Membrane proteomic analysis of pancreatic cancer cells

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Abstract

Background: Pancreatic cancer is one of the most aggressive human tumors due to its high potential of local invasion and metastasis. The aim of this study was to characterize the membrane proteomes of pancreatic ductal adenocarcinoma (PDAC) cells of primary and metastatic origins, and to identify potential target proteins related to metastasis of pancreatic cancer.

Methods: Membrane/membrane-associated proteins were isolated from AsPC-1 and BxPC-3 cells and identified with a proteomic approach based on SDS-PAGE, in-gel tryptic digestion and liquid chromatography with tandem mass spectrometry (LC-MS/MS). X! Tandem was used for database searching against the SwissProt human protein database.

Results: We identified 221 & 208 proteins from AsPC-1 and BxPC-3 cells, respectively, most of which are membrane or membrane-associated proteins. A hundred and nine proteins were found in both cell lines while the others were present in either AsPC-1 or BxPC-3 cells. Differentially expressed proteins between two cell lines include modulators of cell adhesion, cell motility or tumor invasion as well as metabolic enzymes involved in glycolysis, tricarboxylic acid cycle, or nucleotide/lipid metabolism.

Conclusion: Membrane proteomes of AsPC-1 (metastatic) and BxPC-3 (primary) cells are remarkably different. The differentially expressed membrane proteins may serve as potential targets for diagnostic and therapeutic interventions.

Introduction

Pancreatic cancer is one of the most aggressive human malignancies. Despite the advances in therapeutic strategies including surgical techniques as well as local and systemic adjuvant therapies, the overall survival in patients with pancreatic cancer remains dismal and has not improved substantially over the past 30 years. Median survival from diagnosis is typically around 3 to 6 months, and the 5-year survival rate is less than 5%. As a result, in 2003, pancreatic cancer surpassed prostate cancer as the 4th leading cause of cancer-related death in the US [1]. The main reason for the failure of current conventional therapy to cure pancreatic cancer and the major cause for cancer-related mortality in general, is the ability of malignant cells to detach from the primary tumor site and to develop metastasis in different regions of the same organ and in distant organs [2,3]. Pancreatic cancer usually causes no symptoms early on, leading to locally advanced or metastatic disease at time of diagnosis [4]. In this regard, it is important to identify the functional proteins that regulate/promote metastasis in pancreatic cancer. This would facilitate the development of strategies for therapeutic interventions and improved management of cancer patients.

The purpose of this study is to compare the membrane proteins expressed in pancreatic cancer cells of primary and metastatic origins using a proteomics approach. Membrane proteomics can be defined as analysis and characterization of entire complement of membrane proteins present in a cell under a specific biological condition [5,6]. In fact, membrane proteins account for more than two-thirds of currently known drug targets. Defining membrane proteomes is therefore important for finding potential drug targets. Membrane proteomics can also serve as a promising approach to human cancer biomarker...
discovery because membrane proteins are known to have implication in cell proliferation, cell adhesion, cell motility and tumor cell invasion [7-9].

Materials and methods

Cell culture

AsPC-1 and BxPC-3 cell lines were obtained from American Tissue Culture Collection (ATCC, Rockville, MD). These cell lines were initially generated from patients with pancreatic ductal adenocarcinoma (PDAC) [10-12]. The cells were maintained at 5% CO2-95% air, 37°C, and with RPMI 1640 (ATCC) containing 10% FBS, 100 μg/ml penicillin G and 100 mg/ml streptomycin. When the confluence reached 80-90%, the cells were harvested and washed with PBS for three times.

Sample preparation

Membrane proteins from AsPC-1 and BxPC-3 cells were isolated with the ProteoExtract Native Membrane Protein Extraction Kit (EMD Chemicals, Gibbstown, NJ). In brief, the cell pellet was washed three times with the Washing Buffer, and then incubated with ice-cold Extract Buffer [at 4°C for 10 min under gentle agitation. After the pellet was centrifuged at 16,000 g for 15 min (4°C), the supernatant was discarded and 1 mL ice-cold Extract Buffer [was added to the pellet. This membrane protein extraction step was allowed for 30 min at 4°C under gentle agitation. Then the supernatant was collected after centrifugation at 16,000 g for 15 min 4°C.

SDS-PAGE and proteolytic cleavage

Total membrane protein concentration was measured with the 2-D Quant Kit (GE Healthcare, Piscataway, NJ). In total, 20 μg of membrane proteins from each cell line were loaded into a 4-12% NuPAGE Bis-Tris gel (Invitrogen, Carlsbad, CA) for SDS-PAGE separation. The gel was stained with the Simply Blue staining solution (Invitrogen) to visualize the proteins. Each gel was then cut into 15 sections evenly and proteolytic cleavage of proteins in each section was performed with enzyme-grade trypsin (Promega, Madison, WI) as previously described.

Tandem MS and database searching

Liquid chromatography (LC) with tandem MS (LC/MS/ MS) of peptides was performed using a NanoLC system (Eksigent Technologies, Dublin, CA) and a LTQ mass spectrometer (Thermo Fisher, Waltham, MA). Aliquots (5 μL) of the peptide digest derived from each gel slice were injected using an autosampler at a flow rate of 3.5 μL/min. The peptides were concentrated and desalted on a C18 IntegraFrit Nano-Precolumn (New Objective, Woburn, MA) for 10 min, then eluted and resolved using a C18 reversed-phase capillary column (New Objective). LC separation was performed at 400 nL/min with the following mobile phases: A, 5% acetonitrile/0.1%formic acid (v/v); B, 95% acetonitrile/0.1% formic acid (v/v). The chosen LC gradient was: from 5% to 15% B in 1 min, from 15% to 100% B in 40 min, and then maintained at 100%B for 15 min.

Database searches were performed using the X! Tandem search engine against the SwissProt protein sequence database. The search criteria were set with a mass accuracy of 0.4 Da and semi-style cleavage by trypsin. Proteins with two unique peptides are considered as positively identified.

Western blot analysis

AsPC-1 and BxPC-3 cells were lysed with a lysis buffer containing 8 M urea, 2 M Thiourea and 4% CHAPS. Cell lysates with a total protein amount of 40 μg were separated with 8-12% NuPAGE gels at 100 V for about 2 hours and then transferred to polyvinylidene difluoride membrane using an iBlot system (Invitrogen, Carlsbad, CA, USA). After saturating with 2% skim milk, the blots were sequentially incubated with primary antibody (1:100 dilution) and horseradish peroxidase-conjugated antimouse IgG secondary antibody (1:1000 dilution, Applied Biological Materials Inc, Richmond, Canada). Anti-annexin A1 was obtained from Abcam (Cambridge, MA, USA) whereas anti-phosphoglycerate kinase 1 was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Finally, the bands were visualized by enhanced chemiluminescence detection (Applied Biological Materials).

Results

The purpose of this study was to demonstrate a membrane proteomic analysis of PDAC cells and to identify differentially expressed membrane proteins between primary and metastatic PDAC cells, which may have a potential role in metastasis of pancreatic cancer. Two PDAC cell lines, AsPC-1 and BxPC-3, were used in this study. AsPC-1 is a cell line of metastatic origin from a 62 year-old female Caucasian whereas BxPC-3 is a cell line of primary PDAC from a 61 year-old female Caucasian [10-12]. Membrane proteins of AsPC-1 and BxPC-3 cells were isolated and then resolved with SDS-PAGE (Figure 1A). Proteins in each gel slices were proteolytically cleaved and the resulting peptides were analyzed with LC-MS/MS. In total, we identified 221 and 208 membrane or membrane-associated proteins from AsPC-1 and BxPC-3 cells, respectively, based on at least 2 unique peptides. A hundred and nine proteins were present in both cell lines but others were only found in AsPC-1 or in BxPC-3 cells (Figure 1B). All the identified proteins and matched peptides from the two cell lines are summarized in Additional file 1, Tables S1 and S2. Proteins with single matched peptide were not tabulated although previous publications reported identification of
membrane proteins based on single unique peptide [13,14]. The identified proteins were then sorted according to the Gene Ontology Annotation database (Figure 2). A hundred and four proteins were assigned as membrane proteins in AsPC-1 cells whereas 101 proteins were assigned as membrane proteins in BxPC-3 cells. Table 1 lists the “integral to membrane” proteins found in AsPC-1 and BxPC-3 cells. Besides the membrane proteins, the proteomic analysis also identified many membrane-associated proteins, e.g., extracellular matrix (ECM) proteins. To confirm the proteomic finding, we verified the differential levels of Annexin A1 and PGK1 between AsPC-1 and BxPC-3 cells using Western blot analysis. In fact, the differential expression of membrane proteins between AsPC-1 and BxPC-3 can be observed from the SDS-PAGE patterns of membrane proteins from the two cell lines (Figure 1). The proteins showing differential levels include cadherins, catenin, integrins, galectins, annexins, collagens and many others, which are known to have roles in tumor cell adhesion or motility. Cadherins are a class of type-1 transmembrane proteins that depend on calcium ions to function. They play important roles in cell adhesion, ensuring that cells are bound together within tissues. Catenins, which are proteins found in complexes with cadherins, also mediate cell adhesion. Our study identified cadherins (protocadherin-16 and protocadherin alpha-12) and alpha-2 catenin in primary tumor cells (BxPC-3) but not in metastatic tumor cells (AsPC-1), suggesting a defect in cell-to-cell adhesion in metastatic AcPC-1 cells.

Integrins are members of a glycoprotein family that form heterodimeric receptors for ECM molecules. These proteins are involved in an adhesive function, and they provide traction for movement in cell motility [25]. In total, there are 18 α-subunits and 8 β-subunits, which are paired to form 24 different integrins through non-covalent bonding. Among these proteins, integrin-β1, α2, α5, and α6 represent major adhesion molecules for the adhesion of pancreatic cancer cells to ECM proteins [26]. In our study, integrin-β1 and integrin-β4 was found in both tumor cell lines while integrin α2 and α5 only identified in BxPC-3 cells. Collagens are major ECM proteins. Cell surface-expressed portion of collagens (uPA/uPAR) system. As modulators of metastatic growth, these molecules can affect the local ECM, stimulate cell migration, and promote cell proliferation and tumor cell survivals [15]. Furthermore, hypoxia can drive genomic instability and lead to a more aggressive tumor phenotype [16,17], which may partially explain the highly metastatic nature of PDAC [18]. Last but not least, angiogenesis plays a critical role in invasion and metastasis in terms of tumor cell dissemination. Based on these new insights in mechanism of tumor invasion and metastasis, novel therapies are currently investigated for therapy of patients with pancreatic cancer [19-21]. Nevertheless, proteomic analysis of primary and metastatic PDAC is required to reveal additional functional proteins that regulate or promote tumor metastasis, as detailed in previous studies [22-24]. These signature molecules are predictors of metastatic risk and also provide a basis for the development of anti-metastatic therapy.

Our proteomic analysis has revealed a large number of differentially expressed membrane/surface proteins between metastatic and primary PDAC cells, and the validity of such a proteomic approach has been verified by Western blot analysis. In fact, the differential expression of membrane proteins between AsPC-1 and BxPC-3 can be observed from the SDS-PAGE patterns of membrane proteins from the two cell lines (Figure 1). The proteins showing differential levels include cadherins, catenin, integrins, galectins, annexins, collagens and many others, which are known to have roles in tumor cell adhesion or motility. Cadherins are a class of type-1 transmembrane proteins that depend on calcium ions to function. They play important roles in cell adhesion, ensuring that cells are bound together within tissues. Catenins, which are proteins found in complexes with cadherins, also mediate cell adhesion. Our study identified cadherins (protocadherin-16 and protocadherin alpha-12) and alpha-2 catenin in primary tumor cells (BxPC-3) but not in metastatic tumor cells (AsPC-1), suggesting a defect in cell-to-cell adhesion in metastatic AcPC-1 cells.

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Discussion
Metastasis is a highly organ-specific process, which requires multiple steps and interactions between tumor cells and the host. These include detachment of tumor cells from the primary tumor, intravasation into lymph and blood vessels, survival in the circulation, extravasation into target organs, and subsequent proliferation and induction of angiogenesis. Many proteins are critically involved in this process, such as cell-cell adhesion molecules (CAMs), members of the cadherins and, integrins, metalloproteinases (MMPs) and the urokinase plasminogen activator/urokinase plasminogen activator receptor
may serve as ligands for integrins, mediating cell-to-cell adhesion. Twelve members of collagen family were found in the BxPC-3 cells whereas only four members found in AsPC-1 cells.

Conversely, galectin-3 and galectin-4 were found in AsPC-1 but not in BxPC-3 cells. Galectins are carbohydrate-binding proteins and have an extremely high affinity for galactosides on cell surface and extracellular glycoproteins. Galectins, especially galectin-3, are modulators of cancer cell adhesion and invasiveness. Galectin-3 usually exists in cytoplasm, but can be secreted and bound on the cell surface by a variety of glycoconjugate ligands. Once localized to the cell surface, galectin-3 is capable of oligomerization, and the resultant cross-linking of surface glycoproteins into multimolecular complexes on the endothelial cell surface is reported to mediate the adhesion of tumor cells to the vascular endothelium [27]. Lysosome-associated membrane glycoprotein 1 (LAMP1) is a receptor for galectin-3, and was found on the cell surface of highly metastatic tumor cells [28]. Our study revealed LAMP1 in AsPC-1 cells but not in BxPC-3 cells. The cell surface-expressed portion of LAMP1 maybe serve as a ligand for galectin 3, mediating cell-cell adhesion and indirectly tumor spread. FKBP12-rapamycin complex-associated protein (a.k.a., mTOR) was also identified in AsPC-1 cells but not in BxPC-3 cells. mTOR is a downstream serine/threonine protein kinase of the phosphatidylinositol 3-kinase/Akt pathway that regulates cell proliferation, cell motility, cell survival, protein synthesis, and transcription. Rapamycin, a specific inhibitor of mTOR, suppresses lymphangiogenesis and lymphatic metastasis in PDAC cells [29].

The described proteomic approach is reproducible for analysis of membrane proteins in cultured pancreatic cancer cells. We observed consistent SDS-PAGE gel patterns for membrane proteins isolated from cultured AsPC-1 or BxPC-3 cells. To examine the reproducibility of LC-MS/MS for identification of membrane proteins, we repeated LC-MS/MS analysis of the peptides yielded from 3 gel bands. Compared to single LC-MS/MS, which identified 45 proteins in total, the duplicate LC-MS/MS analyses identified 47 proteins (~4% increase).

Figure 2 Sorting of the identified proteins according to their subcellular localization.

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This suggested that the observed difference in membrane protein profiles between the two PDAC cell lines is meaningful. Our adopted approach is valid to identify large membrane proteins, which are usually difficult to analyze with 2-D gel electrophoresis (2-DE) method. In AsPC-1 cells, 35% of the identified proteins have a molecular weight above 70 kDa, whereas 43% of the proteins are larger than 70 kDa in BxPC-3 cells. In addition to the proteins either present in AsPC-1 or in BxPC-3 cells, many other proteins were found in both cell types with a differential number of peptides matched. This may reflect the differential level of a

Table 1 Integral to membrane proteins identified in AsPC-1 & BxPC-3 cells

| Accession # | Protein name | Accession # | Protein name |
|-------------|--------------|-------------|--------------|
| 1A25_HUMAN  | HLA class I histocompatibility antigen, A-25 alpha chain | 4F2_HUMAN | 2F cell-surface antigen heavy chain |
| 4F2_HUMAN   | 4F2 cell-surface antigen heavy chain | ACSL3_HUMAN | Long-chain-fatty-acid-CoA ligase 3 |
| AAA5_HUMAN  | Neutral amino acid transporter B(0) | ACSL4_HUMAN | Long-chain-fatty-acid-CoA ligase 4 |
| ACSL5_HUMAN | Long-chain-fatty-acid-CoA ligase 5 | ADT2_HUMAN | ADP/ATP translocase 2 |
| ADT2_HUMAN  | ADP/ATP translocase 2 | ALK_HUMAN | ALK tyrosine kinase receptor precursor |
| ANP_HUMAN   | Atrial natriuretic peptide clearance receptor | APOH_HUMAN | Adipocyte plasma membrane-associated protein |
| AOCB_HUMAN  | Amine oxidase flavin-containing B | AT1A1_HUMAN | Sodium/potassium-transporting ATPase subunit alpha-1 |
| APMA_HUMAN  | Adipocyte plasma membrane-associated protein | CALX_HUMAN | Calnexin |
| AT1A1_HUMAN | Sodium/potassium-transporting ATPase subunit alpha-1 precursor | CEAM1_HUMAN | Carinoembryonic antigen-related cell adhesion molecule 1 |
| ATP7B_HUMAN | Copper-transporting ATPase 2 | CEAM6_HUMAN | Carinoembryonic antigen-related cell adhesion molecule 6 |
| CALX_HUMAN  | Calnexin | CKA4_HUMAN | Cytoskeleton-associated protein 4 |
| CEAM1_HUMAN | Carinoembryonic antigen-related cell adhesion molecule 1 | CLCN1_HUMAN | Chloride channel protein |
| CEAM6_HUMAN | Carinoembryonic antigen-related cell adhesion molecule 6 | CMC2_HUMAN | Calcium-binding mitochondrial carrier protein Aralar2 |
| CMC2_HUMAN  | Calcium-binding mitochondrial carrier protein Aralar2 | CODA1_HUMAN | Collagen alpha-1(XII) chain |
| CY1_HUMAN   | Cytochrome c1, heme protein | CSM2_HUMAN | CUB and sushi domain-containing protein 2 |
| EGFR_HUMAN  | Epidermal growth factor receptor precursor | EAA1_HUMAN | Excitary amino acid transporter 1 |
| FLNB_HUMAN  | Filamin-B | GP124_HUMAN | Probable G-protein coupled receptor 124 |
| FLRT1_HUMAN | Leucine-rich repeat transmembrane protein FLRT1-3 | GRP78_HUMAN | 78 kDa glucose-regulated protein |
| FZDB_HUMAN  | Frizzled-8 precursor | HNRPM_HUMAN | Heterogeneous nuclear ribonucleoprotein M |
| GRP78_HUMAN | 78 kDa glucose-regulated protein | ITAV_HUMAN | Integrin alpha-V |
| IL4A_HUMAN  | Interleukin-4 receptor alpha chain | KCNQ3_HUMAN | Potassium voltage-gated channel subfamily KQT member 3 |
| IMMT_HUMAN  | Mitochondrial inner membrane protein | L2DH_HUMAN | L-2-hydroxyglutarate dehydrogenase |
| KANK3_HUMAN | Potassium channel subfamily K member 3 | M2OM_HUMAN | Mitochondrial 2-oxoglutarate/malate carrier protein |
| KTN1_HUMAN  | Kinectin | MUC16_HUMAN | Myoferlin |
| LAMPT1_HUMAN | Lysosome-associated membrane glycoprotein 1 | MYOF_HUMAN | Myoferlin |
| LRC59_HUMAN | Leucine-rich repeat-containing protein 59 | OST48_HUMAN | Dolichyl-diphosphooligosaccharide-protein biosynthetic 48 kDa subunit |
| MTC2_HUMAN  | Mitochondrial carrier homolog 2 | PCD16_HUMAN | Protocadherin-16 precursor |
| MUC16_HUMAN | Mucin-16 | PGR1_HUMAN | Membrane-associated progesterone receptor component 1 |
| MYOF_HUMAN  | Myoferlin | PHB_HUMAN | Prohibitin |
| OST48_HUMAN | Dolichyl-diphosphooligosaccharide-protein biosynthetic 48 kDa subunit | PK11_HUMAN | Polycystic kidney disease protein 1-like 1 |
| PHB_HUMAN   | Prohibitin | PTP12_HUMAN | Receptor-type tyrosine-protein phosphatase zeta |
| S12A1_HUMAN | Solute carrier family 12 member 1 | SSRD_HUMAN | Translocon-associated protein subunit delta precursor |
| SF313_HUMAN | Sideroflexin | TFR1_HUMAN | Transferrin receptor protein 1 |
| VAT1_HUMAN  | Synaptic vesicle membrane protein VAT-1 homolog | TMEDA_HUMAN | Transmembrane emp24 domain-containing protein 10 |
| VDAC2_HUMAN | Voltage-dependent anion-selective channel protein 2 | TM40_HUMAN | Mitochondrial import receptor subunit TM40 homolog |
| VMAT2_HUMAN | Synaptic vesicular amine transporter |
protein between the two cell lines, although further verification is needed. Around 50% of the proteins identified in AsPC-1 and BxPC-3 cells are directly classified as membrane proteins, including a number of integral to membrane proteins and plasma membrane proteins. In addition, many mitochondrial inner membrane proteins were also identified from AsPC-1 (n = 21) and BxPC-3 (n = 13) cells. The mitochondrial inner membrane forms internal compartments known as cristae, which allow greater space for the proteins such as cytochromes to function properly and efficiently. The inner mitochondrial membrane contains mitochondria fusion and fission proteins, ATP synthases, transporter proteins regulating metabolite flux as well as proteins that perform the redox reactions of oxidative phosphorylation, many of which were identified in this study. Among the proteins that are not classified as membrane proteins, many are either membrane-associated proteins (e.g., kinases, G proteins, or enzymes) or proteins associated with other subcellular compartments such as mitochondria, endoplasmic reticulum (ER) or nucleus (e.g., histones, elongation factors, translation initiation factor and transcription factors) (Additional file 1, Table S1). It is commonly assumed that a protein is predominantly localized in a given cellular compartment where it exerts its specific function. However, a same protein may be localized at different cell compartments or travel between different organelles and therefore exert multiple cellular functions [30]. In fact, many proteins identified in mitochondria or ER are membrane or membrane-associated proteins.

In addition, many metabolic enzymes were identified from the two PDAC cell lines, reflecting the functional role of pancreas (Tables 2 and 3). These metabolic enzymes are involved in glycolysis, tricarboxylic acid cycle, gluconeogenesis, metabolism of nucleotides, lipids/fatty acids and amino acids, protein folding/ unfolded protein response, and pantose phosphate shunt. Table 4 lists the small, membrane associated G proteins identified in AsPC-1 and BxPC-3 cells. Small GTPases regulate a wide variety of cellular processes, including growth, cellular differentiation, cell movement and lipid vesicle transport. RhoA, Rab-1A and Rab-10 were present in AsPC-1 cells whereas Rab-14 was found in BxPC-3 cells. As a proto-oncogene, RhoA regulates a signal transduction pathway linking plasma membrane receptors to the assembly of focal adhesions and actin stress fibers. On the other hand, Rab-1A regulates the ‘ER-to-Golgi’ transport, a bidirectional membrane traffic between the ER and Golgi apparatus which mediates the transfer of proteins by means of small vesicles or tubular-saccular extensions. Rab-10 is also involved in vesicular trafficking, particularly the directed movement of substances from the Golgi to early sorting endosomes. Mutated KRAS is a potent oncogene in PDAC. KRAS protein is usually tethered to cell membranes because of the presence of an isoprenyl group on its C-terminus. However, KRAS protein was not identified in this study, which might result from numerous mutations of the gene, hindering the matching of peptides based on molecular weight.

Some of the proteins identified from the current study may be further verified in clinical specimens as biomarkers for diagnostic/prognostic applications. Particularly, protein biomarkers may be used to classify pancreatic cancer patients for a better treatment decision. Cancer biomarker discovery is an intensive research area. Despite the fact that a large number of researchers are searching for cancer biomarkers, only a handful of protein biomarkers have been approved by the US Food and Drug Administration (FDA) for clinical use [31]. Interestingly, most of the FDA-approved protein biomarkers for human cancers are membrane proteins, including cancer antigen CA125 (ovarian), carcinoembryonic antigen (colon), epidermal growth factor receptor (colon), tyrosine-protein kinase KIT (gastrointestinal), HER2/NEU, CA15-3, CA27-29, Oestrogen receptor and progesterone receptor (breast) and bladder tumour-associated antigen (bladder) [31]. Similarly, most of the reported protein biomarkers in PDAC are of membrane origin or membrane-associated, including CA 19-9, CEA, CA 242, CA 72-4, KRAS, KAI1, CEA-related cell adhesion molecule 1 (CEACAM1), MUC1, MUC4, among many others [32-39]. For instance, CA 19-9 is a membrane carbohydrate antigen and the most commonly used biomarker in pancreatic cancers. As a cell adhesion molecule, CEA actually mediates the collagen binding of epithelial cells [40]. KAI1, a metastasis suppressor protein, belongs to the transmembrane 4 superfamily. It is up-regulated in early PDAC and down-regulated in metastatic PDAC [34]. The present study also identified CEA-related cell
### Table 2 Metabolic enzymes identified in AsPC-1 cells

| Protein name                                                                 | Accession #   | Unique peptides | Total peptides | Mr (Kda) | PI   | Biological process                                                                 |
|------------------------------------------------------------------------------|---------------|----------------|----------------|----------|------|------------------------------------------------------------------------------------|
| 2-oxoglutarate dehydrogenase E1 component, mitochondrial precursor           | ODO1_HUMAN    | 8              | 18             | 115.9    | 6.39 | Glycolysis                                                                         |
| 3,2-trans-enoyl-CoA isomerase, mitochondrial precursor                       | D3D2_HUMAN    | 3              | 13             | 32.8     | 8.8  | Fatty acid metabolism; Lipid metabolism                                            |
| 3-hydroxyacyl-CoA dehydrogenase type-2                                       | HCD2_HUMAN    | 6              | 10             | 26.9     | 7.65 | Lipid metabolic process; tRNA processing                                            |
| 3-hydroxyisobutyrate dehydrogenase, mitochondrial precursor                  | 3HIDH_HUMAN   | 7              | 16             | 35.3     | 8.38 | Pentose-phosphate shunt; valine metabolic process                                  |
| 3-ketoacyl-CoA thiolase, peroxisomal precursor                               | THIK_HUMAN    | 3              | 4              | 44.3     | 8.76 | Fatty acid metabolism; Lipid metabolism                                            |
| 3-mercaptopyruvate sulfurtransferase                                         | THTM_HUMAN    | 7              | 12             | 72.3     | 5.07 | ER-associated protein catabolic process; ER unfolded protein response; ER regulation of protein folding |
| 78 KDa glucose-regulated protein                                             | GRF78_HUMAN   | 7              | 12             | 72.3     | 5.07 | ER-associated protein catabolic process; ER unfolded protein response; ER regulation of protein folding |
| Acetyl-CoA acetyltransferase, mitochondrial precursor                        | THIL_HUMAN    | 2              | 6              | 45.2     | 8.98 | Ketone body metabolism                                                              |
| Aconitate hydratase, mitochondrial                                           | ACON_HUMAN    | 2              | 3              | 85.4     | 7.36 | Tricarboxylic acid cycle                                                            |
| Acyl-protein thioesterase 1                                                 | LYPA1_HUMAN   | 2              | 2              | 24.7     | 6.29 | Fatty acid metabolism; Lipid metabolism                                            |
| Adenylate kinase 2, mitochondrial                                           | KAD2_HUMAN    | 7              | 20             | 26.5     | 7.67 | Nucleic acid metabolic process                                                     |
| ADP/ATP translocase 2                                                        | ADT2_HUMAN    | 5              | 11             | 32.9     | 9.76 | Transmembrane transporter activity                                                  |
| Aldehyde dehydrogenase, mitochondrial                                       | ALDH2_HUMAN   | 3              | 7              | 56.3     | 6.63 | Alcohol metabolic process                                                          |
| Alpha-enolase                                                                | ENOA_HUMAN    | 2              | 2              | 47.1     | 7.01 | Glycolysis                                                                         |
| Amine oxidase B                                                              | AOFB_HUMAN    | 2              | 2              | 58.7     | 7.2  | Oxidation reduction                                                                |
| Aspartate aminotransferase, mitochondrial                                    | AATM_HUMAN    | 4              | 6              | 47.4     | 9.14 | Lipid transport                                                                   |
| ATP synthase subunit alpha, mitochondrial                                    | ATPA_HUMAN    | 21             | 52             | 59.7     | 9.16 | ATP synthesis                                                                      |
| ATP synthase subunit d, mitochondrial                                        | ATPSH_HUMAN   | 3              | 7              | 18.5     | 5.21 | ATP synthesis; ion transport                                                       |
| ATP synthase subunit b, mitochondrial                                        | ATSF1_HUMAN   | 2              | 3              | 28.9     | 9.37 | ATP synthesis                                                                      |
| ATP synthase subunit beta, mitochondrial                                     | ATPB_HUMAN    | 28             | 95             | 56.5     | 5.26 | ATP synthesis                                                                      |
| ATP synthase subunit f, mitochondrial                                        | ATPK_HUMAN    | 2              | 2              | 10.9     | 9.7  | ATP synthesis; ion transport                                                       |
| ATP synthase subunit gamma, mitochondrial                                    | ATPG_HUMAN    | 3              | 6              | 33.8     | 9.23 | ATP synthesis; proton transport                                                    |
| ATP synthase subunit Q, mitochondrial                                        | ATPO_HUMAN    | 6              | 11             | 23.3     | 9.97 | ATP synthesis; ion transport; ATP catabolic process                                 |
| Calcium-binding mitochondrial carrier protein Aralar2                        | CMC2_HUMAN    | 7              | 16             | 74.1     | 7.14 | Mitochondrial aspartate and glutamate carrier                                     |
| Citrate synthase, mitochondrial precursor                                    | CISY_HUMAN    | 2              | 3              | 51.7     | 8.45 | Tricarboxylic acid cycle                                                           |
| Cytochrome b5 type B                                                         | CYB5B_HUMAN   | 2              | 4              | 16.3     | 4.88 | Electron transport                                                                |
| Cytochrome b-c1 complex subunit 1, mitochondrial                            | QCR1_HUMAN    | 6              | 12             | 52.6     | 5.94 | Electron transport                                                                |
| Cytochrome b-c1 complex subunit 2, mitochondrial                            | QCR2_HUMAN    | 3              | 4              | 48.4     | 8.74 | Aerobic respiration; electron transport chain; oxidative phosphorylation           |
| Cytochrome c oxidase subunit 2                                               | COX2_HUMAN    | 2              | 6              | 25.5     | 4.67 | Electron transport chain                                                          |
| Cytochrome c1, heme protein, mitochondrial                                   | CY1_HUMAN     | 5              | 10             | 35.4     | 9.15 | Electron transport chain                                                          |
| Cytochrome c1, heme protein, mitochondrial                                   | CY1_HUMAN     | 2              | 3              | 35.4     | 9.15 | Electron transport chain                                                          |
| D-beta-hydroxybutyrate dehydrogenase, mitochondrial                         | BDH_HUMAN     | 2              | 3              | 38.1     | 9.1  | Oxidation reduction                                                               |
| Delta(3,5)-Delta(2,4)-dieneoyl-CoA isomerase, mitochondrial                | ECH1_HUMAN    | 4              | 10             | 35.8     | 8.16 | Fatty acid metabolism; Lipid metabolism                                           |
| Delta-1-pyrroline-5-carboxylate synthetase                                   | PSCS_HUMAN    | 2              | 4              | 87.2     | 6.66 | Amino-acid biosynthesis; Proline biosynthesis                                     |
| Dihydrolipoyl dehydrogenase, mitochondrial                                   | DLDH_HUMAN    | 7              | 16             | 54.1     | 7.95 | Cell redox homeostasis                                                            |
| Dihydrolipoicylsine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial | DOD2_HUMAN    | 3              | 5              | 65.7     | 7.96 | Glycolysis                                                                       |
| Dihydrolipoicylsine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial | DOD2_HUMAN    | 4              | 7              | 48.6     | 9.01 | Tricarboxylic acid cycle                                                          |
| Enzyme Name                                      | Accession | Description                             | Function                                                                                     |
|-------------------------------------------------|-----------|-----------------------------------------|---------------------------------------------------------------------------------------------|
| Electron transfer flavoprotein subunit alpha, mitochondrial | ETFA_HUMAN |                                         | Electron transport                                                                           |
| Electron transfer flavoprotein subunit beta      | ETFB_HUMAN |                                         | Electron transport                                                                           |
| Endoplasmin                                     | ENPL_HUMAN |                                         | ER-associated protein catabolic process; protein folding/transport; response to hypoxia     |
| Enoyl-CoA hydratase, mitochondrial              | ECHM_HUMAN |                                         | Metabolic process phosphoglycolate phosphatase activity                                    |
| Glutamate dehydrogenase 1, mitochondrial,       | DHE3_HUMAN |                                         | Fatty acid metabolism; Lipid metabolism                                                    |
| Glycerol-3-phosphate dehydrogenase, mitochondrial precursor | GPDM_HUMAN |                                         | Fatty acid metabolism; Lipid metabolism                                                    |
| Isoleucyl-tRNA synthetase, mitochondrial precursor | SYIM_HUMAN |                                         | Protein biosynthesis                                                                        |
| Isovaleryl-CoA dehydrogenase, mitochondrial      | IVD_HUMAN |                                         | Leucine catabolic process; Oxidation reduction                                              |
| LDHA_HUMAN                                      | L-lactate dehydrogenase A chain                |                                         | Glycolysis                                                                                  |
| LONM_HUMAN                                      | Lon protease homolog, mitochondrial            |                                         | Required for intramitochondrial proteolysis                                                |
| ACSL5_HUMAN                                     | Long-chain-fatty-acid-CoA ligase 5             |                                         | Fatty acid metabolism; Lipid metabolism                                                    |
| MTH2_HUMAN                                      | Malate dehydrogenase, mitochondrial            |                                         | Tricarboxylic acid cycle; Glycolysis                                                       |
| MTCH2_HUMAN                                     | Mitochondrial carrier homolog 2               |                                         | Transmembrane transport                                                                    |
| IMMT_HUMAN                                      | Mitochondrial inner membrane protein          |                                         | Protein binding; Cell proliferation-inducing                                               |
| NBSR3_HUMAN                                     | NADH-cytochrome b5 reductase 3                |                                         | Cholesterol biosynthesis; Lipid/steroid synthesis                                           |
| PRDX5_HUMAN                                     | Peroxiredoxin-5                               |                                         | Cell redox homeostasis                                                                      |
| PPCKM_HUMAN                                     | Phosphoenolpyruvate carboxykinase, mitochondrial |                             | Gluconeogenesis                                                                              |
| PGK1_HUMAN                                      | Phosphoglycerate kinase 1                     |                                         | Glycolysis                                                                                  |
| PDA1_HUMAN                                      | Protein disulfide-isomerase                    |                                         | Cell redox homeostasis                                                                      |
| PDA3_HUMAN                                      | Protein disulfide-isomerase A3                 |                                         | Cell redox homeostasis                                                                      |
| PDA4_HUMAN                                      | Protein disulfide-isomerase A4                 |                                         | Cell redox homeostasis; Protein secretion                                                  |
| PDA6_HUMAN                                      | Protein disulfide-isomerase A6                 |                                         | Cell redox homeostasis; Protein folding                                                    |
| ETHE1_HUMAN                                     | Protein ETHE1, mitochondrial                  |                                         | Metabolic homeostasis in mitochondria                                                       |
| SC16A_HUMAN                                     | Protein transport protein Sec16A               |                                         | ER-Golgi transport; Protein transport                                                       |
| ODPA_HUMAN                                      | Pyruvate dehydrogenase E1 component alpha subunit, mitochondrial | | Glycolysis                                                                                  |
| ODPAT_HUMAN                                     | Pyruvate dehydrogenase E1 component subunit alpha, mitochondrial precursor | | Glycolysis; Tricarboxylic acid cycle                                                       |
| ODPC_HUMAN                                      | Serine hydroxymethyltransferase, mitochondrial |                                         | Glycolysis; L-serine metabolic process; Glycine metabolic process; One-carbon metabolic process |
| DSHA_HUMAN                                      | Succinate dehydrogenase flavoprotein subunit, mitochondrial | | Electron transport; Tricarboxylic acid cycle                                               |
| SUCB2_HUMAN                                     | Succinyl-CoA ligase (GDP-forming) beta-chain, mitochondrial precursor | | Succinyl-CoA metabolic process; Tricarboxylic acid cycle                                  |
### Table 2 Metabolic enzymes identified in AsPC-1 cells (Continued)

| Protein name                                                                 | Accession # | Unique peptides | Total peptides | Mr (KDa) | PI  | Biological process                                                                 |
|------------------------------------------------------------------------------|-------------|----------------|----------------|----------|-----|-----------------------------------------------------------------------------------|
| Succinyl-CoA ligase [GDP-forming] subunit alpha, mitochondrial precursor     | SUCA_HUMAN  | 2              | 5              | 35       | 9.01 | Tricarboxylic acid cycle                                                           |
| Superoxide dismutase [Mn], mitochondrial                                      | SODM_HUMAN  | 2              | 5              | 24.7     | 8.35 | Elimination of radicals                                                             |
| Thioredoxin-dependent peroxide reductase                                      | PRDX3_HUMAN | 4              | 10             | 27.7     | 7.68 | Cell redox homeostasis; Hydroperoxide catabolism                                   |
| Thiosulfate sulfurtransferase                                                 | THTR_HUMAN  | 2              | 3              | 33.4     | 6.77 | Cyanate catabolic process                                                           |
| Trifunctional enzyme subunit alpha, mitochondrial                           | ECHA_HUMAN  | 17             | 46             | 82.9     | 9.16 | Fatty acid metabolism; Lipid metabolism                                            |
| Trifunctional enzyme subunit beta, mitochondrial                            | ECHB_HUMAN  | 6              | 12             | 51.3     | 9.45 | Fatty acid metabolism                                                              |
| Trimethyllysine dioxygenase, mitochondrial                                    | TMLH_HUMAN  | 2              | 3              | 49.5     | 7.64 | Carnitine biosynthesis                                                             |
| Very long-chain specific acyl-CoA dehydrogenase, mitochondrial              | ACADV_HUMAN | 2              | 3              | 70.3     | 8.92 | Fatty acid metabolism; Lipid metabolism                                            |

### Table 3 Metabolic enzymes identified in BxPC-3 cells

| Protein name                                                                 | Accession # | Unique peptides | Total peptides | Mr (KDa) | PI  | Biological process                                                                 |
|------------------------------------------------------------------------------|-------------|----------------|----------------|----------|-----|-----------------------------------------------------------------------------------|
| 2-oxoglutarate dehydrogenase E1 component, mitochondrial                    | ODO1_HUMAN  | 4              | 4              | 115.9    | 6.39| Glycolysis                                                                         |
| 3-ketoacyl-CoA thiolase, mitochondrial                                       | THIM_HUMAN  | 2              | 4              | 41.9     | 8.32| Fatty acid metabolism; Lipid metabolism                                            |
| 78 kDa glucose-regulated protein                                            | GRP7B_HUMAN | 31             | 91             | 72.3     | 5.07| ER-associated protein catabolic process ER unfolded protein response ER regulation of protein folding |
| Adenylate kinase 2, mitochondrial                                            | KAD2_HUMAN  | 4              | 7              | 26.5     | 7.67| Nucleotide/nucleic acid metabolic process                                           |
| ADP/ATP translocase 2                                                       | ADT2_HUMAN  | 2              | 5              | 32.9     | 9.76| Transmembrane transporter activity                                                  |
| Alpha-aminoacidic semialdehyde dehydrogenase                               | ALTA1_HUMAN | 2              | 2              | 55.3     | 6.44| Cellular aldehyde metabolic process; oxidation reduction                           |
| Alpha-enolase                                                              | ENOA_HUMAN  | 3              | 5              | 47.1     | 7.01| Glycolysis                                                                         |
| Annexin A1                                                                 | ANXA1_HUMAN | 4              | 5              | 38.7     | 6.57| Anti-apoptosis; Exocytosis; Lipid metabolic process                                |
| Aspartate aminotransferase, mitochondrial precursor                         | AATM_HUMAN  | 2              | 7              | 47.4     | 9.14| Lipid transport                                                                    |
| ATP synthase subunit alpha, mitochondrial                                   | ATPA_HUMAN  | 3              | 6              | 59.7     | 9.16| ATP synthesis                                                                      |
| ATP synthase subunit beta, mitochondrial                                     | ATPB_HUMAN  | 4              | 13             | 56.5     | 5.26| ATP synthesis                                                                      |
| ATP synthase subunit d, mitochondrial                                       | ATPSH_HUMAN | 2              | 4              | 18.5     | 5.21| ATP synthesis; Ion transport                                                      |
| ATP synthase subunit gamma, mitochondrial                                   | ATPG_HUMAN  | 2              | 3              | 33       | 9.23| ATP synthesis; Ion transport                                                      |
| ATP synthase subunit Q, mitochondrial                                       | ATPO_HUMAN  | 2              | 3              | 23.3     | 9.97| ATP synthase; Ion transport ATP catabolnic process                                  |
| Calcium-binding mitochondrial carrier protein Aralar2                       | CMC2_HUMAN  | 2              | 4              | 74.1     | 7.14| Mitochondrial aspartate and glutamate carrier                                       |
| Citrate synthase, mitochondrial;                                            | CISY_HUMAN  | 3              | 5              | 51.7     | 8.45| Tricarboxylic acid cycle                                                           |
| Cytochrome b-c1 complex subunit 1, mitochondrial                           | QCRI_HUMAN  | 3              | 5              | 52.6     | 5.94| Electron transport                                                                |
| Cytochrome b-c1 complex subunit 2, mitochondrial                           | QCR2_HUMAN  | 2              | 2              | 48.4     | 8.74| Respiratory gaseous exchange                                                        |
| Cytochrome c oxidase subunit 2                                              | COX2_HUMAN  | 2              | 4              | 25.5     | 4.67| Electron transport chain                                                           |
| Cytochrome c oxidase subunit 5B, mitochondrial precursor                    | COX5B_HUMAN | 2              | 2              | 13.7     | 9.07| Amino-acid biosynthesis                                                           |
| Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase, mitochondrial precursor        | ECH1_HUMAN  | 2              | 6              | 35.8     | 8.16| Fatty acid metabolism; Lipid metabolism                                            |
| Delta-1-pyrroline-5-carboxylate synthetase                                  | PSCS_HUMAN  | 2              | 3              | 87.2     | 6.66| Proline biosynthesis                                                              |
| Dihydrolipoyl dehydrogenase, mitochondrial                                   | DLDH_HUMAN  | 5              | 13             | 54.1     | 7.95| Cell redox homeostasis                                                            |
| Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial | ODO2_HUMAN  | 3              | 6              | 48.6     | 9.01| Tricarboxylic acid cycle                                                       |
| Metabolic Enzyme | Gene Symbol | Chromosome | AF 10-Term | AF 10-Log | Description |
|------------------|-------------|------------|-------------|-----------|-------------|
| Electron transfer flavoprotein subunit alpha, mitochondrial | ETF-A_HUMAN | 3 | 7 | 35.1 | 8.62 | Electron transport |
| Electron transfer flavoprotein subunit beta | ETF-B_HUMAN | 2 | 3 | 27.8 | 8.25 | Electron transport |
| Endoplasmin | ENP-L_HUMAN | 16 | 31 | 92.4 | 4.76 | ER-associated protein catabolic process; protein folding/transport; response to hypoxia |
| Enoyl-CoA hydratase, mitochondrial | ECHM_HUMAN | 3 | 12 | 31.4 | 8.34 | Fatty acid metabolism; Lipid metabolism |
| ERD1-like protein alpha precursor | ERD1A_HUMAN | 2 | 3 | 54.4 | 5.48 | Electron transport |
| Glucosidase 2 subunit beta | GLU-2B_HUMAN | 2 | 5 | 59.4 | 4.33 | ER protein kinase cascade |
| Glutamate dehydrogenase 1, mitochondrial | DHE-3_HUMAN | 2 | 2 | 61.4 | 7.66 | Glutamate metabolism |
| Glyceraldehyde-3-phosphate dehydrogenase | G3P-HUMAN | 2 | 2 | 36 | 8.57 | Glycolysis |
| Glycerol-3-phosphate dehydrogenase, mitochondrial | GPDM-HUMAN | 2 | 4 | 80.8 | 7.23 | Glycolysis |
| Heme oxygenase 2 | HMOX-2_HUMAN | 2 | 4 | 36 | 5.31 | Heme oxidation; Oxidation reduction; Response to hypoxia |
| Hexokinase-1 | HKX-1_HUMAN | 2 | 3 | 102.4 | 6.36 | Glycolysis |
| L-2-hydroxyglutarate dehydrogenase, mitochondrial | L2HGDH_HUMAN | 2 | 2 | 50.3 | 8.57 | Cellular protein metabolic process; Oxidation reduction |
| Lon protease homolog, mitochondrial | LOMM-HUMAN | 2 | 2 | 106.4 | 6.01 | Required for intramitochondrial proteolysis |
| Long-chain-fatty-acid–CoA ligase 3 | ACSL3-HUMAN | 2 | 3 | 80.4 | 8.65 | Fatty acid metabolism; Lipid metabolism |
| Long-chain-fatty-acid–CoA ligase 4 | ACSL4-HUMAN | 2 | 3 | 79.1 | 8.66 | Fatty acid metabolism; Lipid metabolism |
| Malate dehydrogenase, mitochondrial | MDHM-HUMAN | 3 | 4 | 35.5 | 8.92 | TCA glycolysis |
| Medium-chain specific acyl-CoA dehydrogenase, mitochondrial | ACADM-HUMAN | 2 | 3 | 46.6 | 8.61 | Fatty acid metabolism; Lipid metabolism |
| Methylene-tetrahydrofolate reductase | MTHR-HUMAN | 2 | 2 | 74.5 | 5.22 | Methionine metabolic process; Oxidation reduction |
| Mitochondrial 2-oxoglutarate/malate carrier protein | M2OM-HUMAN | 2 | 2 | 34 | 9.92 | Transport |
| Mitochondrial import receptor subunit TOM40 homolog | TOM40-HUMAN | 3 | 3 | 37.9 | 6.79 | Ion transport; Protein transport |
| Neutral alpha-glucosidase A8 | GANA8-HUMAN | 7 | 10 | 106.8 | 5.74 | Carbohydrate metabolic process |
| Neutral cholesterol ester hydrolase 1 | ADCL1-HUMAN | 2 | 4 | 45.8 | 6.76 | Lipid degradation |
| Ornithine aminotransferase, mitochondrial precursor | OAT-HUMAN | 4 | 6 | 48.5 | 6.57 | Mitochondrial matrix protein binding |
| Phosphoeno-polpyruvate carboxykinase, mitochondrial | PPCKM-HUMAN | 2 | 3 | 70.6 | 7.56 | Glucoseogenesis |
| Protein disulfide-isomerase | PDI-1_HUMAN | 8 | 14 | 57.1 | 4.76 | Cell redox homeostasis |
| Protein disulfide-isomerase A3 | PDI-A3_HUMAN | 16 | 25 | 56.7 | 5.98 | Cell redox homeostasis |
| Protein disulfide-isomerase A4 | PDI-A4_HUMAN | 7 | 11 | 72.9 | 4.96 | Cell redox homeostasis; Protein secretion |
| Protein disulfide-isomerase A5 | PDI-A5_HUMAN | 2 | 4 | 48.1 | 4.95 | Cell redox homeostasis; Protein folding |
| Pyruvate kinase isozymes M1/M2 | KPYM-HUMAN | 5 | 7 | 57.9 | 7.96 | Glycolysis; Programmed cell death |
| Serine hydroxymethyltransferase, mitochondrial precursor | GLYM-HUMAN | 2 | 4 | 56 | 8.76 | L-serine metabolic process; Glycine metabolic process; One-carbon metabolic process |
| Sterol regulatory element-binding protein 2 | SREBP2-HUMAN | 2 | 2 | 123.6 | 8.72 | Cholesterol metabolism; Lipid metabolism; Steroid metabolism |
| Succinate dehydrogenase flavoprotein subunit, mitochondrial | DSHA-HUMAN | 3 | 10 | 72.6 | 7.06 | Electron transport; Tricarboxylic acid cycle |
| Succinyl-CoA4-ketoacid-coenzyme A transferase I | SCOT-HUMAN | 2 | 5 | 56.1 | 7.13 | Ketone body catabolic process |
| Sulfide:quinone oxidoreductase, mitochondrial | SQRD-HUMAN | 6 | 9 | 49.9 | 9.18 | Oxidation reduction |
| Superoxide dismutase [Mn], mitochondrial | SODM-HUMAN | 2 | 5 | 24.7 | 8.35 | Elimination of radicals |
| Transmembrane emp24 domain-containing protein 10 | TEDA-HUMAN | 2 | 3 | 25 | 6.98 | ER-Golgi protein transport |
| Trifunctional enzyme subunit alpha, mitochondrial | ECHA-HUMAN | 4 | 7 | 82.9 | 9.16 | Fatty acid metabolism; Lipid metabolism |
| Trifunctional enzyme subunit beta, mitochondrial | ECHB-HUMAN | 2 | 4 | 51.3 | 9.45 | Fatty acid metabolism |
Table 4 A list of small G proteins identified in AsPC-1 and BxPC-3 cells

| G Protein | MW (kDa) | Score | Nearest Human Sequence | AsPC-1 | BxPC-3 |
|-----------|----------|-------|------------------------|--------|--------|
| Ras-related protein Rab-1B | 3 | 7 | 22.2 | RAB1B_HUMAN | VVDNTAKEF ADSLGPFLF TSAK VVDNTAKEF ADSLGPFLF TSAK EFADSLGPFLF LETSAK EFADSLGPFLF LETSAK EFADSLGPFLF LETSAK EFADSLGPFLF LETSAK NATVNEQAFM TMAAEIK |
| Ras-related protein Rab-7a | 3 | 5 | 23.5 | RAB7A_HUMAN | DPNFPPFWL GNKIDLENR DPNFPPFWL GNKIDLENR DPNFPPFWL GNK EAINVEQAFQ TIAR EAINVEQAFQ TIAR |
| Ras-related protein Rab-1A | 3 | 7 | 22.7 | RAB1A_HUMAN | VVDYTTAKEF ADSLGPFLF TSAK VVDYTTAKEF ADSLGPFLF TSAK EFADSLGPFLF LETSAK EFADSLGPFLF LETSAK EFADSLGPFLF LETSAK EFADSLGPFLF LETSAK NATVNEQAFM TMAAEIK |
| Ras-related protein Rab-10; | 2 | 6 | 22.5 | 8.58 | RAB10_HUMAN | LLLGDSGVG K LLLGDSGVG K AFLTLEAIL R AFLTLEAIL R AFLTLEAIL R AFLTLEAIL R |
| Ras-related protein Rab-2A | 3 | 3 | 23.5 | 6.08 | RAB2A_HUMAN | YIIIDTGVG K TSNVEFAFI NTAK IQPQHAATNA THAGNQQGQQ AGGQCC |
| Ras GTPase-activating-like protein IQGAP1 | 2 | 2 | 189.1 | IQGA1_HUMAN | IPQHAATNA THAGNQQGQQ AGGQCC |
| Transforming protein RhoA | 2 | 3 | 21.8 | RHOA_HUMAN | QVELALWDTAGQEDYD QVELALWDTAGQEDYD HFCPVPILVGK |
| Ras-related protein Rab-2A | 2 | 3 | 23.5 | 6.08 | RAB2A_HUMAN | GAAGALLYD ITR TSNVEFAFI NTAK TSNVEFAFI NTAK |
| Ras-related protein Rab-1B | 3 | 8 | 22.2 | 5.55 | RAB1B_HUMAN | VVDNTAKEF ADSLGPFLF TSAK VVDNTAKEF ADSLGPFLF TSAK VVDNTAKEF ADSLGPFLF TSAK EFADSLGPFLF LETSAK EFADSLGPFLF LETSAK EFADSLGPFLF LETSAK EFADSLGPFLF LETSAK NATVNEQAFM TMAAEIK |
| Ras-related protein Rab-7a | 2 | 3 | 23.5 | 6.39 | RAB7A_HUMAN | DPNFPPFWL GNK EAINVEQAFQ TIAR EAINVEQAFQ TIAR |
| Ras-related protein Rab-14 | 2 | 2 | 23.9 | 5.85 | RAB14_HUMAN | TGENVEDAFL EAA AAKK TGENVEDAFL EAAK |
adhesion molecule 1, CE A-related cell adhesion molecule 6, 4F2 cell-surface antigen heavy chain (a.k.a., CD98), epidermal growth factor receptor (EGFR), hypoxia up-regulated protein 1, MUC16 and mTOR, which may be further verified in clinical specimens as biomarkers for PDAC.

In summary, we have demonstrated a proteomic approach for analysis and identification of membrane proteins in primary and metastatic PDAC cells. Many of the identified proteins are known to be modulators of cell-to-cell adhesion and tumor cell invasion. With the potential targets derived from the present study, we will next focus on promising candidates and explore their functional role in cell proliferation, apoptosis or metabolism in PDAC. Similar membrane proteomics approach can be applied to tissue specimens from patients with primary and metastatic tumors to reveal membrane protein targets for prognostic application or therapeutic intervention.

Additional material

Additional file 1: Membrane and membrane-associated proteins identified in AsPC-1 cells (Table S1) and BxPC-3 cells (Table S2). Highlighted proteins were only found in AsPC-1 cells (Table S1) and BxPC-3 cells (Table S2).

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Authors’ contributions

SH conceived of the study, participated in its design and coordination and drafted the manuscript. XLJ and MZ participated in the study design. VLGW participated in the study design and critically reviewed the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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