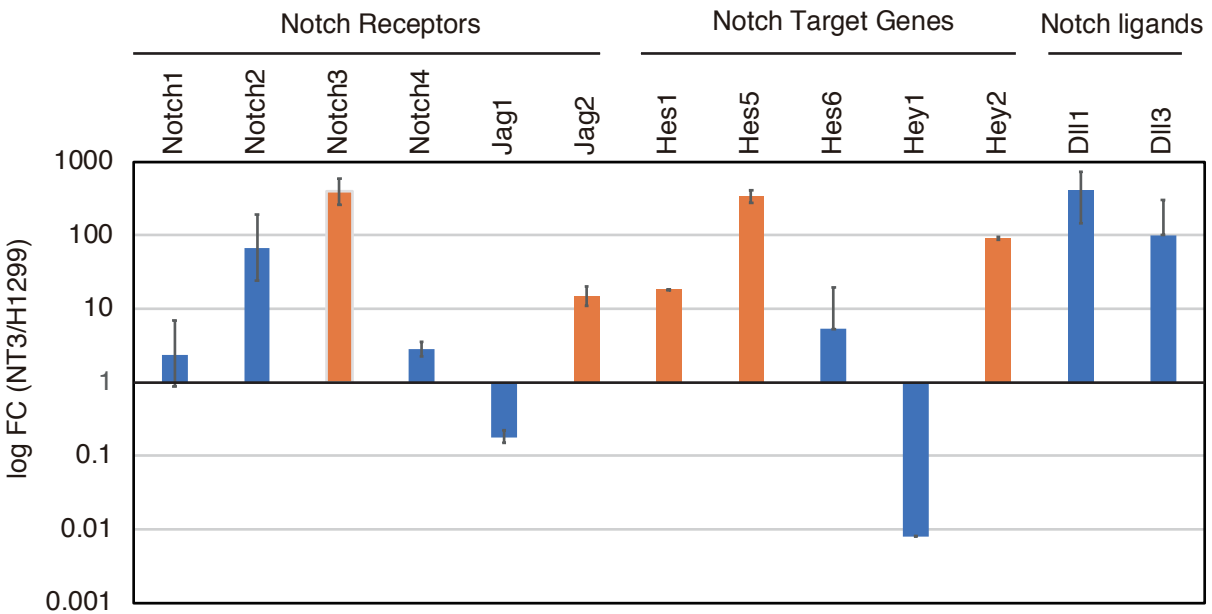

Figure S4

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