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ORIGINAL PAPER

Pancreas-specific protein disulfide isomerase has a cell type-specific expression in various mouse tissues and is absent in human pancreatic adenocarcinoma cells: implications for its functions

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Abstract Members of the protein disulfide isomerase (PDI) family play a critical role in catalyzing the formation of disulfide bonds in secretory proteins, and most of these enzymes have a wide tissue distribution. However, the pancreas-specific PDI homolog was previously suggested to be exclusively expressed in the pancreas (thus commonly referred to as PDIp). In the present study, we found that PDIp was also highly expressed in several other tissues in mice, including the stomach, cecum, ileum, adrenal glands, epididymis, and prostate. Notably, in the digestive organs, such as the stomach and pancreas, very high levels of PDIp were selectively expressed in the digestive enzyme-secreting cells (e.g., gastric chief cells and pancreatic acinar cells). This observation suggests that PDIp may function as a protein-folding catalyst for secretory digestive enzymes. In ileum, PDIp was exclusively expressed in Paneth cells. In addition, high levels of PDIp expression were also detected in normal human pancreas, but its expression was mostly absent in human pancreatic duct adenocarcinoma and pancreatic cancer cell lines. The absence of PDIp expression in pancreatic adenocarcinoma may serve as an additional biomarker for pancreatic cancer.

Keywords PDI · PDIp · Pancreas · Stomach · Ileum · Paneth cells · Disulfide bond · Isomerase · Chaperone

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Abbreviations

PDI	Protein disulfide isomerase
PDIp	Pancreas-specific PDI homolog

Introduction

Formation of correct disulfide bonds in secretory proteins plays an essential role in the maintenance of their structural stability and functional integrity. In eukaryotes, the synthesis of polypeptides destined for secretory pathways usually takes place in endoplasmic reticulum (ER), where formation of disulfide bonds is mostly catalyzed by members of the protein disulfide isomerase (PDI) family (Gruber et al. 2006; Appenzeller-Herzog and Ellgaard 2008). PDI, the most studied member in this protein family, is known to have the ability to catalyze the formation, isomerization, and reduction of disulfide bonds [reviewed in ref. (Wilkinson and Gilbert 2004; Gruber et al. 2006)]. In addition, PDI can also serve as a molecular chaperone (Wang and Tsou 1993). Besides PDI, other members of the PDI family proteins, including ERp57, ERp72, Erp29, PDIp, PDIr and PDILT, have also been characterized in recent years, and it is generally thought that these proteins can interact with different sets of client proteins in vivo (Lumb and Bulleid 2002; Wilkinson and Gilbert 2004; Ellgaard and Ruddock 2005; Gruber et al. 2006).

Most members of the PDI family proteins are ubiquitously expressed in various tissues or cells (Maattanen et al. 2006), but two of them, namely, PDILT and PDIp, were reported to be selectively expressed in testis (van Lith et al. 2005) and pancreas (Desilva et al. 1996; Klappa et al. 1998), respectively. Since PDIp was found to be able to

interact with newly synthesized peptides in the in vitro translation system (Elliott et al. 1998) or with misfolded proteins (Desilva et al. 1996; Klappa et al. 1998), its expression in pancreas had led to the speculation that PDIp might be able to preferentially recognize pancreatic zymogens (Freedman et al. 2002; Wilkinson and Gilbert 2004). Notably, recent studies reported that PDIp mRNA was also detected in SH-SY5Y human neuroblastoma cells (Conn et al. 2004); similarly, its protein was detected in the mouse stomach (Niki et al. 2006). These observations suggest that PDIp may have a wider tissue distribution than previously known.

In the present study, therefore, we sought to examine further the expression and cellular distribution of PDIp in various mouse tissues by jointly using Western blotting and immunohistochemical staining methods. We found that besides pancreas, PDIp was also highly expressed in several other tissues, and its expression in these tissues was cell type-specific. In addition, while high levels of PDIp expression were detected in normal human pancreas, its expression was mostly absent in human pancreatic adenocarcinoma cells and several pancreatic cancer cell lines.

Materials and methods

Reagents, tissues, and cell lines

Specific rabbit antibody against PDI was obtained from Sigma–Aldrich (St. Louis, MO, catalog No. P7372, at 1:2000 dilution for Western blotting), and the anti-GAPDH IgG was from Cell Signaling (Danvers, MA, catalog No. 2118, at 1:2000 dilution for Western blotting). The recombinant human PDIp protein selectively expressed in *E. coli* cells was purified by a joint use of affinity chromatography and ion exchange chromatography, and the purified PDIp protein was used to raise the mouse anti-PDIp antiserum as described recently (Fu and Zhu 2009a, b). The antiserum was diluted at 1:2500 for Western blotting analysis and at 1:200 for immunohistochemical and immunocytochemical stainings.

For studying the tissue distribution of PDI and PDIp, 8-week-old female C57BL/6J mice were obtained from Harlan (Indianapolis, Indiana), and maintained in the animal facility at University of Kansas Medical Center (KUMC). The animal use procedures were approved by the Institutional Animal Care and Use Committee of KUMC, and the NIH guidelines for humane treatment of animals were strictly followed. The animals were killed with CO_2 overdose followed by decapitation. Various tissues (e.g., kidney and prostate) were immediately collected, dissected, and washed in phosphate-buffered saline (PBS) and then processed for immunohistochemical staining. The rhesus monkey pancreas tissue was purchased from the US Biomax (Rockville, MD, catalog is RhFTS151). The human normal pancreas tissue specimens were obtained from the National Disease Research Interchange (catalog is 0060960-13). The human pancreatic tissue array was purchased from the US Biomax (catalog No. PA721).

All cell lines used in this study were obtained from the ATCC (Manassas, VA). They included the human breast cancer cells (MCF-7), human pancreatic cancer cells (Mia Paca-2, Bx PC-3 and Capan-2), rat pancreatic cells (RIN-m5F), mouse hippocampal cells (HT22), monkey kidney cells (cos-7), and human neuroblastoma cells (SK-N-SH). These cell lines were cultured according to the instructions of the supplier.

Transfection of mammalian cells

As described earlier (Fu and Zhu 2009a), the pcDNA3.1-PDIp plasmids (with or without the endoplasmic reticulum-targeting signal peptide) were transfected into cos-7 cells seeded in 24-well plates by using Lipofectamine 2000 and Opti-MEM I reduced serum medium (both obtained from Invitrogen). At 36 h after transfection, cells were harvested and subjected to analysis.

Preparation of nuclear, cytosolic, and microsome fractions from mammalian cells

Cos-7 cells were harvested by trypsin digestion and then subjected to subcellular fractionation by using a Nuclear/Cytosol Fractionation kit (catalog no. is K266-100) from BioVision (Mountain View, CA) according to manufacturer's instructions. The obtained cytosol was further centrifuged at 100,000*g* for 90 min at 4°C to prepare the microsomal fraction. The nuclear, cytosolic, and microsomal fractions were then subjected to Western blotting analysis to determine the levels of specific proteins of interest.

Western blotting

Animal and human tissues were homogenized in the RIPA lysate buffer, consisting of 50 mM Tris–HCl (pH 7.4), 0.1 mM EDTA, 0.1% SDS, 0.15 M NaCl, 1% sodium deoxycholate, and protease inhibitors. After centrifugation at 10,000*g* for 10 min (at 4°C), supernatants were collected. Protein concentrations were determined by using the Protein Assay kit (from Bio-Rad, catalog no. #500-0006) based on the Bradford assay. Bovine serum albumin (BSA) was used as standard. Cultured cells were lysed with the E1500 lysis buffer (Promega, Madison, WI), and the

cellular proteins were separated using the SDS–polyacrylamide gel electrophoresis. The gels were then transferred onto the Sequi-Blot PVDF membrane (from Bio-Rad), the membranes were then blocked in a buffered saline solution (0.05 M phosphate, 0.8% NaCl, and 0.02% KCl, pH 7.4) containing 5% nonfat milk (w/v) for 1 h at room temperature, and then incubated with the primary antibody for 1.5 h. The membranes were subsequently rinsed three times (10 min each) in saline solution, incubated with HRP-conjugated anti-mouse IgG or HRP-conjugated anti-rabbit IgG for 45 min at room temperature, and then rinsed three times. Secondary antibodies on the membranes were detected by using an enhanced chemiluminescence (ECL) detection system (from Amersham Bioscience).

Detection of the cellular distribution of PDIp by immunohistochemical staining

To determine PDIp tissue distribution, various mouse tissues were fixed in 10% (w/v) neutral buffered formalin for 24 h. Formalin-fixed tissues were imbedded in paraffin, cut into 5-μm sections, and mounted on plain slides. The tissue sections were dried for 45 min at 45°C, dewaxed in xylene, and rehydrated through ethanol-graded solutions. For antigen retrieval, slides were immersed in a citric acid buffer solution (pH 6.0) and boiled in microwave oven for 15 min. Endogenous peroxidase was quenched with incubation with 3% H_2O_2 in PBS for 30 min. Slides were blocked for 1 h with 5% goad anti-mouse serum. The primary antibody (mouse anti-human PDIp antiserum or the normal BALB/c mouse serum) was diluted (at 1:200, v/v) in PBS with 0.1% Tween 20 before use and incubated for 1 h at room temperature. Then the secondary antibody (HRP-conjugated goat anti-mouse IgG, diluted at 1:1000, v/v) was incubated for 1 h. Diaminobenzidine was used as a chromogen. Tissue sections were counterstained with hematoxylin QS, mounted in a mounting medium, and visualized under a light microscope.

Detection of the subcellular localization of PDIp by immunocytochemical staining

The cultured cells in 24-well plates were washed once with PBS and fixed in 100% methanol for 15 min at room temperature. Cells were rinsed three times in PBS, permeabilized with 0.2% Triton-X 100 in PBS for 10 min, and then blocked by 10% normal horse serum or goat serum for 2 h at room temperature. Cells were incubated with the primary antibody overnight at 4°C in PBS, rinsed three times with PBS, and then incubated with the Texas red-conjugated anti-mouse horse IgG (TI-2000, at 1:100 dilution; from Vector Laboratory, Southfield, MI) and/or with the FITC-conjugated anti-rabbit goat IgG (FI-1000, at 1:200 dilution; from Vector Laboratory) for 1 h at room temperature. Finally, cells were stained with ProLong Gold Antifade reagent (Invitrogen, P-36931) containing 300 nM 4,6-diamidino-2-phenylindole (DAPI) for 10 min and rinsed three times with PBS before imaging analysis using a fluorescence microscope.

Results

Levels of PDIp protein in various mouse tissues

We validated the specificity of the antiserum against PDIp produced in our laboratory using Western blotting analysis. Firstly, we compared the expression pattern of PDI and PDIp in cos-7 cells transfected with a control vector or a PDIp-expression vector. As shown in Fig. 1a, only a single band corresponding to PDIp was observed in the cos-7 cells transfected with a PDIp-expression vector (lane 2), whereas no band was detectable in the control cells (lane 1). By contrast, the PDI protein was detected in both cells. This result was consistent with our earlier observation showing that cos-7 cells expressed PDI but not PDIp (Fu et al. 2008; Fu and Zhu 2009a). Secondly, only a single band corresponding to PDIp was detected in human and monkey pancreatic tissue lysates (Fig. 1a, lane 3, 4). These observations suggest that the anti-PDIp antiserum could specifically recognize PDIp but had no cross-reactivity with PDI or other cellular proteins.

PDIp protein levels in various mouse tissues were analyzed using Western blotting and were compared with those of PDI. As expected, PDIp was found to be highly expressed in mouse pancreas (lane 6, Fig. 1b). Besides pancreas, high levels of PDIp protein were also detected in the stomach (lane 9, Fig. 1b). This observation is in line with a recent report showing that PDIp was present in mouse stomach (Niki et al. 2006). In addition, moderate levels of PDIp were detected in several other tissues, including cecum, ileumn, liver, kidney, and uterus. Notably, one smaller band for PDIp was observed in some of the tissues (lanes 2, 3, 5, 10, 11, 12, and 13, as indicated with a shaded arrow in Fig. 1b). Given that PDIp was shown to be glycosylated in vivo and the deglycosylated form had a lower mobility on the SDS acrylamide gel than the glycosylated form (Desilva et al. 1997; Klappa et al. 1998), the smaller band of PDIp observed here likely was the unglycosylated form in these tissues. Different from PDIp, PDI was almost ubiquitously expressed in all the tissues examined (Fig. 1b), which agreed with earlier studies [summarized in a review by (Maattanen et al. 2006)].

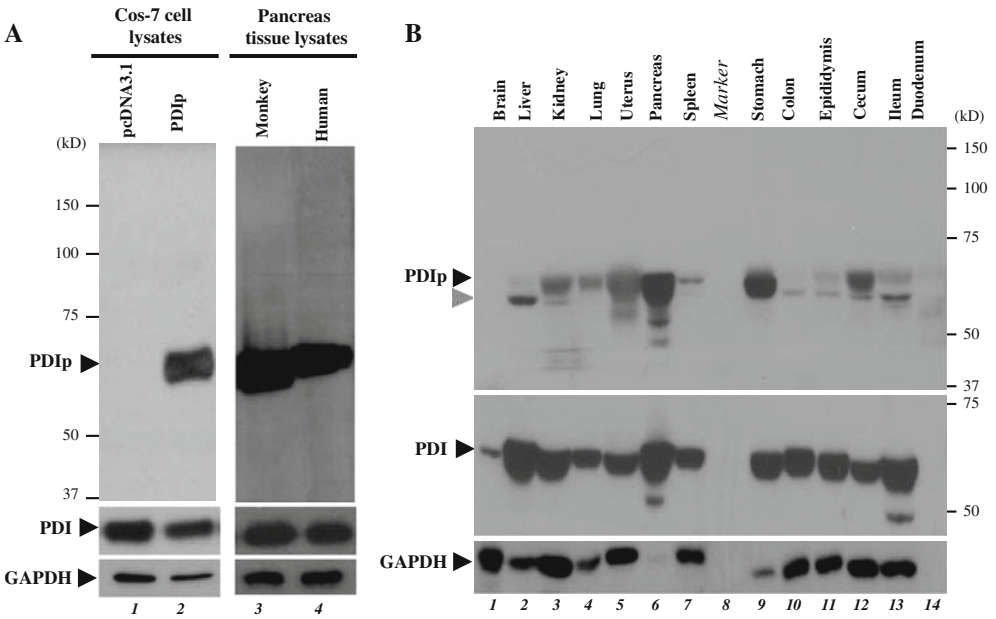

Fig. 1 Comparison of PDIp and PDI in their protein expression levels in different mouse tissues by Western blotting. **a** Cos-7 cells were transfected with pcDNA3.1 (*lane 1*) or pnDNA-PDIp plasmid (*lane 2*) for 36 h before cell lysis and Western blotting analysis. Pancreatic tissues were homogenized and then subjected to Western blotting analysis. **b** The mouse tissue proteins (*lanes 1–14*) were analyzed, and 5 μg protein was loaded in each lane. *Lane 8* represents the protein marker for molecular size calibration

Cellular localization of PDIp in various mouse tissues

Pancreas, stomach, and GI tract

To determine the cell type-specific distribution of PDIp in various mouse tissues, immunohistochemical analysis was performed. Firstly, we probed the cellular expression pattern of PDIp in the pancreas. Overall, PDIp was found to be predominantly expressed in pancreatic acinar cells but little or no staining was found in pancreatic islet cells (Fig. 2b–d). This observation is in agreement with previous reports (Desilva et al. 1997; Dias-Gunasekara et al. 2005). Notably, at a high magnification (630×, oil lens), the subcellular distribution of PDIp in pancreatic acinar cells showed a clearly dotted pattern (Fig. 2e–g). A similar pattern of subcellular distribution was also seen in normal human pancreatic acinar cells (Fig. 2h).

Next, we determined the cellular expression pattern of PDIp in stomach and ileum (Fig. 3). In the stomach, PDIp was selectively expressed in mucosal cells, but not in cells in submucosal and muscle layers (Fig. 3b–d). It appeared that the PDIp expression level was highest in the fundus of mucosa and became markedly lower in the apical part. Notably, at a high magnification (630×), the distribution of PDIp in mucosa cells also showed a dotted pattern (Fig. 3e, f), as seen in pancreatic acinar cells. Some of the stained cells appeared to be chief cells (as indicated with arrows) that secrete pepsinogen, when their morphological features were compared with those observed in an earlier study (Xie et al. 2005). Additional slides showing the staining of chief cells were presented in Fig. S4. In ileum, PDIp was only detected in a single layer of epithelial cells (Fig. 3h, I). Based on the morphological characteristics seen at a higher magnification (Fig. 3j, k), these stained cells appeared to be at the fundus of the intestinal crypts, and the staining likely was associated with the Paneth cells when comparing with other report (Kelly et al. 2004). Paneth cells secrete a number of unique molecules (e.g., alpha-defensins, lysozyme, and phospholipase A2) into the lumen of the crypt following exposure to bacteria or bacterial antigens. Such cell type-specific expression pattern of PDIp was not observed in other segments of the small intestine (e.g., duodenum and jejunum, data not shown).

Other tissues

The expression of PDIp in various segments of the gastrointestinal mucosa prompted use to more systematically analyze its cellular distribution in other tissues as well (e.g., adrenal glands, epididymis, liver, lung, heart, kidney, and thymus). Of the tissues analyzed, cell type-specific expression of PDIp was also detected in the adrenal glands, epididymis, and prostate (shown in Fig. 4). In the adrenal gland, PDIp was expressed in cells of the zona fasciculate of adrenal cortex as well as adrenal medulla (Fig. 4a), which are responsible for secreting steroid hormones and epinephrine, respectively (Young et al. 2006). In epididymis, PDIp was expressed in pseudostratified epithelium cells (Fig. 4b), which are known

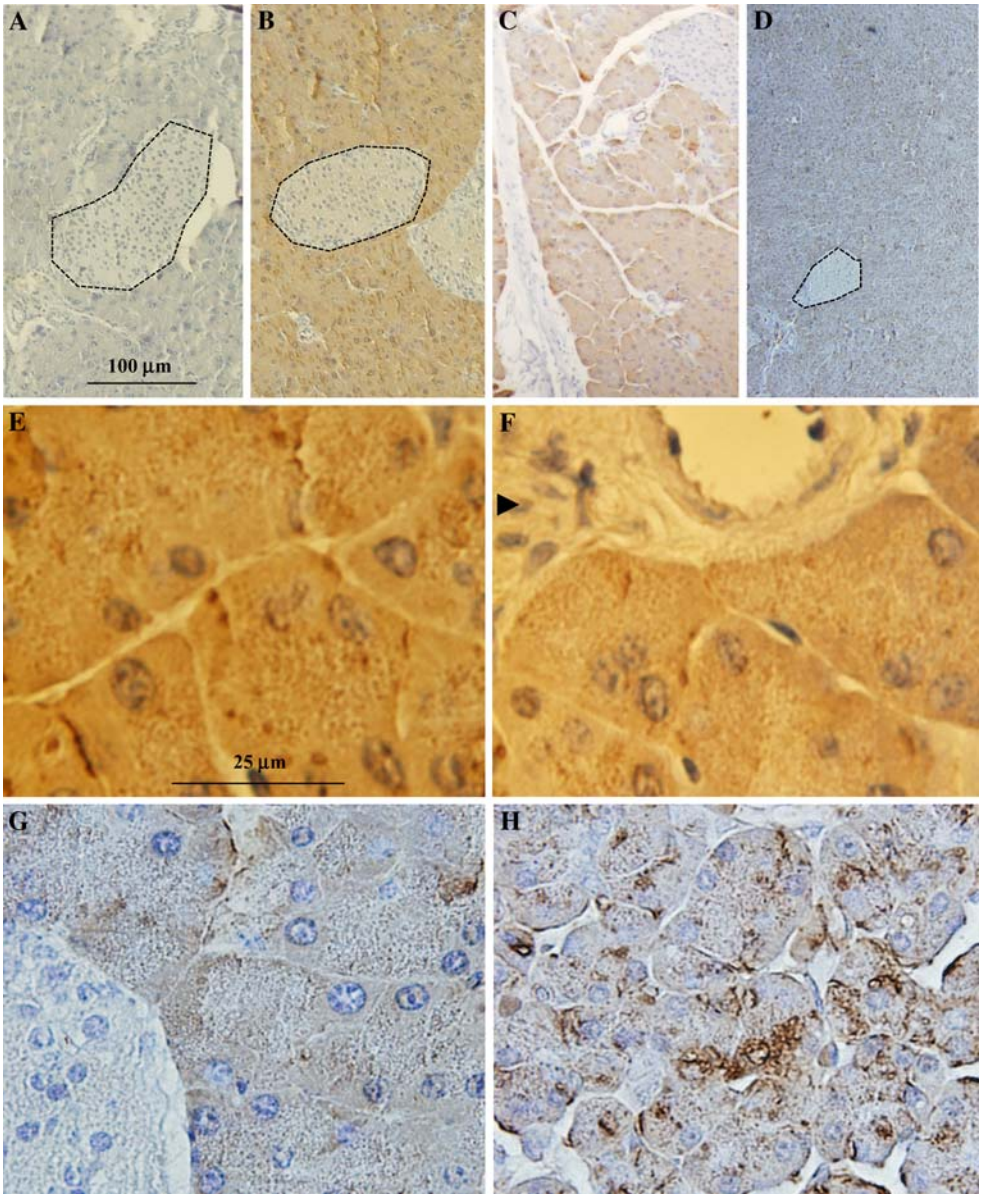

Fig. 2 Selective expression of PDIp in pancreatic acinar cells. Representative images (×100) for the immunohistochemical staining of PDIp in mouse pancreas (**b**, **c**, **d**) from different experiments. Slide **a** was probed with the normal mouse serum, and used as a negative control. Slides **e**, **f,** and **g** show the high magnification images (×630), and the islet cells (typically indicated by *circles* in Slide **a**, **b**, **d**) with little or no staining are included in slides **f** and **g**. Slide **h** is a representative image for the immunohistochemical staining of PDIp protein in normal human pancreatic tissue

to be involved in secretion of certain proteins (Young et al. 2006). In prostate, PDIp was not detected in stroma cells but selectively detected in secretory epithelial cells (Fig. 4c), which are known to secrete proteolytic enzymes, such as fibrinolysins (Young et al. 2006).

Absence of PDIp expression in human pancreatic cancer tissues and cell lines

PDIp expression was not detected in a number of human pancreatic cancer cell lines examined (Fig. 5a), although PDI was found to be constitutively expressed in all of these cell lines (Fig. 5a). This observation prompted us to also examine PDIp in a human pancreatic tissue array to further determine whether its expression was altered during the development of pancreatic cancer. The human pancreatic tissue array analyzed in this study included 18 cases of duct adenocarcinoma, 4 cases of islet carcinoma, 1 case of acinar cell carcinoma, and 2 cases of normal pancreatic tissues. The results for all cancer specimens are shown in Fig. S1, and some of the representative results are shown in Fig. 5b. We found that PDIp, which was highly expressed

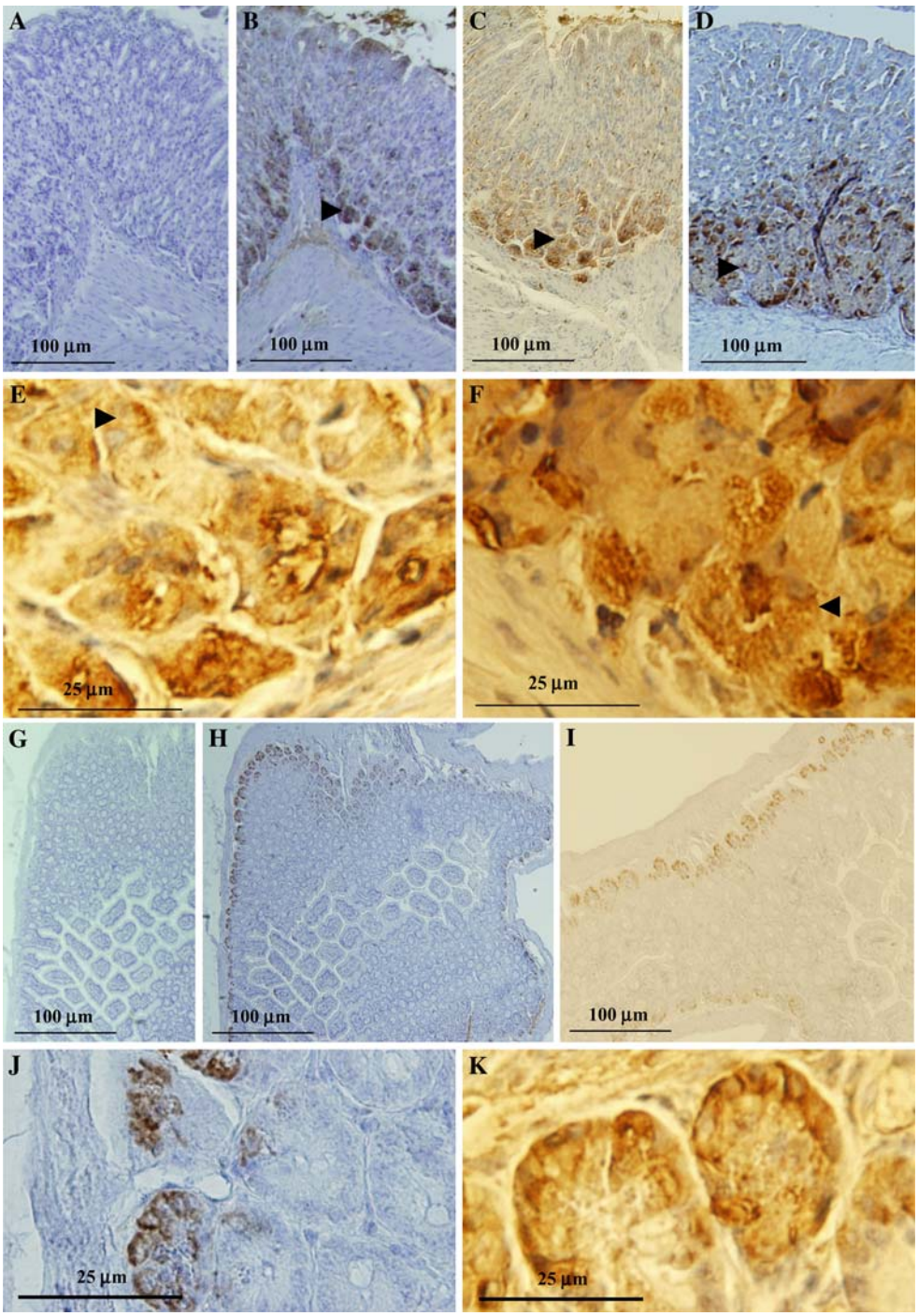

Fig. 3 Cell-specific expression of PDIp in mouse stomach and ileum. Representative images (×100) of the immunohistochemical staining of PDIp in the mouse stomach (**b**, **c**, **d**) from multiple experiments. Slides **a** was probed with the normal mouse serum, and used as a negative control. Slides **e** and **f** show the higher magnification images (×630). The chief cells were indicated by the *arrows*. Slides **h**, **i**, **j,** and **k** show the representative results for the immunohistochemical staining of PDIp in mouse ileum at ×100 magnification (**h** and **i**) and ×630 magnification (**j** and **k**). Slides **g** was probed with the normal mouse serum, and used as a negative control

in normal pancreatic tissue (slide 1 in Fig. 5b, or slides H4–H9 in Fig. S1), was not detected in 17 of the 18 duct adenocarcinoma samples (pathological grade I, II, and III, as represented by slides 2, 3, 4 in Fig. 5b, respectively; see Fig. S1 and Table S1 for details). In comparison, PDIp was still abundantly expressed in normal tissues adjacent to the adenocarcinoma (represented by slide 5 in Fig. 5b, or see slides B7, B8, B9, and F3 in Fig. S1). These data implied

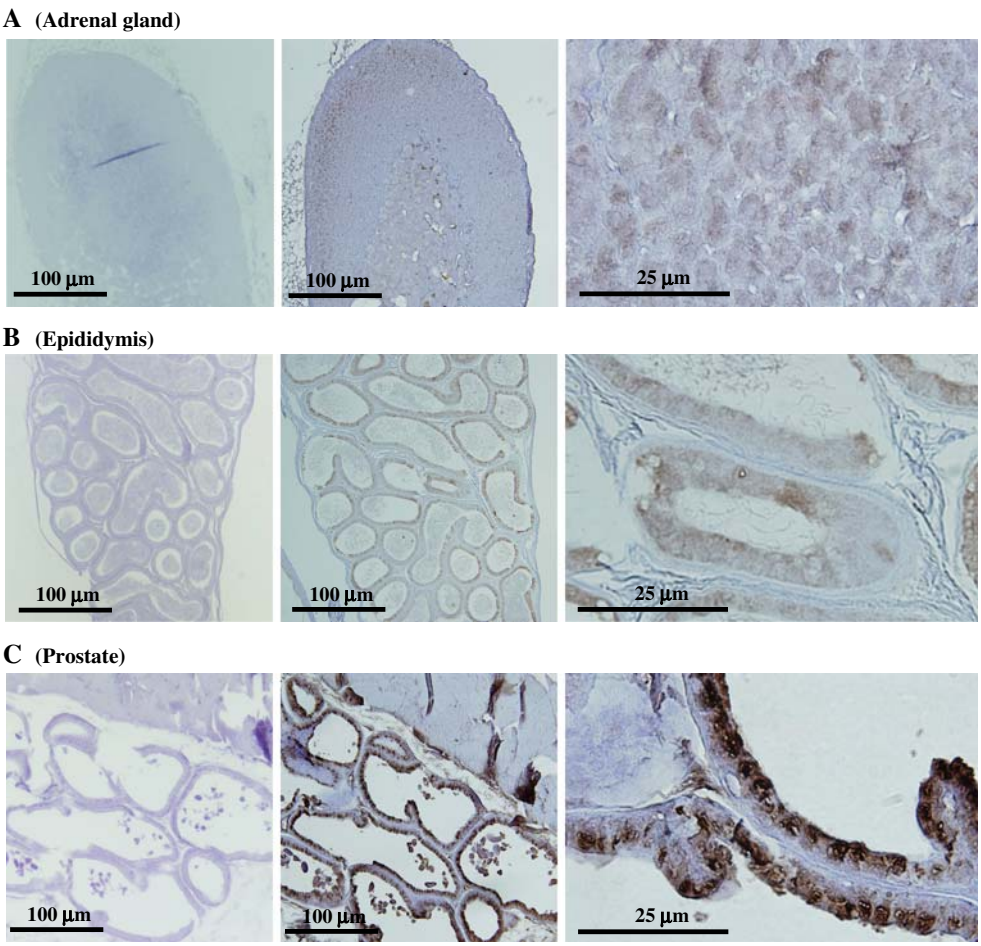

Fig. 4 Cell type-specific expression of PDIp in several mouse tissues. The sections of different mouse tissues were probed with the anti-PDIp antiserum. The positively stained cells were photographed under ×100 magnification (*middle part* for each tissue) and ×630 magnification (*right part* for each tissue). The *left part* for each tissue was probed with the normal mouse serum, and used as a negative control

that the disappearance of PDIp was associated with the development of pancreatic duct adenocarcinoma. Notably, there was a detectable expression of PDIp in one of the eighteen adenocarcinoma samples (slide E4 in Fig. S2, and slide E6 in Fig. S3). The positively stained cells may be the adjacent normal cells based on their morphological characteristics seen at a higher magnification (Fig. S2B, S2C, S2D, S3B, S3C). In addition, little or no PDIp expression was detected in islet cell carcinoma (slide 6 in Fig. 5b or slides G1–G9 in Fig. S1), and it was also not detected in acinar cell carcinoma (slide 7 in Fig. 5b or slides H1, H2, and H3 in Fig. S1).

Subcellular localization of PDIp in cultured mammalian cells

Our recent study (Fu and Zhu 2009a) showed that the over-expressed PDIp carrying a full signal peptide targeting to the ER was predominantly present in the microsomal fraction from the cultured mammalian cells, suggesting its potential localization in the ER. To validate its subcellular localization, we over-expressed PDIp in cos-7 cells and then determined its subcellular localization by using immunocytochemical staining. As shown in Fig. 6a, PDIp was not localized in the nuclei but was exclusively present in the cytoplasmic compartment, particularly in the perinuclear region. To further confirm its localization in the endoplasmic reticulum, we performed double staining of PDIp with calnexin, which was a commonly used endoplasmic reticulum-localized marker protein (Bergeron et al. 1994; Schultz-Norton et al. 2006). As shown in Fig. 6b, PDIp was co-localized with the endogenous calnexin.

To further demonstrate the role of the PDIp signal peptide in its subcellular localization, we conducted subcellular fractionation study by analyzing PDIp distribution when compared with representative marker proteins. The cos-7 cells that over-expressed the PDIp proteins with or without the ER-targeting signal peptide (first 21 amino acids of PDIp) were subjected to fractionation to separate the nuclear, cytosolic, and microsomal fractions. As shown in Fig. 6c, both forms of the PDIp protein appeared to predominantly co-exist with PDI and calnexin in the cytosolic fraction (lanes 1-2 vs. lanes 3-4) but not in the nuclear fraction. The TATA-binding protein (TBP), a commonly

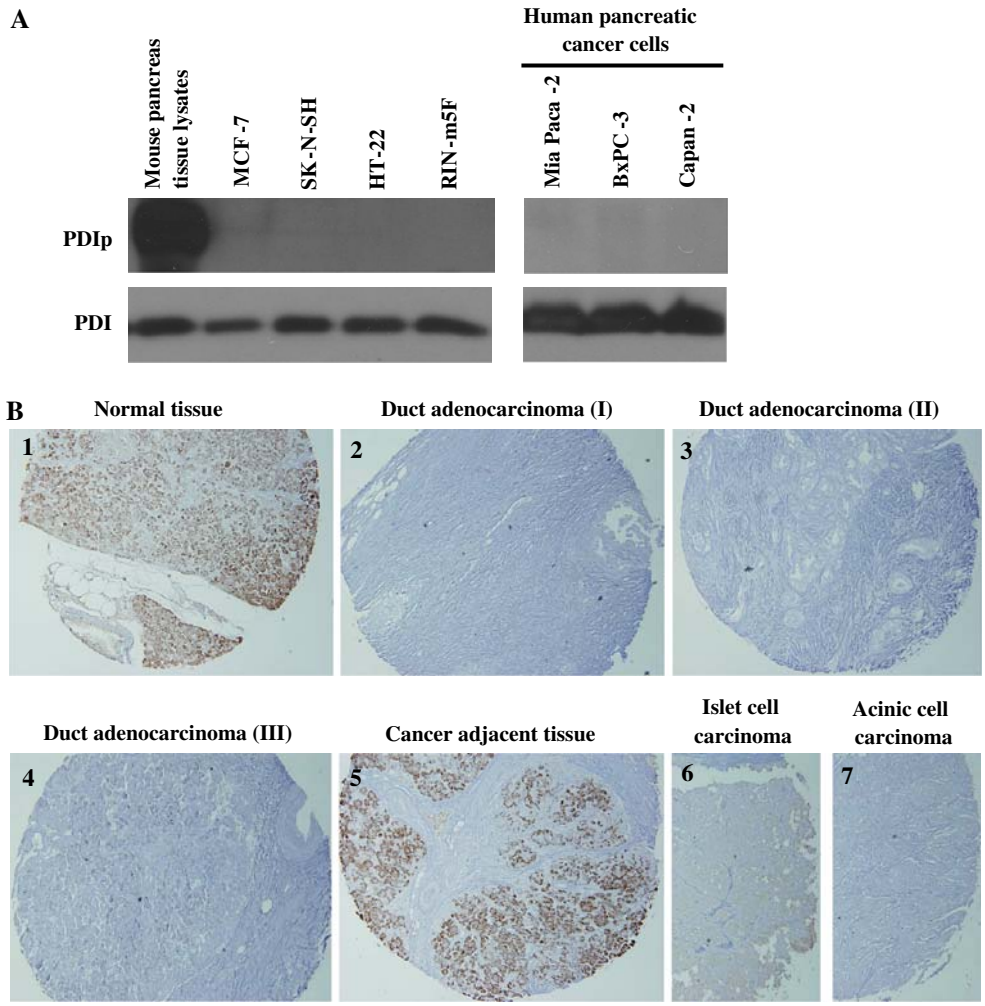

Fig. 5 Loss of PDIp expression in human pancreatic cancer cells and duct adenocarcinoma. **a** Western blotting analysis of PDI and PDIp protein levels in several cancer cell lines (*right part*). Five microgram proteins for each well were loaded. **b** Representative results for the immunohistochemical staining of PDIp in a human pancreas tissue array, including the normal tissue (**1**), duct adenocarcinoma (**2**, **3**, **4**), islet cell carcinoma (**6**), acinar cell carcinoma (**7**), and cancer adjacent tissue in a duct adenocarcinoma (**5**). For details, also see Fig. S1 and Table S1

used nuclear loading control (Rosati et al. 2009), was predominantly detected in the nuclear fraction although it was also marginally detected in the cytosolic fraction, suggesting that nuclear proteins were mostly separated from the cytosolic proteins although the separation was incomplete. Further analysis of the microsomes containing the ER membrane fraction showed that the PDIp protein containing the ER-targeting signal peptide co-existed with PDI and calnexin (lane 6 in Fig. 6c). By contrast, much lower protein levels were detected for the PDIp protein without the ER-targeting signal peptide (lane 5 in Fig. 6c), suggesting that the signal peptide helped guide PDIp into the ER. In addition, it appeared that PDIp without the signal peptide had a lower mobility than the form carrying the signal peptide (lane 1 vs. lane 2, or lane 5 vs. lane 6). Given the fact that PDIp is known to be glycosylated in vivo (Desilva et al. 1997; Klappa et al. 1998), this observation was expected because the over-expressed PDIp carrying a signal peptide would be glycosylated in ER whereas the form without a signal peptide would not be glycosylated due to its localization in the cytosol.

Discussion

The results of our present study showed that besides pancreas, PDIp was also expressed in several other tissues, including the stomach, ileum, adrenal glands, prostate, and epididymis. The expression of PDIp in these tissues was cell type-specific (Figs. 2, 3, 4). In particular, PDIp protein displayed a dotted pattern of distribution in various digestive enzyme-producing cells, such as the acinar cells of the

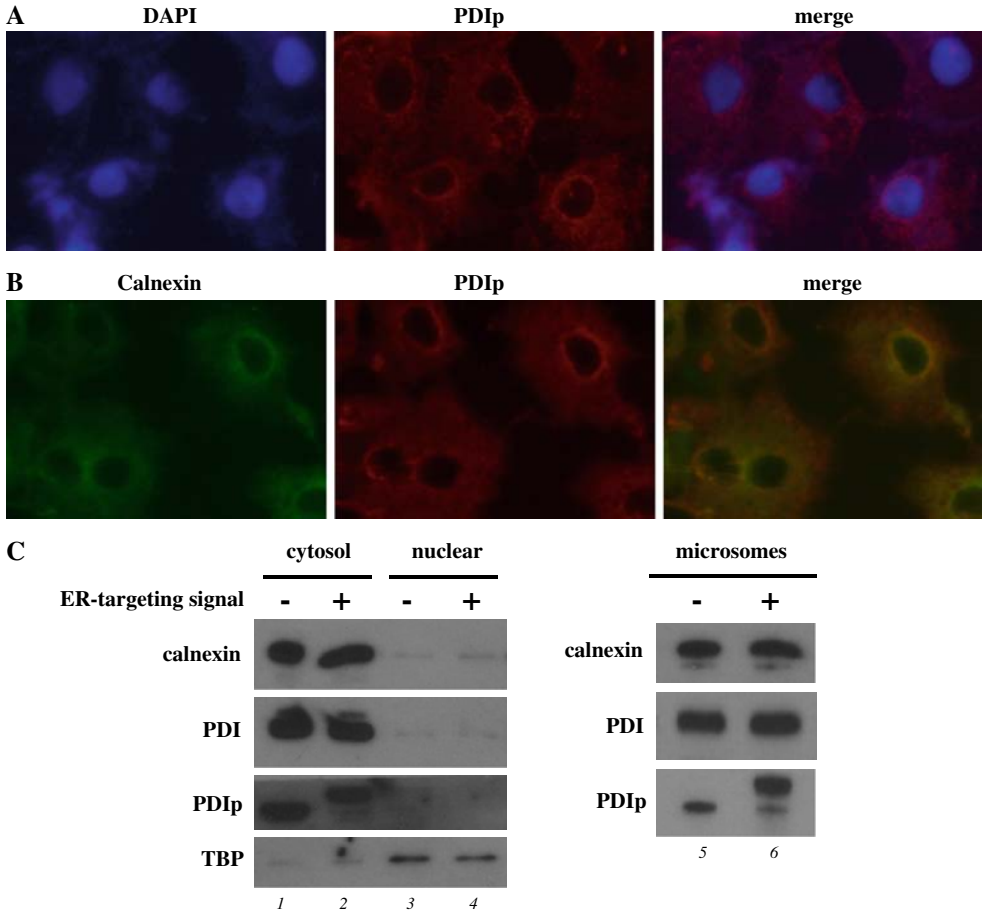

Fig. 6 Subcellular localization of PDIp in cos-7 cells. Cos-7 cells were transfected with a PDIp expression vector for 36 h before it was subjected to analysis. **a** Double immunocytochemical staining of the nuclei (left part, shown by the DNA-binding dye DAPI) and PDIp (middle part) in cos-7 cells. **b** Double immunocytochemical staining of calnexin (left part) and PDIp (middle part) in cos-7 cells. Calnexin is a commonly used marker protein for ER (Bergeron et al. 1994; Schultz-Norton et al. 2006). **c** Western blotting of PDIp present in the nuclear, cytosolic, and microsomal subcellular fractions prepared from cos-7 cells over-expressing PDIp with an ER-targeting signal peptide (*lanes 2*, *4*, *6*) or without an ER-targeting signal peptide (*lanes 1*, *3*, *5*). PDI and calnexin were analyzed for comparison. TBP was used as the nuclear loading control (Rosati et al. 2009)

pancreas (Fig. 2e, f, g, h) and chief cells of the stomach (Fig. 3e, f). In addition, PDIp protein was also present in a dotted pattern in Paneth cells of the ileum (Fig. 3h, i, j, k), a group of cells that are known to specifically secrete anti-bacterial alpha-defensins, lysozyme, and phospholipase A2 (Ghoos and Vantrappen 1971; Harwig et al. 1995; Wilson et al. 1999; Ayabe et al. 2000). Taken together, these observations suggest the possibility that PDIp protein may be physically associated with storage vesicles for these secretory proteins in these cells.

PDIp has both disulfide isomerase activity (Desilva et al. 1996; Fu and Zhu 2009a, b) and also chaperone activity (Elliott et al. 1998; Klappa et al. 1998; Fu and Zhu 2009b). Earlier studies have shown that PDIp could interact with newly synthesized peptides in the in vitro translation system (Elliott et al. 1998) or with misfolded proteins (Desilva et al. 1996; Klappa et al. 1998). It has been suggested that one of the physiological functions of PDIp is related to the production and secretion of digestive enzymes (Klappa et al. 1998; Freedman et al. 2002). This suggestion was supported by our observations that PDIp was selectively expressed in various types of cells in the pancreas, stomach, and ileum that are capable of producing secretory enzymes. It is possible that PDIp may have highly specified functions in assisting the proper folding of zymogens (the inactive precursor of some digestive enzymes) to keep them in the inactive state while they are inside the cells.

It is also of interest to note that PDIp was found to be highly and selectively expressed in Paneth cells of the ileum. Paneth cells are known to specifically secrete alpha-defensins, lysozyme, and phospholipase A2 that have antimicrobial activity against a wide range of bacterial cells (Ghoos and Vantrappen 1971; Harwig et al. 1995; Wilson et al. 1999; Ayabe et al. 2000). These secretory

molecules contain multiple intra-subunit disulfide bonds that are critical for their functions (Yao et al. 1997; Szyk et al. 2006). It is tempting to suggest that PDIp may also be involved in protecting the host against bacterial infection via assisting the proper folding of antimicrobial secretory molecules in Paneth cells of the ileum.

PDIp was found to be highly expressed in normal human pancreatic tissues but its expression was mostly absent in human pancreatic carcinoma or pancreatic cancer cell lines (Fig. 5). Although the mechanism for the loss of PDIp expression during the development of pancreatic adenocarcinoma is unclear at present, this observation suggests that PDIp may be considered as an additional diagnostic biomarker for human pancreatic cancer, along with other biomarkers that were recently developed (Goggins 2005, 2007). It is suspected that PDIp may also be absent in certain cancerous issues of the stomach, as the case in the pancreatic adenocarcinoma.

In addition to serving as a disulfide isomerase and a chaperone protein, it was recently shown that PDIp, like PDI, is an intracellular estrogen-binding protein and can accumulate estrogens in live cells in culture and also in pancreatic tissues (Fu et al. 2008; Fu and Zhu 2009a). The PDIp-bound estrogen can help augment estrogen receptor-mediated transcriptional activity (Fu and Zhu 2009a). Since pancreas (Morales-Miranda et al. 2007), stomach (Sakata et al. 2006), adrenal glands (Young et al. 2006), prostate (Ellem and Risbridger 2007), and epididymis (Parlevliet et al. 2006; Pearl et al. 2007) are all considered to be estrogen-sensitive organs, the expression of PDIp in these organs suggests that it may function as a modulator of the estrogenic activity at these target sites. In this context, it is also of interest to suggest that the very high levels of PDIp expressed in estrogen-producing cells in the zona fasciculate of the adrenal cortex may serve as an intracellular protein for binding and storing estrogens.

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