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Role of Wnt5b-Ror1 signaling in the proliferation of pancreatic ductal adenocarcinoma cells

Natsuko Yamauchi[1, 2], Mako Otsuka[1], Tomohiro Ishikawa[1], Yoshihiro Kakeji[2], Akira Kikuchi[3], Atsuhiro Masuda[4], Yuzo Kodama[4], Yasuhiro Minami[1*], Koki Kamizaki[1*]

[1.]Division of Cell Physiology, Department of Physiology and Cell Biology, Graduate School of Medicine, Kobe University, Kobe, Japan

[2.]Division of Gastrointestinal Surgery, Department of Surgery, Graduate School of Medicine, Kobe University, Kobe, Japan

[3.]Center for Infectious Disease Education and Research, Osaka University, Suita, Japan

[4.]Division of Gastroenterology, Department of Internal Medicine, Kobe University Graduate School of Medicine, Kobe, Japan

***Corresponding authors:** Y. Minami (7-5-1, Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan; E-mail: yminami@med.kobe-u.ac.jp, Tel: +81-78-382-5561, Fax: +81-78-382-5579) and K. Kamizaki (7-5-1, Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan; E-mail: pelican@med.kobe-u.ac.jp, Tel: +81-78-382-5561, Fax: +81-78-382-5579)

Short title: Role of Wnt5b-Ror1 signaling in PDAC

Key words: Pancreatic ductal adenocarcinoma (PDAC), Wnt5b, Ror1, Proliferation

Abstract

Pancreatic ductal adenocarcinoma (PDAC) is one of the most refractory cancers with the worst prognosis. Although several molecules are known to be associated with the progression of PDAC, the molecular mechanisms underlying the progression of PDAC remain largely elusive. The Ror-family receptors, Ror1 and Ror2, which act as a receptor(s) for Wnt-family ligands, particularly Wnt5a, are involved in the progression of various types of cancers. Here, we show that higher expression of *Ror1* and *Wnt5b*, but not *Ror2*, are associated with poorer prognosis of PDAC patients, and that Ror1 and Wnt5b are expressed highly in a type of PDAC cell lines, PANC-1 cells. Knockdown of either *Ror1* or *Wnt5b* in PANC-1 cells inhibited their proliferation significantly *in vitro*, and knockout of *Ror1* in PANC-1 cells resulted in a significant inhibition of tumor growth *in vivo*. Furthermore, we show that Wnt5b-Ror1 signaling in PANC-1 cells promotes their proliferation in a cell-autonomous manner by modulating our experimental setting *in vitro*. Collectively, these findings indicate that Wnt5b-Ror1 signaling might play an important role in the progression of some if not all of PDAC by promoting proliferation.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies with the worst prognosis among all cancer types. It has the poorest prognosis due to late diagnosis, high invasiveness, frequent metastasis formation, and resistance to all treatment modalities, with a 5-year survival rate of approximately 12%, one of the lowest of all cancer entities (Siegel *et al.* 2023). One reason for the lack of improvement in poor prognosis is that the molecular pathogenesis of PDAC remains largely unclear. Although several reports have been made of

factors, including the intra-tumoral microbiome, tertiary lymphoid structure, and so on, associated with the prognosis in PDAC (Ashina *et al.* 2023; Tanaka *et al.* 2023; Abe *et al.* 2024), the comprehensive molecular mechanism has not yet been fully elucidated. Therefore, elucidating molecular mechanisms and discovering diagnostic and therapeutic target molecules are essential to improve the poor prognosis of PDAC.

Receptor tyrosine kinase-like orphan receptor (Ror)-family receptors, consisting of Ror1 and Ror2, are the type I transmembrane receptors that act as receptors for their cognate Wnt-family ligands. It is well known that Wnt5a binds to the cysteine-rich domain located in the extracellular region of Ror1 and Ror2, and thereby activating Wnt5a-Ror signaling that mediates non-canonical Wnt signaling to regulate cellular polarity, migration, and proliferation (Nishita *et al.* 2010; Endo *et al.* 2022). Accumulating evidence demonstrates that the other Wnt ligands, such as Wnt5b, Wnt9a, and Wnt11, can also bind to the Ror-family receptors (Harada *et al.* 2017; Weissenböck *et al.* 2019; Menck *et al.* 2021; Zhang *et al.* 2024). It has become evident that Wnt-Ror signaling plays the crucial roles in regulating the progression of various types of cancers, including gastric cancer, lung adenocarcinoma, osteosarcoma, head and neck squamous cell carcinoma, and so on (Takiguchi *et al.* 2016; Nishita *et al.* 2017; Ikeda *et al.* 2020; Avincsal *et al.* 2021; Nishita *et al.* 2023). It has recently been shown that the Ror-family receptors are also involved in the progression of PDAC (Liu *et al.* 2021; Yamazaki *et al.* 2023). However, the molecular mechanisms of how the Ror-family receptors regulate PDAC progression remain largely unclear.

Here, we show that higher expression of Ror1 is associated with poorer prognosis of patients with PDAC. Our *in vitro* analysis revealed that Ror1 is expressed highly in PANC-1 cells, one of the PDAC cell lines, and that Ror1 plays an important role in promoting the proliferation of PANC-1 cells. Consistently, *in vivo* xenograft transplantation experiments with

PANC-1 and their derived *Ror1* KO cells further emphasize an important role of Ror1 in the growth of PDAC. We further show that Wnt5b, rather than Wnt5a, is expressed highly in PANC-1 cells, and provide evidence indicating that Wnt5b-Ror1 signaling in PANC-1 cells plays an important role in promoting their proliferation. These findings suggest that Wnt5b and Ror1 can be suitable candidates for treating some if not all of PDAC.

Results and Discussion

Expression of Ror1 in patients with PDAC.

Although it has recently been reported that Ror-family receptors play an important role in the progression of PDAC (Liu *et al.* 2021; Yamazaki *et al.* 2023), the molecular mechanism of how the Ror-family receptors can regulate the property of PDAC cells remains to be determined. To clarify the issue, we first examined whether higher or lower expression levels of *Ror1* and *Ror2* can be associated with the prognosis of patients with PDAC using the TCGA dataset stored in the Human Protein Atlas (https://www.proteinatlas.org/). As shown, higher expression of *Ror1*, but not *Ror2*, was associated with poorer prognosis of PDAC patients (Figure 1A). Therefore, we focused on the role of Ror1 in PDAC.

We next examined whether Ror1 is indeed expressed in patients with PDAC using an immunohistochemical approach. To this end, sections of formalin-fixed paraffin-embedded (FFPE) specimens from 19 patients with PDAC specimens were prepared. The sections were stained with anti-Ror1 antibodies, and we found that Ror1 was expressed highly in the ductal structures within tissues of PDAC (Figure 1B and S1). These findings suggest that Ror1-mediated signaling might play a role in regulating the progression of PDAC.

Ror1 plays an important role in the proliferation of PANC-1 cells.

As an attempt to investigate the role of Ror1 in PDAC cells, we first compared Ror1 expression among three PDAC cell lines (PANC-1, AsPC-1, and CFPAC-1 cells) by Western blotting. Since the highest expression levels of Ror1 were detected in PANC-1 cells among the cell lines examined (Figure 2A), we primarily used PANC-1 cells in this study hereafter. It has been reported that Ror1 promotes the proliferation of various cancer cells (Ikeda *et al.* 2020; Nishita *et al.* 2023), and thus we investigated the function of Ror1 in the proliferation of PANC-1 cells by transfection with siRNAs against *Ror1* (si*Ror1#1*, si*Ror1#2*) or control siRNA (siControl) (Figure 2B). We performed the WST-8 assay, and found that *Ror1* knockdown resulted in significant inhibition of PANC-1 cell proliferation (Figure 2C). We then performed the EdU assay as described in Experimental procedures and found that *Ror1* knockdown resulted in decreased proportion of EdU-positive PANC-1 cells (Figure 2D), indicating that Ror1 is required for cell-cycle progression of PANC-1 cells. We further confirmed the role of Ror1 in the proliferation of PANC-1 cells by treatment with Strictinin, a Ror1 inhibitor (Fultang *et al.* 2019). As shown, Strictinin treatment inhibited the proliferation of PANC-1 cells in a concentration-dependent manner (Figure 2E). These results indicate that Ror1 plays an important role in promoting proliferation of PANC-1 cells.

Next, we investigated whether Ror1 plays an important role in the growth of PANC-1 cells *in vivo*. To this end, we established *Ror1* knockout PANC-1 cells using the CRISPR-Cas9 system as described in Experimental procedures (Figure S2), and subcutaneously transplanted them into the NOG mice (Ito *et al.* 2002). Seven weeks after their transplantation, the tumor tissues were dissected, followed by measuring the tumor weight. We found that the tumor

weight was significantly decreased by *Ror1* knockout of PANC-1 cells when compared with their parental ones (Figure 2F). Therefore, our *in vitro* and *in vivo* analyses suggest that Ror1 might play an important role in the progression of some if not all of PDAC as exemplified by PANC-1 and their derived *Ror1* knockdown (KD) or *Ror1* KO cells.

Wnt5b-Ror1 signaling promotes the proliferation of PANC-1 cells.

Ror1 is known to acts as a receptor of Wnt5a to activate non-canonical Wnt signaling (Nishita *et al.* 2010; Endo *et al.* 2022). We then examined the expression levels of *Wnt5a* in the three PDAC cell lines along with PC-9 cells, that are one of the representative lung adenocarcinoma cell lines expressing both Wnt5a and Wnt5b at higher levels (Zhang *et al.* 2020; Nishita *et al.* 2023). It was found that *Wnt5a* in PDAC cell lines is expressed much lower than that in PC-9 cells. However, the expression of *Wnt5b*, the closest relative of Wnt5a, is significantly higher in PANC-1 cells than in other PDAC cells (Figure 3A). It can be assumed that binding of Wnt5b, rather than Wnt5a, to Ror1 might be required to elicit Ror1-mediated signaling in PANC-1 cells. In fact, TCGA analysis revealed that higher expression of *Wnt5b*, was associated with poorer prognosis of PDAC (Figure 3B). Thus, we investigated the role of Wnt5b in PANC-1 cells by transfection with siRNAs against *Wnit5b* (si*Wnt5b#1*, si*Wnt5b#2,* and *Wnt5b#3*) or control siRNA (siControl) (Figure 3C). Similar to the result with *Ror1* knockdown (Figure 2C), the *Wnt5b* knockdown also inhibited comparably the proliferation of PANC-1 cells (Figure 3D), indicating that both Wnt5b and Ror1 expressed in PANC-1 cells, might play an important role in promoting their cellular proliferation. Finally, we investigated whether or not Wnt5b-Ror1 signaling indeed promotes the proliferation of PANC-1 cells. Since recombinant Wnt5b commercially available was not biologically active enough to restore the

proliferation of *Wnt5b* KD PANC-1 cells at least partly (data not shown) and *Ror1* KD PANC-1 cells by themselves cultured in DMEM containing fresh FBS seemed to secrete Wnt5b endogenously, we improved our experimental setting where *Ror1* KD PANC-1 cells were cultured in DMEM containing concentrated Wnt5b conditioned medium (Wnt5b-CM) or control conditioned medium (control CM) as described in Experimental procedures. As shown in Figure 3E, three days culture of siControl-transfected PANC-1 cells with Wnt5b CM promotes cell proliferation when compared to that with control CM. On the other hand, any apparent difference in cell proliferation of *Ror1* KD PANC-1 cells (si*Ror1*#1- or si*Ror1*#2-transfected PANC-1 cells) cultured with Wnt5b CM or control CM for 3 days (Figure 3E). These results suggest that Wnt5b-Ror1 signaling might promote the proliferation of PANC-1 cells.

Here we show that the expression levels of Ror1 and Wnt5b in PANC-1 cells were higher than those in the other PDAC cell lines, AsPC-1 and CFPAC-1 cells. At present the reason why the expression levels of Ror1 and Wnt5b are different among the three PDAC cell lines remains unclear. With this respect, it has recently been shown that *Ror1* expression can be induced by YAP/BRD4 in PDAC cells (Yamazaki *et al.* 2023). On the other hand, it has been reported that *Ror1* expression can also be induced transcriptionally by other transcriptional factors, including STAT3, NF-kB, HIF1a, Notch, and Nkx2-1 in other types of cancer cells (Li *et al.* 2010; Yamaguchi *et al.* 2012; Kamizaki *et al.* 2017; Huang *et al.* 2023; Ishikawa *et al.* 2023), yet the role(s) of these factors in *Ror1* expression in PDAC cells is still elusive. The molecular mechanism regulating *Wnt5b* expression in some if not all of PDAC cells has also yet to be elucidated. Future study will be required to clarify the molecular mechanisms that upregulate expression *Wnt5b* and *Ror1* in a subset of PDAC cells. In our present study, we found that Wnt5b-Ror1 signaling promotes the proliferation of PANC-1 cells in a cell-autonomous manner. Since the downstream effectors of Wnt5b-Ror1 signaling remain largely unidentified, it is of

importance revealing the molecular mechanism of how Wnt5b-Ror1 signaling promotes the proliferation of PDAC cells in the future.

Experimental procedures

Cell culture and transfection

PANC-1 and CFPAC-1 cells were provided by T. Kobayashi (Nara Institute of Science and Technology). AsPC-1 cells were obtained from the Japanese Collection of Research Bioresources Cell Bank (Osaka, Japan). L-cells expressing Wnt5b stably were prepared as described previously (Harada *et al.* 2017). PANC-1 and L-cells were cultured in DMEM (Fujifilm Wako Pure Chemicals Corporation, Osaka, Japan) supplemented with 10% (v/v) FBS. AsPC-1 cells and CFPAC-1 cells were maintained in RPMI-1640 and IMDM (Nacalai Tesque, Tokyo, Japan) containing 10% (v/v) FBS, respectively. It was confirmed that PANC-1 cells were not contaminated by analyzing the short tandem repeats (BEX CO., LTD, Tokyo, Japan).

Cells were transfected with the respective siRNAs using Lipofectamine RNAiMax (Thermo Fisher Scientific, Waltham, MA), according to the manufacturer's instructions. The following sequence of siRNA were used: si*Ror1#1,* 5'-GCAAGCAUCUUUACUAGGA-3' and 5'-UCCUAGUAAAGAUGCUUGC-3'; si*Ror1#2*, 5'-GAGCAAGGCUAAAGAGCUA-3' and 5'-UAGCUCUUUAGCCUUGCUC-3'; si*Wnt5b#1,* 5'-GGGUCUUGUCCAGAAUGUA -3' and 5'-UACAUUCUGGACAAGACCC-3'; si*Wnt5b#2,* 5'-GCUUCAACCUCGAUGUCUU-3' and 5'-AAGACAUCGAGGUUGAAGC-3'; si*Wnt5b#3,* 5'-CUGUCUUUGGGAGAGUCAU-3' and 5'-AUGACUCUCCCAAAGACAG- 3'.

Cell proliferation assay

Cell proliferation was monitored by WST-8 assay or EdU assay. For the WST-8 assay, the cells were plated at a density of 2,000 cells/well in a 96-well plate in triplicate, and the WST-8 assay was performed on the next day of seeding as day 0 using Cell Counting Kit 8 (Dojindo, Kumamoto, Japan). When the effect of Wnt5b stimulation in the proliferation of PANC-1 cells was examined, the medium was changed into conditioned medium prepared as described below section at the day 0. The absorbance of 450 nm was measured one hour after incubation with WST-8 assay solution by Microplate reader Model550 (BioRad, Hercules, CA). To analyze the proportion of EdU-positive cells, the cells were cultured with EdU (at a final concentration of 10 μM, Thermo Fisher Scientific) for 15 minutes. Subsequently, the cells were fixed by 4% (w/v) PFA. The EdU-positive cells were visualized using a Click-it EdU Cell Proliferation Kit for Imaging (Thermo Fisher Scientific) according to the manufacturer's instructions. Proportion of EdU-positive cells was automatically calculated by BZ-X700 and Hybrid Cell Count (Keyence, Osaka, Japan).

Preparation of concentrated Wnt5b-conditioned medium

L-cells stably expressing Wnt5b or and control ones were obtained as described previously (Harada *et al.* 2017) and were seeded in a 10 cm ϕ dish (2.0×10^6 cells/dish). One to two days after seeding cells, the medium was changed into a fresh one without FBS, and the cells were cultured for 48 hours. The medium was concentrated using Vivaspin 20-10K (Cytiva, Marlborough, MA). The concentrated Wnt5b or control conditioned medium was added to the PANC-1 cells culturing in medium.

RNA isolation and quantitative RT-PCR

Total RNA was isolated using Sepasol-RNA I SuperG (Nacalai Tesque) and reverse transcribed using PrimeScript RT Reagent Kit (Takara Bio, Shiga, Japan) to synthesize cDNA. Quantitative RT-PCR was performed with a LightCycler 480 II system (Roche Diagnostics, Basel, Switzerland) using SYBR Green (Roche Diagnostics). The mRNA levels were standardized compared to the mRNA levels of *18S* ribosomal RNA. The following primers were used: *Ror1*: Fw_ 5'-TGCCAGCCCAGTGAGTAATCT-3', Rv_ 5'-GCCAATGAAACCAGCAATCTG-3', *Wnt5a*: Fw_5'-TAAGCCCAGGAGTTGCTTTG-3', Rv_5′-GCAGAGAGGCTGTGCTCCTA-3′, *Wnt5b*, Fw_5'-TTCTGACAGACGCCAACTCC-3', Rv_5'-TGGCATTCCTTGATGCCAGT-3', *18S*: Fw_5'-CGCTGAGCCAGTCAGTGTAG-3', Rv_5'-CGCTGAGCCAGTCAGTGTAG-3'.

Western blotting

The cells were solubilized in lysis buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% (v/v) Nonidet P-40, 10 mM NaF, 5 mM EDTA, 10 mM Na_3VO_4, 10 μg/mL aprotinin, 10 μg/mL leupeptin, and 0.25 mM p-APMSF). Proteins obtained by lysis (10 μg per lane) were separated by SDS-PAGE and transferred to the membrane. The membranes were immunoblotted with the primary antibodies (anti-Ror1 antibody (#16540, 1:500, Cell signaling Technology, Danvers, MA) and anti-α-tubulin (PM054, 1:500, MBL, Tokyo, Japan)) overnight at 4°C. After the reaction with the primary antibodies, the membranes were washed by PBST, followed by incubation with HRP-conjugated secondary antibodies (BioRad). Immunoreactive bands were detected using ImmunoStar LD (Fujifilm Wako Pure Chemicals Corporation) and ChemiDoc Touch MP (BioRad).

Immunohistochemical analysis

The fixed tissue specimens resected from 19 patients with PDAC at the Department of Gastroenterology, Kobe University Hospital, were embedded in paraffin. The paraffin blocks were cut into 4 μm sections and incubated with Ror1 antibody (#4102, 1:50, Cell Signaling Technology) or control IgG (Jackson Immuno Research, Philadelphia, PA) overnight at 4°C. Subsequently, the sections were incubated with HRP-labeled polymer-conjugated anti-rabbit IgG antibody (ImmPRESS Reagent kit Peroxidase; Vector Laboratories, Burlingame, CA) for 60 minutes at room temperature followed by reaction with DAB Chromogen (Dako Cytomation, Glostrup, Denmark). Nuclei were counterstained with hematoxylin. Specimens were observed using a BZ-X700 microscope (Keyence).

Generation of *Ror1* KO PANC-1 cells

Ror1-deficient PANC-1 cells were generated using the Alt-R CRISPR-Cas9 system (Integrated DNA Technologies, San Jose, CA) according to the manufacturer's protocol. In brief, PANC-1 cells were transfected with an RNP complex consisting of Cas9 nuclease V3 (Integrated DNA Technologies) and guide RNA against the *Ror1* gene (Design ID: Hs.Cas9.ROR1.1. AA). After transfection, monoclonal PANC-1 cells were obtained by limiting dilution.

Xenograft assay

Six-week-old male NOG mice (*NOD/Shi-scid, IL-2RγKO Jic*) were obtained from In-

Vivo Science Inc (Tokyo, Japan). PANC-1 cells and PANC-1 *Ror1*KO cells in 50% (v/v) Matrigel (Corning, Corning, NY) diluted by PBS were subcutaneously transplanted into the mice (5×10^6 cells/mouse). Tumor weights were measured 7 weeks after transplantation. Animal experiments in this study were approved by the Institutional Animal Experimentation Committee (Permit No.P230607) and conducted by the Kobe University Animal Experimentation Regulations.

TCGA data analysis

The TCGA dataset of PDAC patients was downloaded from the Human Protein Atlas (https://www.proteinatlas.org/). Kaplan-Meier curves for overall survival based on *Ror1*, *Ror2*, and *Wnt5b* expression were generated by GraphPad Prism 9.0 (GraphPad Software, Boston, MA). The cut-off value was determined using the minimum p-value approach described in the Human Protein Atlas.

Statistical analysis

Statistical significance was determined by one-way ANOVA followed by Dunnett's or Holm's test in case more than three groups were analyzed. When two groups were compared, a Student's *t*-test was used. Significance was described as $*p<0.05$, $**p<0.01$, $***p<0.001$.

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Figure Legend

Figure 1. Higher level of *Ror1* expression is associated with poorer prognosis in patients with PDAC.

A. Kaplan-Meier survival analysis of *Ror1* high and low expression groups (left) and *Ror2* high and low expression groups (right) in patients with PDAC. (*Ror1* high: n = 46, *Ror1*

low: n = 130, *Ror2* high: n = 49, *Ror2* low: n = 127. *p<0.05, ns: not significant, log-rank test.)

B. Immunohistochemical analysis of Ror1 with surgical specimens from patients with PDAC. Representative images of HE, control IgG and anti-Ror1 immunohistochemistry of PDAC tissue sections are shown. The scale bar is 200 μm. The boxed region is magnified on the right of each image. The scale bar in the magnified image is 100 μm.

Figure 2. Ror1 plays an important role in the proliferation of the PANC-1 cells.

A. Protein expression levels of Ror1 and αTubulin in PDAC cell lines (PANC-1, AsPC-1, and CFPAC-1 cells). The graph on the right shows the relative band intensity of Ror1, which is normalized by the intensity of αTubulin. Data are shown as mean ± S. D. (n = 3, **p<0.01, ns: not significant, Holm's test).

B. Protein expression levels of Ror1 and αTubulin in PANC-1 cells treated with the indicated siRNAs. Images are representative of at least three independent experiments.

C. Proliferation of PANC-1 cells treated with the indicated siRNAs was evaluated using WST-8 assay at the indicated time points as described in Experimental procedures. Data are shown as mean ± S. D. (n = 4, *p<0.05, **p<0.01, ***p<0.001, Holm's test).

D. The proportion of EdU-positive PANC-1 cells treated with the indicated siRNAs was evaluated as described in Experimental procedures. Data are shown as mean ± S. D. (n = 4, *p<0.05, **p<0.01, Dunnett's test).

E. Proliferation of PANC-1 cells treated with Strictinin (at a final concentration of 0, 25, 50, 100, 200 μM, respectively) was evaluated using WST-8 assay at the indicated time points. Data are shown as mean ± S. D. (n = 3, **p<0.01, ***p<0.001, ns: not significant, Holm's

test).

F. Image of tumor tissues dissected from the xenograft model. Seven weeks after the subcutaneous transplantation of PANC-1 cells, the tumor tissues were dissected, and the weight was measured. The right graph shows the weight of the tumor tissues. (n = 4, **p*<0.05, Student's *t*-test)

Figure 3. Wnt5b-Ror1 signaling promotes the proliferation of PANC-1 cells.

A. Expression levels of *Wnt5a* and *Wnt5b* in PDAC cell lines (PANC-1, AsPC-1, and CFPAC-1 cells) and PC9 cells, a LUDA cell line, as a positive control for *Wnt5a* and *Wnt5b*. Data are shown as mean ± S. D. (n = 3, ***p*<0.01, ns: not significant, Holm's test).

B. Kaplan-Meier survival analysis of *Wnt5b* high and low expression groups in patients with PDAC. (*Wnt5b* high: n = 36, *Wnt5b* low: n = 140. **p*<0.05, log-rank test.)

C. Expression level of *Wnt5b* in PANC-1 cells treated with the indicated siRNAs. Data are shown as mean ± S. D. (n = 3, ****p*<0.001, Dunnett's test).

D. Proliferation of PANC-1 cells treated with the indicated siRNAs was evaluated by WST-8 assay at the indicated time points. Data are shown as mean ± S. D. (n = 3, ****p*<0.001, Holm's test).

E. Proliferation of PANC-1 cells treated with the indicated siRNAs and concentrated conditioned medium (CM) was evaluated by WST-8 assay at the indicated time points. Data are shown as mean ± S. D. of triplicates from one representative of two independent experiments. (****p*<0.001, Holm's test).

Figure S1. Immunohistochemical analysis of Ror1 in patients with PDAC.

Immunohistochemical analysis of Ror1 with surgical specimens from patients with PDAC. Images of HE and anti-Ror1 immunohistochemistry of PDAC tissue sections are shown. The scale bar is 200 μm.

Figure S2. Confirmation of the ablated expression of Ror1 in *Ror1* knockout (KO) PANC-1 cells.

Protein expression of Ror1 and αTubulin in PANC-1 cells (wild-type and *Ror1* KO #1 and #2) is shown. The #1 and the #2 are different clones of *Ror1* KO cells. Since the #2 clone grew better than the #1, it was used for the xenograft assay.

Figure 1

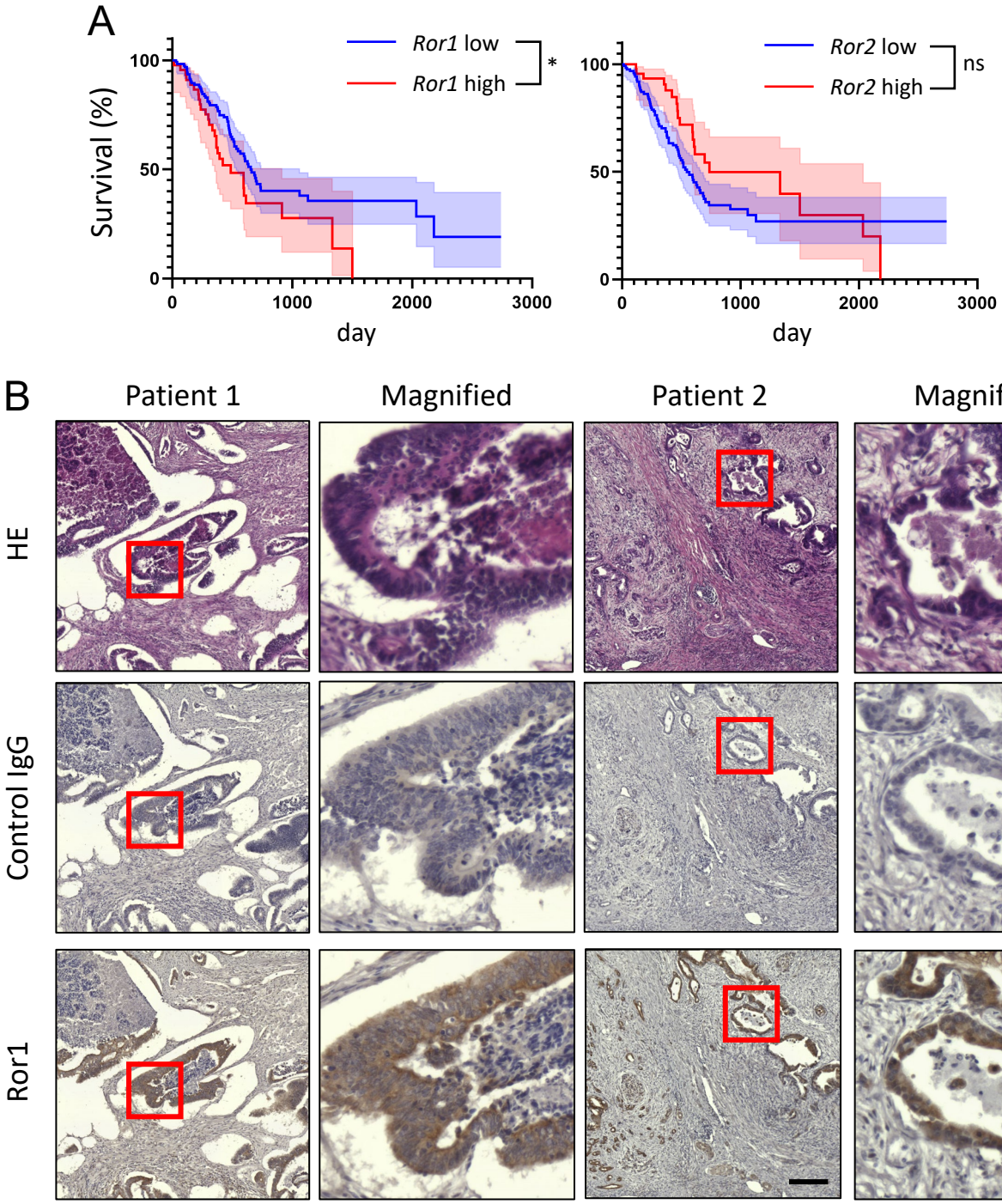
A
Survival (%)
100
50
0
0
1000
2000
3000
day
Ror1 low
Ror1 high
*
Ror2 low
Ror2 high
ns
B
Patient 1
Magnified
Patient 2
Magnified
HE
Control IgG
Ror1

Figure 2

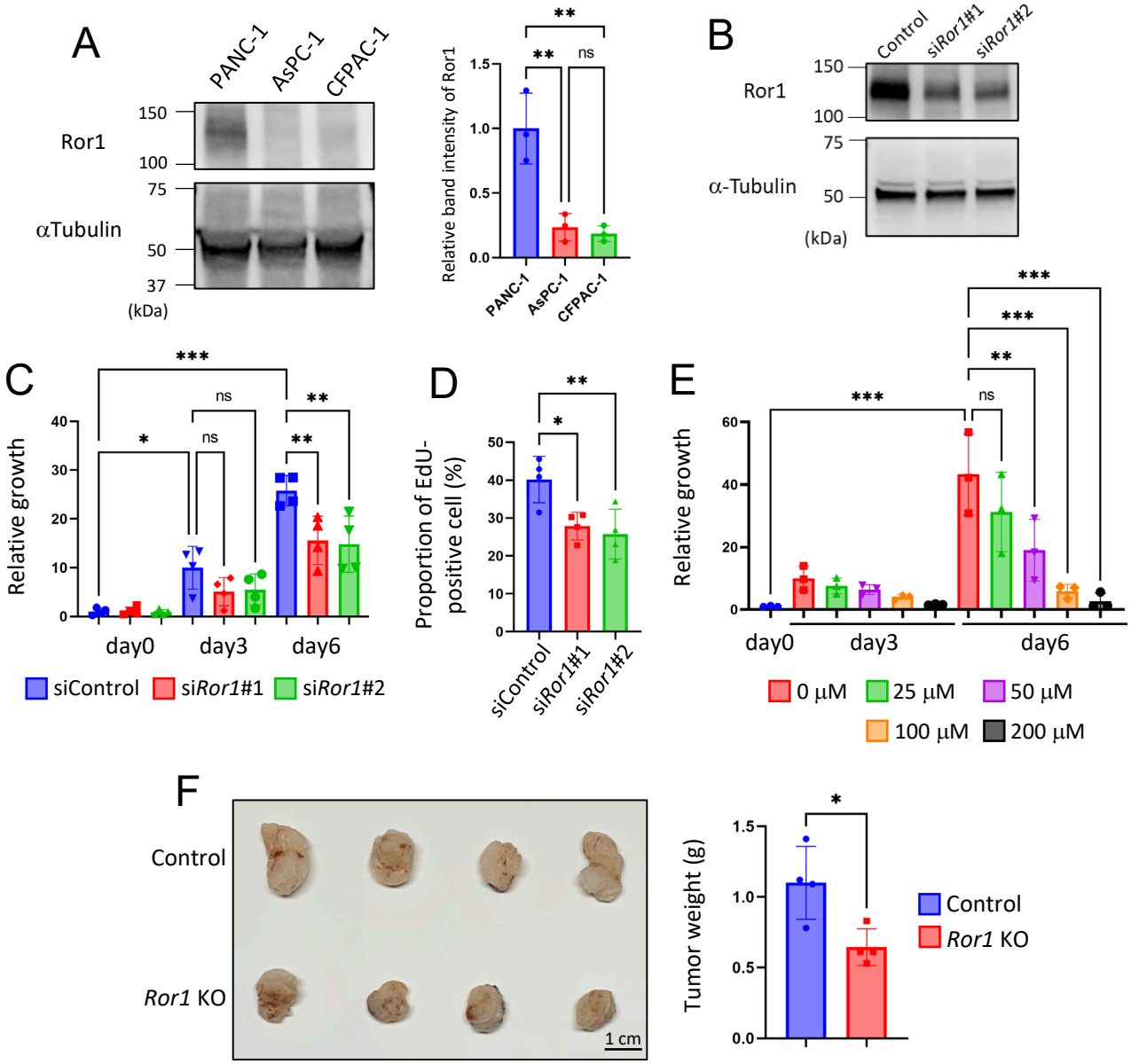
A
PANC-1
AsPC-1
CFPAC-1
Ror1
αTubulin
150
100
75
50
37
(kDa)
Relative band intensity of Ror1
1.5
1.0
0.5
0.0
**
ns
B
Control
siRor1#1
siRor1#2
Ror1
α-Tubulin
150
100
75
50
(kDa)
C
Relative growth
40
30
20
10
0
***
*
ns
**
day0
day3
day6
siControl
siRor1#1
siRor1#2
D
Proportion of EdU-positive cell (%)
50
40
30
20
10
0
**
*
siControl
siRor1#1
siRor1#2
E
Relative growth
60
40
20
0
***
ns
**
day0
day3
day6
0 μM
25 μM
50 μM
100 μM
200 μM
F
Control
Ror1 KO
1 cm
Tumor weight (g)
1.5
1.0
0.5
0.0
*
Control
Ror1 KO

Figure 3

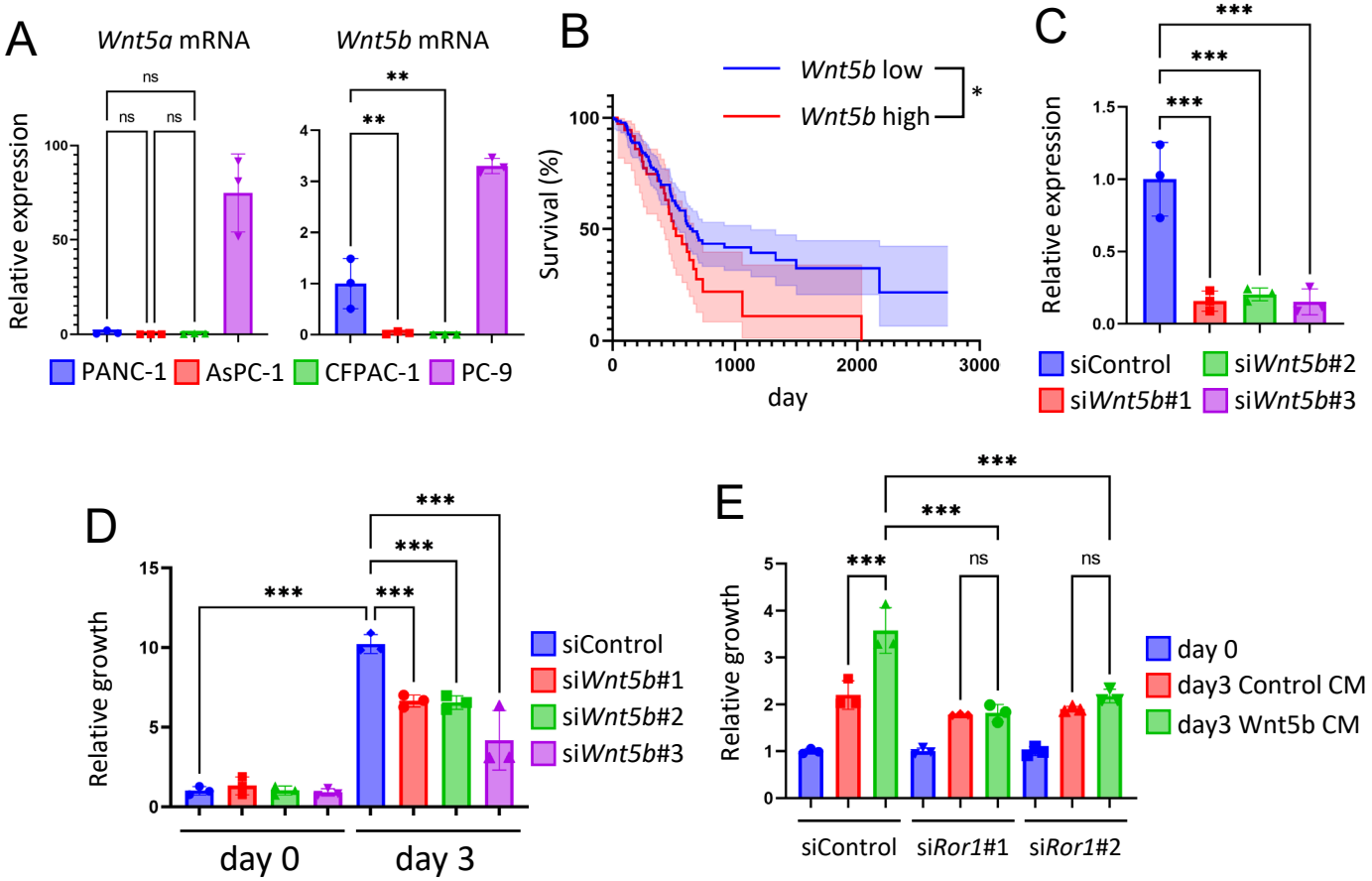
A
Wnt5a mRNA
Wnt5b mRNA
Relative expression
ns
ns
ns
**
**
PANC-1
AsPC-1
CFPAC-1
PC-9
B
Wnt5b low
Wnt5b high
*
Survival (%)
day
C
Wnt5b mRNA
***
***
***
Relative expression
siControl
siWnt5b#1
siWnt5b#2
siWnt5b#3
D
***
***
***
***
Relative growth
siControl
siWnt5b#1
siWnt5b#2
siWnt5b#3
day 0
day 3
E
***
***
***
ns
ns
Relative growth
day 0
day3 Control CM
day3 Wnt5b CM
siControl
siRor1#1
siRor1#2

Figure S1

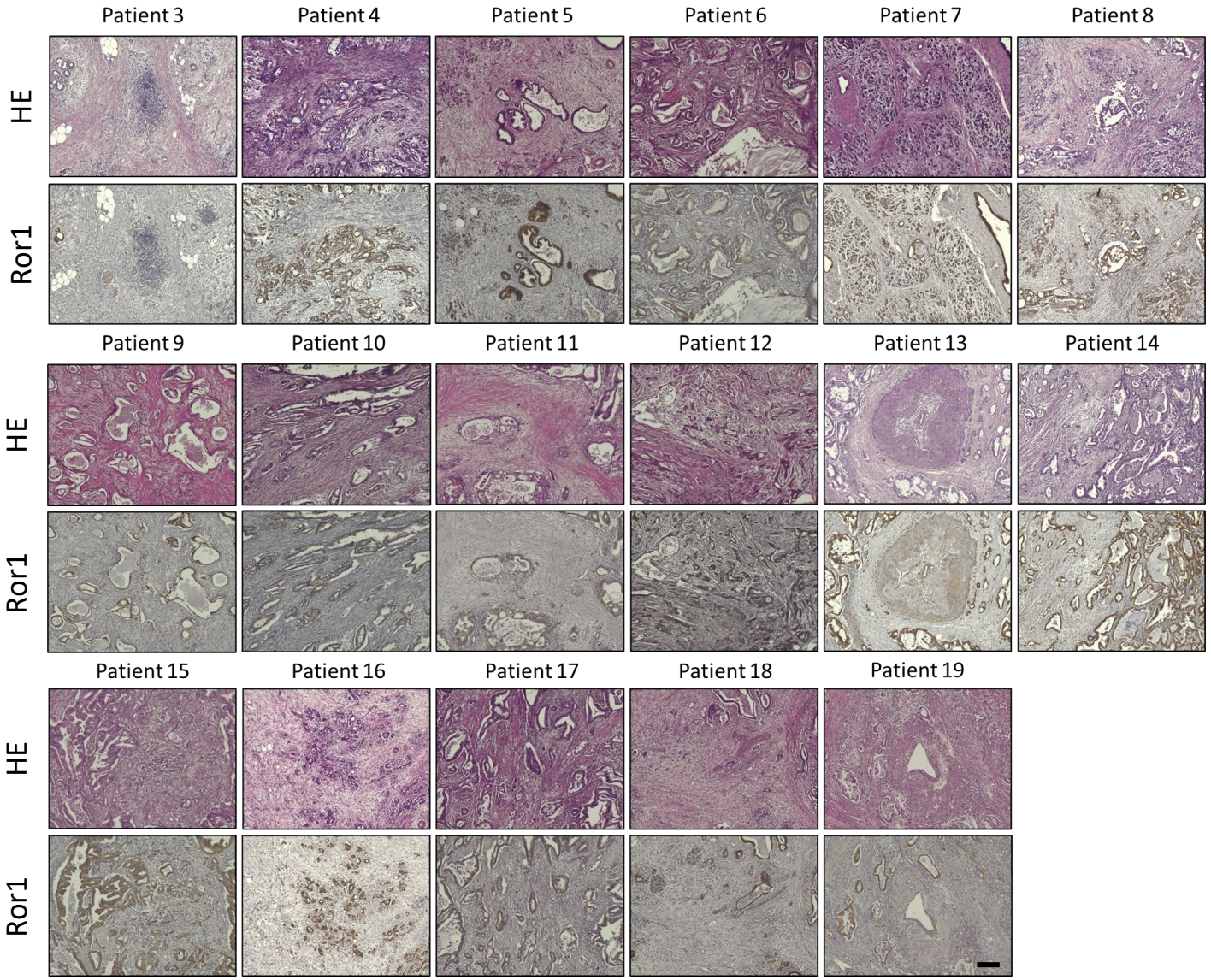
Patient 3
Patient 4
Patient 5
Patient 6
Patient 7
Patient 8
HE
Ror1
Patient 9
Patient 10
Patient 11
Patient 12
Patient 13
Patient 14
HE
Ror1
Patient 15
Patient 16
Patient 17
Patient 18
Patient 19
HE
Ror1

Figure S2