An Oligopeptide Transporter Is Expressed at High Levels in the Pancreatic Carcinoma Cell Lines AsPc-1 and Capan-2

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ABSTRACT

Carcinomas of the exocrine pancreas are poorly understood and have a poor prognosis because of their highly malignant nature. Using two human pancreatic cancer cell lines, AsPc-1 and Capan-2, we have investigated avenues that might be useful in targeting the delivery of antineoplastic agents to such cancers. Qualitative RNA PCRs established the presence of the oligopeptide transporter PEPT 1 in these pancreatic cancer cell lines. Northern analysis confirmed the presence of a 3.3-kb transcript. The transporter is normally expressed primarily in small intestinal epithelial cells for nutrient absorption. It is also expressed in a human intestinal cell line, Caco-2. High levels of PEPT 1 protein expression in AsPc-1 and Capan-2, as multiple glycosylated forms (M Mr ~90,000–120,000), were confirmed by Western immunoblotting, when compared with Caco-2 cell cultures. Absorption of the model dipeptide glycyl-L-sarcosine by AsPc-1 and Capan-2 cells was similar to glycyl-I-sarcosine absorption by Caco-2 cells and a Chinese hamster ovary cell line expressing human PEPT 1 (CHO-PEPT 1). Uptake was pH-dependent and inhibited by several di/tripeptides and bestatin, but it remained unaffected by glycine and tetraglycine. Peptide solute transport by AsPc-1 and Capan-2 cells exhibited binding affinities (Kms) similar to those previously reported for PEPT 1, whereas the transport maximal velocity (Vmax) of the AsPc-1 cells was much greater than those of the Capan-2 and Caco-2 cells. Immunomicroscopy demonstrated PEPT 1 protein localized at the plasma membrane and in intracellular vesicular structures, similar to that observed for Caco-2 and CHO-PEPT 1 cells. These data suggest that the pancreatic cancer cell lines AsPc-1 and Capan-2 express surprisingly high levels of a solute transporter that was previously thought to be restricted in function to the absorption of nutrients from the small intestine.

INTRODUCTION

Pancreatic cancer is the fifth most common cause of cancer deaths in the United States (1). Current estimates suggest that most pancreatic cancers originate from epithelial duct cells and that 80% of those cancers have a growth-permissive point mutation in the Ki-ras protooncogene (2, 3). It has also been suggested that activation of the Ki-ras gene and an inactivation of the p53 gene are strongly and cooperatively associated with pancreatic carcinogenesis (4, 5). A number of pancreatic cancer cell lines derived from primary and metastatic tumors have been used as model systems for the in vitro study of this disease. We have obtained two such human malignant pancreatic cancer cell types, AsPc-1 and Capan-2, which are ductal in origin and demonstrate a mutant Ki-ras genotype (5). AsPc-1 and Capan-2 cells have been described as being derived from a moderately well-defined adenocarcinoma and an adenocarcinoma, respectively (6). Our focus has been to identify unique properties of pancreatic cancers, which might provide a method of targeting therapeutic agents.

The solute transporter, referred to as PEPT 1, is expressed predominantly in the duodenum and jejunum of the small intestine, where it functions in the absorption of oligopeptides from the lumen of the gut (7). Much lower levels of PEPT 1 appear to be expressed in the ileum of the small intestine (7). PEPT 1 mRNA has also been found in liver and pancreatic tissues, but at lower levels than that observed in the ileum of the small intestine (8). The protein expression of PEPT 1 in these tissues is uncertain because the liver is not known to absorb peptides from the circulation (9). A similar oligopeptide transporter, PEPT 2, functions in the kidney to reabsorb peptide solutes from glomerular filtrate (10), and both transporters exhibit a similarly broad range of solute selectivity. The PEPT 1 transporter has been suggested to provide the uptake pathway in the intestine for several classes of therapeutic agents besides oligopeptides: ß-lactam and cephalosporin antibiotics (11), angiotensin-converting enzyme inhibitors (12), renin inhibitors, and the ß-amino acid bestatin [(2S,3R)-3-amino-2-hydroxy-4-phenylbutanoyl]-L-leucine; Ref. 13]. Thus, the ability of the PEPT 1 transporter to accept a broad spectrum of substrates of oligopeptide size and structure has made the transporter an attractive candidate for drug delivery (14). In fact, an aspartic acid conjugate of N-phosphonacetic acid has been shown to be a promising adjunct therapy for patients with pancreatic cancer (15), and conjugation of either glutamic or valine with melphalan to form dipeptide-like structures has recently been shown to improve the uptake of cytotoxic agent into Caco-2 cells (16).

The human colon adenocarcinoma cell line, Caco-2, has served as a model for the study of intestinal drug absorption (17). This cell line expresses PEPT 1 at levels comparable to that of the ileum and transports oligopeptides with characteristics similar to those observed in the frog oocyte microinjection system expressing PEPT 1 (7). Recently, a stably transfected Chinese hamster ovary cell line that expresses high levels of the human form of PEPT 1 (CHO-PEPT 1) has been established (18). We have used a comparison of Caco-2 and CHO-PEPT 1 cells to evaluate the expression and function of PEPT 1 in the Ki-ras mutant pancreatic cancer cells AsPc-1 and Capan-2. Our observations, which demonstrate that these pancreatic cancer cell lines express functional PEPT 1 at surprisingly high levels, may have impact on future therapeutic strategies in the treatment of pancreatic cancer and pancreatic cancer-derived metastases.

MATERIALS AND METHODS

Materials. [14C]Gly-Sar3 (specific activity, 55 mCi/mmol) was custom synthesized by American Radiolabeled Chemicals, Inc. (St. Louis, MO). Gly-Sar, bestatin, I-phenylglycine, Gly-Gly-Gly, and Gly-Gly-Gly-Gly were purchased from Sigma Chemical Co. (St. Louis, MO). Gly-Pro was from Peninsula Laboratories (Belmont, CA). D-Phenyl-L-glutamic acid and PEPT 1-specific RT-PCR oligonucleotide primers were synthesized at Genentech, Inc. (South San Francisco, CA). All cells, except for the CHO-PEPT 1 cells, were obtained from American Type Culture Collection (Rockville, MD). An affinity-purified rabbit polyclonal antisemiserum recognizing human PEPT 1 was prepared by Quality Control Biochemical (Hopkinton, MA) from animals injected with a peptide corresponding to the last 18 amino acid residues of the protein (LEKSNYPFMSGANSQKQM). The mouse monoclonal antibody to

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3 The abbreviations used are: Gly-Sar, glycyl-L-sarcosine; RT-PCR, reverse transcription-PCR; CEA, carcinoembryonic antigen; G3PDH, glyceraldehyde 3-phosphate dehydrogenase.
human CEA was from Zymed Laboratories (South San Francisco, CA). Cy3- and Cy5-labeled secondary antibodies were from Amersham (Arlington Heights, IL). [3H]Iodinated peptides were purchased from New England Nuclear (Boston, MA). G3PDH probe DNA was purchased from Clontech (Palo Alto, CA). The pBluescript vector containing the human PEPT I 2.2-kb cDNA was provided by Dr. F. H. Leibach (Dept. of Biochemistry and Molecular Biology, Medical College of Georgia, Augusta, GA).

Cell Culture. Capan-2 and AsPC-1 cells were grown in McCoy's 5A containing 10% heat-inactivated fetal bovine serum, 2 mM glutamine, and 100 units/ml penicillin-100 mg/ml streptomycin mixture. Caco-2 cells were grown in DMEM supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine, and 100 units/ml penicillin-100 µg/ml streptomycin mixture and nonessential amino acids. Chinese hamster ovary cells, CHO-K1, were grown in Ham's F12 supplemented with 10% fetal bovine serum, 100 units/ml penicillin-100 µg/ml streptomycin, and 2 mM glutamine. CHO-PEPT I cells were also grown in similarly supplemented Ham's F12, but with the addition of 300 µg/ml geneticin (Life Technologies, Inc., Gaithersburg, MD). All cells were cultured at 37°C in a humidified atmosphere with 5% CO2.

RNA PCR (RT-PCR). Poly(A)+ RNA samples from CHO-K1, CHO-PEPT I, Caco-2, AsPC-1, and Capan-2 cells were subjected to RT-PCR using the GeneAmp RNA-PCR kit (PE Applied Biosystems, Foster City, CA). The primers used were specific for PEPT I and corresponded to bases 717-736 and 1791-1810 of the published sequence of a human intestinal oligopeptide transporter (PEPT I). The RT-PCR products were analyzed by agarose gel electrophoresis.

Northern Analysis. Poly(A)+ RNA was extracted from cells using the FastTrack mRNA Isolation Kit (Invitrogen, Carlsbad, CA). The EcoRV-Norl-2.2-kb cDNA fragment excised from the plBluescript vector (8) and the G3PDH probe DNA were 32P-labeled using Ready-to-Go dCTP DNA Labeling Beads (Pharmacia Biotech, Piscataway, NJ). RNA samples (4 µg) were fractionated on a 1.2% agarose-2.2 M formaldehyde gel. The mRNA was then transferred to a nylon membrane (Zeta-Probe GT; Bio-Rad, Richmond, CA) by capillary action and probed under low-stringency conditions. Briefly, the blots were incubated at 42°C overnight with the 32P-labeled cDNA probe in hybridization buffer consisting of 0.9 M NaCl, 0.09 M sodium citrate (pH 7.0), 0.5% (v/v) SDS, 0.1% (w/v) Ficoll, 0.1% (w/v) polyvinylpyrrolidone, 0.1% (w/v) BSA, 100 µg/ml sheared, denatured salmon sperm DNA, and 40% formamide (Life Technologies, Inc.). The blot was washed twice (20 min/wash) in buffer consisting of 0.3 M NaCl, 0.03 M sodium citrate (pH 7.0), 0.1% SDS, 1.5 mM sodium citrate, at room temperature. The blot was stripped and reprobed with a human G3PDH probe under similar conditions to assess RNA loading and transfer efficiency. High-stringency Northern analysis was also performed on the stripped blots. After probing as above with previously mentioned probes, blots were washed three times at 65°C in buffer composed of 0.015 M NaCl and 0.1% sodium citrate (pH 7.0), and 0.1% SDS. Autoradiograms of the high-stringency results and short exposure times were used in the densitometric analysis to compare expression levels.

Western Analysis. Membrane-enriched fractions of individual cell types were prepared as described previously (17). The protein concentration of each membrane fraction was determined by Bradford assay (Bio-Rad). Membrane fractions (40 µg protein) were separated on an 8% SDS-PAGE and electroblotted onto a polyvinylidene fluoride membrane (Bio-Rad). Blots were probed under low-stringency conditions. Briefly, the blots were incubated at 42°C overnight with the 32P-labeled cDNA probe in hybridization buffer consisting of 0.9 M NaCl, 0.09 M sodium citrate (pH 7.0), 0.5% (v/v) SDS, 0.1% (w/v) Ficoll, 0.1% (w/v) polyvinylpyrrolidone, 0.1% (w/v) BSA, 100 µg/ml sheared, denatured salmon sperm DNA, and 40% formamide (Life Technologies, Inc.). The blot was washed twice (20 min/wash) in buffer consisting of 0.3 M NaCl, 0.03 M sodium citrate (pH 7.0), 0.1% SDS, 1.5 mM sodium citrate, at room temperature. The blot was stripped and reprobed with a human G3PDH probe under similar conditions to assess RNA loading and transfer efficiency. High-stringency Northern analysis was also performed on the stripped blots. After probing as above with previously mentioned probes, blots were washed three times at 65°C in buffer composed of 0.015 M NaCl and 0.1% sodium citrate (pH 7.0), and 0.1% SDS. Autoradiograms of the high-stringency results and short exposure times were used in the densitometric analysis to compare expression levels.

RESULTS

RT-PCR was performed on poly(A)+ RNA isolated from the two pancreatic cancer cell lines (AsPC-1 and Capan-2), the colonie cancer cell line with a small intestinal phenotype (Caco-2), a Chinese hamster ovary cell line stably transfected with human PEPT I cDNA (CHO-PEPT I), and the untransfected Chinese hamster ovary cell line (CHO-K1) as a negative control. Agarose gel electrophoresis of the RT-PCR products (Fig. 1A) demonstrated the presence of a PCR product of PEPT 1 (hPepT1) was 1094 bp. Lane 5. positive control for the PCR. B, samples of poly(A)+ RNA (4 µg) isolated from CHO-K1, CHO-PEPT I, AsPC-1, Capan-2, and Caco-2 cells (corresponding to Lanes 1–5) were subjected to RT-PCR using primers specific for human PEPT I. The RT-PCR products were analyzed by agarose gel electrophoresis. The expected size of the product of PEPT I (hPepT1) was 1094 bp. Lane 6, positive control for the PCR. C, samples of poly(A)+ RNA (4 µg) isolated from CHO-K1, CHO-PEPT I, AsPC-1, Capan-2, and Caco-2 cells (corresponding to Lanes 1–5) were size-fractionated, transferred to a nylon membrane, and hybridized with a 2.2-kb 32P-labeled human PEPT I cDNA probe. The expected transcript of 3.3 kb was seen in AsPC-1, Capan-2, and Caco-2 cell preparations (top). A transcript of smaller size was detected in CHO-PEPT I cells, consistent with the size of the cDNA used in the stable transfection of these cells. The membrane was stripped and reprobed with a human 32P-labeled G3PDH probe to assess loading and transfer efficiency (bottom). The results shown are those obtained under conditions of low stringency. Semiquantitative determinations were performed with blots prepared using high stringency (see text).
product of the expected size (~1094 bp) in the reactions with RNA from AsPc-1, Capan-2, Caco-2, and CHO-PEPT 1 but not with RNA from CHO-K1. These results suggested that, qualitatively, these two pancreatic cancer cell lines contain mRNA for PEPT 1.

A semiquantitative determination of the mRNA levels in these cell types was performed using equal loadings (4 𝜇g) of total poly(A)+ RNA (Fig. 1B). Hybridization with a radiolabeled 2.2-kb human PEPT 1 cDNA probe demonstrated a greater expression level of PEPT 1 in AsPc-1 and Capan-2 cells than in the intestinal cell line Caco-2. Densitometry measurements were made on the results (data not shown) obtained under high-stringency wash conditions (normalized to the G3PDH loading standard). These suggest that AsPc-1 and Capan-2 cells express PEPT 1 levels approximately 5 and 2.6 times, respectively, greater than Caco-2 cells. These levels do not, however, take into consideration that G3PDH expression levels may vary among different cell types and, as such, are merely approximations. The 3.3-kb transcript identified with our probe is the expected size for the human PEPT 1 message (8). A minor transcript of about 7.2 kb was also observed in all three of these cell types. CHO-PEPT 1 cells were similarly examined as a positive control for the oligopeptide transporter, and a transcript of approximately 2.8 kb was identified. The apparent size discrepancy between transcripts from the CHO-PEPT 1 and the other three cell lines is likely due to the fact that the human cDNA used to transfect the CHO-PEPT 1 cells has a truncated 3′ noncoding region (8). As expected, Northern analysis of the control cells CHO-K1 did not result in any hybridization.

Because a number of mechanisms are known to control mRNA transcription, protein translation, and protein stability, elevated mRNA expression levels are not necessarily indicative of correspondingly high levels of protein expression. To assess protein expression levels, Western immunoblot analysis of cell membrane lysates were performed using polyclonal antibodies that recognized the COOH-terminal region of human PEPT 1. These blots confirmed that AsPc-1 and Capan-2 cells do, indeed, express PEPT 1 at high levels (Fig. 2) but not to the same extent as suggested by the Northern blot data. Human PEPT 1 cDNA has an open reading frame of 2127 bp and encodes a protein of 708 amino acids (8). Based upon the encoded amino acid sequence, human PEPT 1 should have a core molecular size of 𝑀 manoeur 78,810. In addition, it also contains seven putative N-linked glycosylation sites. Recognition of the COOH-terminal region of PEPT 1 in Western immunoblots, therefore, provided the opportunity for an analysis of various glycosylated forms of PEPT 1, and the detection of multiple bands greater than 𝑀 manoeur 78,000 was not unexpected. AsPc-1, Capan-2, and Caco-2 cells all exhibited similar band patterns, with prominent forms having apparent 𝑀 manoeur s of approximately 88,000, 93,000, 106,000, 110,000, 120,000, and 128,000. In a previous study, in which the intestinal transporter was functionally expressed in frog oocytes, photoaffinity labeling of membrane proteins with a photoreactive β-lactam antibiotic identified a protein of 𝑀 manoeur ~130,000 (14). The band pattern for the CHO-PEPT 1 preparation was slightly different and may reflect differences in the glycosylation pathways of hamster versus human epithelial cells. All bands shown, except for the minor band at 𝑀 manoeur ~144,000 were blocked completely when the polyclonal antibody was preincubated with the immunization peptide prior to analysis (data not shown). Densitometric analysis of Western immunoblots showed 2.2- and 1.5-fold increases in levels of protein expression in AsPc-1 and Capan-2 cells, respectively, compared with Caco-2 cells. This crude measurement, however, does not take into account any potential differences in the efficiency of transfer for the various glycosylated forms of PEPT 1 and the possibility that these various forms interact differently with the polyclonal antibody used in our studies.

To determine whether the PEPT 1 protein expressed by AsPc-1 and Capan-2 cells was functional at the plasma membrane of these pancreatic cancer cells, we studied several parameters of oligopeptide transport. The time course for the accumulation of Gly-Sar in AsPc-1
and Capan-2 cells was measured. As the data in Fig. 3 indicates, uptake was found to be linear over the 30-min incubation period in both cell types. The incubation times for all subsequent experiments were chosen to be within the linear range of uptake. The uptake of compounds by PEPT 1 has been shown to be proton dependent and specific to di/tripeptides and structurally similar compounds (7, 8, 20). The uptake of $[^{14}C]$Gly-Sar by both AsPc-1 and Capan-2 cells was blocked by competing unlabeled Gly-Sar and was significantly greater at acidic pH than it was at neutral pH (Fig. 4). At pH 7.4, peptide uptake was decreased in Capan-2 and AsPc-1 cells by 52%, indicating a requirement for a proton gradient. The pH-dependent uptake profiles of these two pancreatic cancer cells were very similar to that observed previously with Caco-2 cells (21, 22), frog oocytes expressing functional human PEPT 1 (8), and CHO-PEPT 1 cells (18). The uptake specificities of AsPc-1 and Capan-2 cells, as assessed by determining the inhibition of $[^{14}C]$Gly-Sar in the presence of competing peptides, were similar for the two cell types (Table 1) and comparable to previously published values for human PEPT 1 (22). The transporter in both cell types was specific for di/tripeptides. The amino acid glycine and the tetrapeptide Gly-Gly-Gly-Gly, at a competing concentration of 20 mM, had minimal inhibitory effects on Gly-Sar uptake, whereas dipeptides, tripeptides, and bestatin were very potent inhibitors.

To determine the kinetic parameters of the oligopeptide transport system in AsPc-1 and Capan-2 cells, the dependence of Gly-Sar concentration on uptake rate was investigated. The relationship between concentration and uptake rate was hyperbolic, indicating saturability of the transport systems in AsPc-1 (Fig. 5A) and Capan-2 cells (Fig. 5B). Michaelis-Menten kinetic parameters for rapid equilibrium at a single class of sites were calculated by fitting the data to a one-site binding model using GraphPad Prism 2.0 for Macintosh using nonlinear regression (GraphPad Software Inc., San Diego, CA). The AsPc-1 transport system exhibited a $K_m$ of 0.80 ± 0.17 mM and a $V_{max}$ of 65 ± 4.4 nmol/mg protein/10 min, whereas the transport system in Capan-2 cells had a $K_m$ of 1.0 ± 0.2 mM and a $V_{max}$ of 10.9 ± 1.0 nmol/mg protein/10 min. Although both cell types appear to have similar $K_m$ values, the $V_{max}$ for the transport system in AsPc-1 cells about 5 times greater than that of Capan-2 cells. A previous study of Gly-Sar uptake in Caco-2 cells, grown on plastic supports, has measured a $K_m$ of 1.1 ± 0.1 mM and a $V_{max}$ of 17.7 ± 0.6 nmol/mg protein/10 min (23). On the basis of the $K_m$ determinations, the transport systems in both the Capan-2 and AsPc-1 cells appear to be similar to that in Caco-2 cells. The difference in the $V_{max}$ of AsPc-1 cells suggests that they express a more functional transporter, consistent with the Western analysis data.
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Fig. 6. AsPc-1 and Capan-2 have PEPT 1 distributions similar to those of Caco-2 and CHO-PEPT 1 cells. Cells were labeled with a polyclonal antibody raised against the COOH-terminal 18 amino acids of the human PEPT 1 and imaged using a laser scanning confocal microscope. Capan-2 and Caco-2 cells demonstrated similar labeling of both plasma membrane and intracellular vesicles. AsPc-1 and CHO-PEPT 1 cells showed a more pronounced perinuclear staining. CHO-K1 cells were labeled similarly as a negative control. Scale bar, 10 μm.

Cellular localization of the oligopeptide transporter in AsPc-1, Capan-2, Caco-2, and CHO-PEPT 1 cells was determined using the polyclonal antibody raised against the COOH-terminal peptide of human PEPT 1 and fluorescence confocal microscopy (Fig. 6). Capan-2 and Caco-2 cells demonstrated similar distributions of label between the plasma membrane and a population of intracellular vesicles. AsPc-1 and CHO-PEPT 1 cells were similar to one another but slightly different in their labeling patterns from Capan-2 and Caco-2 cells. Label in both AsPc-1 and CHO-PEPT 1 cells was also present at the plasma membrane and in a population of intracellular vesicles like Capan-2 and Caco-2 cells, but the intracellular label distribution was observed to be less homogeneous and more localized to a perinuclear region. CHO-K1 cells served as a negative control, and no significant labeling was observed. In addition, to further confirm that PEPT-1 was, indeed, located on the plasma membrane, double-labeling experiments were performed using the anti-PEPT 1 antibody in conjunction with a monoclonal antibody against CEA, a cell surface antigen (24). In both AsPc-1 and Capan-2 cells, PEPT 1 colocalized with CEA at the cell periphery, indicating the presence of PEPT 1 at the plasma membrane (Fig. 7).

DISCUSSION

Pancreatic cancers frequently result from the conversion of an epithelial (ductal) cell to an adenocarcinoma. This conversion is commonly associated with activating mutations in Ki-ras and inhibiting mutations in the tumor suppressor protein p53. Other studies have suggested that these cancers can overexpress molecules such as the HER2/neu gene product (25), epidermal growth factor and transforming growth factor-α receptors (25), Cripto (26), endothelin-A, CA 19-9, and DUPAN-2 (6), which might be useful in targeting antineoplastic agents to pancreatic cancer cells. Our data have demonstrated that pancreatic cancer cell lines AsPc-1 and Capan-2 express high levels of another potential targeting molecule, a functional oligopeptide transporter. Physical and functional characterization of this transport activity was consistent with that of the proton-coupled oligopeptide transporter. Physical and functional characterization of this transport activity was consistent with that of the proton-coupled oligopeptide transporter PEPT 1 (7, 8, 20). Although a substantial portion of the PEPT 1 expressed by these cells appears to reside in an intracellular vesicle pool, sufficient oligopeptide transport capacity exists at the plasma membrane for the potential application of delivering a peptide-like antineoplastic agent. Such a delivery through the PEPT 1 expressed by Caco-2 cells has recently been demonstrated (16).

PEPT 1 can transport a wide range of peptide and peptide-like substances, suggesting that the chemical criteria for substrate recognition are not very discriminating. Also, solute transport by PEPT 1 occurs after the binding and simultaneous transport of a proton (7, 8, 20). Clearly, the oligopeptide transport to AsPc-1 and Capan-2 cells that we have observed is enhanced by a decreased extracellular pH. Although a scenario for maintaining an acidic pH at the apical surface of a polarized intestinal epithelial cells through the activity of a Na+/H+ exchanger has been suggested (27, 28), it is unclear at present if and how such a low extracellular pH might be maintained in pancreatic cancers to optimize the function of this transporter. Thus, an added complexity to this issue is the likelihood that, following
conversion to a cancerous phenotype, pancreatic duct cells probably lose their polarity and ability to maintain ionic gradients. The observation that the apical versus basolateral uptake of peptides by polarized Caco-2 cells is different may be due to difference in surface pH (21).

It is interesting that a number of intracellular vesicles establish and/or maintain a low internal pH and that the PEPT 1 appears to localize to a population of intracellular vesicles. If these vesicles containing PEPT 1 also maintain an internal acidic environment, the associated proton gradient could facilitate the transport of oligopeptides or other PEPT 1 substrates present within these vesicles into the cytoplasm. Thus, PEPT 1 may play a role in the normal reabsorption of small peptides from catabolic pathways and also provide another entry route for therapeutics delivered to the cell cytoplasm through a endocytosis pathway.

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