hENT-1 antibody assessment

Cell lines

Human pancreatic tumor cell lines PANC-1, AsPC-1, BxPC-3, CFPAC-1 were obtained from the ATCC (Manassas, VA, USA) and cultured according to the guidelines. All cell lines were authenticated using a short tandem repeat (STR) DNA test.

Western blot analysis

The cells were lysed in RIPA buffer [25 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, and 0.1% SDS] supplemented with protease inhibitor cocktail (Roche Applied Science, Germany). The protein concentration was measured using the BCA Protein Assay (Thermo Fisher Scientific, Waltham, MA, USA). Total cell lysates (30 μg) were separated on 10% SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Millipore). The membranes were incubated with the following antibodies: anti-ENT-1 (Abcam), anti-β-actin (Cell Signaling). Following washing, the membranes were developed with anti-mouse or rabbit horseradish peroxidase conjugated secondary antibodies (Cell Signaling), and analyzed with ImageQuant LAS4000 system (Fujifilm, Tokyo, Japan).

Quantitative real-time reverse transcriptase-polymerase chain reaction (RT-qPCR)

Total RNA was extracted with TRIzol reagent (Life Technologies), and complementary DNA was synthesized with the SuperScript™III First-Strand Synthesis System (Life Technologies). PCR assay was performed in triplicate using a SYBR Premix Dimer Eraser (Perfect Real Time) kit (Takara Bio, Shiga, Japan) on a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad). RPLP0 gene was used as an endogenous control. The primers were as follows: hENT-1: forward 5'-GTAGCCTCGGCATTTGCTTG-3', reverse 5'-CCTCTGAAGGCACCTGGTT-3'; RPLP0: forward 5'-CAGATTGGCTACCCAACTGTT-3', reverse 5'-GGGAAGGTGTAATCCGTCTCC-3'.

Immunofluorescence

The cells grown on glass coverslips were washed using phosphate-buffered saline (PBS) and fixed in 4% paraformaldehyde for 20 min at room temperature. Then, the cells were blocked with 2% bovine serum albumin (BSA) for 30 min. Next, the cells were incubated with anti-ENT-1 antibody overnight at 4 ℃, washed three times, and incubated with Alexa flour 488-conjugated goat anti-rabbit IgG for 1 h at room temperature. Finally, the cells were examined using a fluorescence microscope (Nikon, Japan).

Results

RT-qPCR and Western blot analysis of hENT-1 expression in four different pancreatic cancer cell lines PANC-1, AsPC-1, BxPC-3, CFPAC-1 showed differential hENT-1 expression levels among cell lines, and PANC-1 cells had significantly higher hENT-1 expression level, while other cells had lower level (Figure S1A,S1B). Immunofluorescence identification of two different cell lines was in accordance with the results from Western blotting and RT-qPCR. Therefore, the anti-ENT-1 antibody was suitable for immunofluorescence assay and could be used to assess hENT-1 expression level (Figure S1C).

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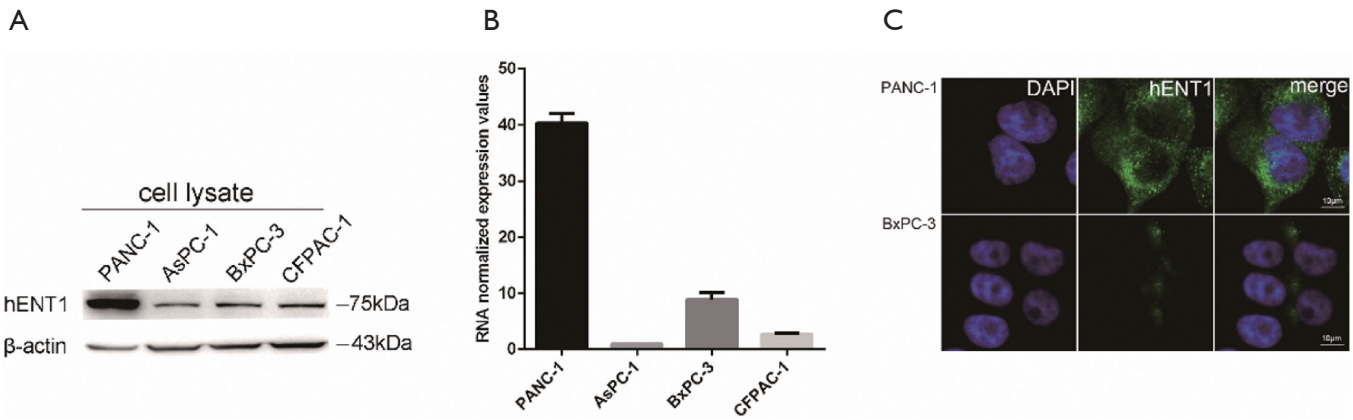

Figure S1 hENT-1 antibody assessment. (A) Western blot analysis of hENT-1 protein expression in four pancreatic cancer cell lines. (B) RT-qPCR of hENT-1 mRNA expression in four pancreatic cancer cell lines. (C) Immunofluorescence identification of two different cell lines PANC-1 and BxPC-3.

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