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Detection and proteomic characterization of extracellular vesicles in human pancreatic juice

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ABSTRACT

Aims: The prognosis of patients with pancreatic cancer has remained virtually unchanged with a high mortality rate compared to other types of cancers. An earlier detection would provide a time window of opportunity for treatment and prevention of deaths.
In the present study we investigated extracellular vesicle (EV)-associated potential biomarkers for pancreatic cancer by directly assessing EV size-based subpopulations in pancreatic juice samples of patients with chronic pancreatitis or pancreatic cancer. In addition, we also studied blood plasma and pancreatic cancer cell line-derived EVs.
Methods: Comparative proteomic analysis was performed of 102 EV preparations from human pancreatic juices, blood, and pancreatic cancer cell lines Capan-1 and MIA PaCa-2. EV preparations were also characterized by electron microscopy, tunable resistive pulse sensing, and flow cytometry.
Results: Here we describe the presence of EVs in human pancreatic juice samples. Pancreatic juice EV-associated proteins that we identified as possible candidate markers for pancreatic cancer included mucins, such as MUC1, MUC4, MUC5AC, MUC6 and MUC16, CFTR, and MDR1 proteins. These candidate biomarkers could also be detected by flow cytometry in EVs found in pancreatic juice and those secreted by pancreatic cancer cell lines.
Conclusions: Together our data show that detection and characterization of EVs directly in pancreatic juice is feasible and may prove to be a valuable source of potential biomarkers of pancreatic cancer.

1. Introduction

Globally pancreatic cancer (PC) is one of the leading causes of cancer mortality due to its poor prognosis. While most types of cancers have seen vast improvements in patient survival within the last decade, only about 5% of PC patients survive beyond 5 years of diagnosis [1,2].

Extracellular vesicles (EVs), nanostructures secreted by most if not all cells [3,4] have been classified based on biogenesis and sizes into different subtypes including exosomes, microvesicles and apoptotic bodies or large oncosomes [3,5–7]. Since no established molecular markers for the individual EV subpopulations are available, here we refer to EV fractions by their size ranges (small, intermediate sized and large EVs with 50–150 nm, 100–1000 nm and >1 μm in diameter, respectively) similar to the terms used by the group of Thery [5]. EVs have been found to carry various molecular cargoes including proteins [8], RNA [9,10], DNA [11], and lipids [12],

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each with corresponding post-synthetic modifications (glycosylation, oxidation, citrullination) [13]. By transferring such molecules, EVs play roles in various processes such as inflammation [14], cancer [8,15,16], and cardiovascular diseases [17,18]. In recent years EVs have attracted significant research interest due to their potential as biomarkers for diseases by carrying molecules from their releasing cells [19]. Exosome-associated glypican-1 was suggested as a biomarker for PC [20] while another study showed exosome-associated miRNA signature to be a superior biomarker of the disease [21]. Additionally, a nanoplasmonic approach was used for detection of PC-associated molecules in EVs in as little as 1 μL of blood plasma [22].

Here we took the approach of characterizing EVs directly in pancreatic juices (PJ) instead of only in the circulation of PC patients as previous reports by our group and others have shown that PJ is a suitable source of EVs [23–26].

Our results show that PJ EVs from various pathologies carry differential proteomic cargos that may serve for biomarker identification and validation.

2. Methods

2.1. Patients and sample collection

Patients with chronic pancreatitis (CP) and adenocarcinomas of pancreatic head (PH-AC) or hepatopancreatic ampulla (A-AC) were treated at the 1st Department of Surgery at Semmelweis University. Supplementary Tables 1 and 2 summarize the data and histological diagnosis of patients enrolled in this study following the AJCC PC Staging System [27–29].

When opening of the pancreatic duct was indispensable for surgery (e.g. partial pancreatic resections, pancreatoduodenectomies, Vater papilla excisions, pancreatic duct decompressions), we punctured it with a cannula (20G) and aspired 1–5 mL PJ without appearance of blood. The surgery was then continued according to routine practices. PJ samples were centrifuged twice at 2,500 g for 15 min and stored at −80 °C until use.

All experimental protocols were approved by the Hungarian Scientific and Research Ethics Committee (TUKEB) and performed in accordance to the guidelines and regulations of the Helsinki Declaration in 1975. All patients signed an informed consent form.

2.2. Cell lines

Capan-1 and MIA PaCa-2 PC cell lines were provided by Dr. Klaus Felix, (Universität Heidelberg, Heidelberg) and were cultured in IMDM containing 20% (v/v) fetal bovine serum (FBS) and DMEM containing 10% (v/v) FBS, respectively. Cell lines were grown with 2 mM glutamine and 1% Ab/AM at 37 °C in 5% CO_2/air, and were regularly tested for Mycoplasma contamination.

2.3. EV isolation

For cell line-derived EVs, cells were washed three times with PBS, and EV production took place for 24 h in serum-free medium to avoid contamination with EVs present in FBS. Viability under serum free conditions for 24 h was >90–95% as confirmed with annexin V-FITC and propidium iodide (both from BD Biosciences) staining as described before [6]. Three size-based EV fractions were isolated. For cell line-derived EVs, cells were first depleted by centrifugation at 300*g* for 10 min. For PJ-derived EVs, samples were diluted 4 times in PBS before EV isolation. Next, samples were filtered by gravity through a 5 μm filter (Millipore) and centrifuged at 2,000 g for 10 min at room temperature resulting in pellets enriched in large EVs. Supernatants were next filtered by gravity through a 0.8 μm filter (Millipore) and centrifuged at 12,600 g for 30 min at room temperature to result in pellets enriched in intermediate EVs. Finally, supernatants were ultracentrifuged in an Optima MAX-XP ultracentrifuge with MLA-55 rotor (Beckman Coulter Inc.) at 100,000 g for 70 min at 4 °C to result in pellets enriched in small EVs.

PJ-derived EVs were also isolated by using anti-CD63 magnetic beads (Thermo Fisher). Briefly, PJ samples were centrifuged at 300 g for 5 min and then 2,000 g 20 min. Magnetic beads were blocked by 1% BSA for 30 min. Then 12 μL beads/sample were incubated with 250 μL of undiluted PJ supernatant overnight with continuous rotation at 4 °C. This was followed by flow cytometric detection after staining of bead-bound EVs with fluorescent anti-CD63 PE (Sigma Aldrich).

2.4. Tunable resistive pulse sensing measurements

EVs were analyzed by qNano (IZON Science) as described previously [6,30,31] with at least 500 particles counts on NP100, NP400, NP800 and NP2000 nanopores. Events were recorded with stable current (115–145 nA), root mean square noise below 12 pA, and linear particle rates using calibration beads CPC100B, CPC400E, CPC800D and CPC2000D (all from IZON).

2.5. Transmission electron microscopy

EV pellets were fixed with 4% paraformaldehyde, washed with PBS, and post-fixed in 1% OsO_4 (Taab) for 30 min. After rinsing with distilled water, pellets were dehydrated in graded ethanol, including block staining with 1% uranyl-acetate in 50% ethanol for 30 min, and were embedded in Taab 812 (Taab). Overnight polymerization of samples at 60 °C was followed by sectioning and analysis using a Hitachi 7100 electron microscope (Hitachi Ltd.).

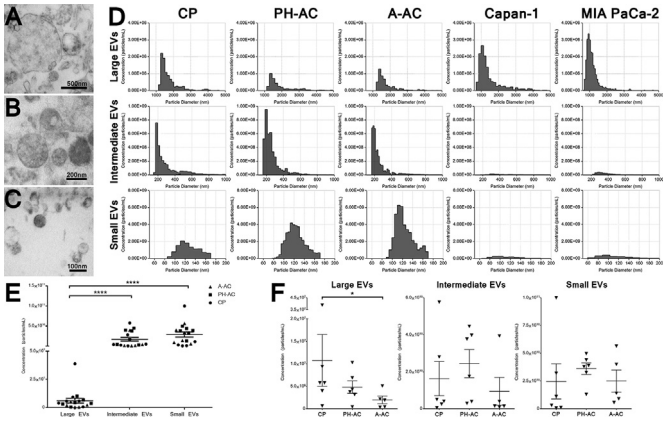


Fig. 1. Morphology, size, and concentration of pancreatic juice and cell line-derived extracellular vesicles.
Panels A, B, and C, show representative electron microscopic images of large, intermediate, and small extracellular vesicles, respectively, from human pancreatic juice. Panel D shows representative size distributions of large, intermediate, and small extracellular vesicles found in human pancreatic juice of patients with chronic pancreatitis (CP), and adenocarcinomas of the pancreatic head (PH-AC) or hepatopancreatic ampulla (A-AC), as well conditioned media of two pancreatic cancer cell lines (Capan-1, MIA PaCa-2). Panel E shows concentration (Mean ± SEM) of large, intermediate, and small extracellular vesicles found in pancreatic juices of all patients while Panel F shows separately those of patients with CP (n = 6), and PH-AC, (n = 6) or A-AC, (n = 5). Mann Whitney tests revealed statistically significant differences when comparing large to intermediate or small extracellular vesicles across all patients but no statistically significant differences between the patient groups except for patients with A-AC having significantly lower amounts of large EVs as compared to patients with CP.

2.6. Flow cytometry of extracellular vesicles

EVs were incubated with 1 μl of each affinity reagent, antibody or isotype control (listed in Supplementary Table 3) for 30 min at room temperature in the dark in 50 μL followed by dilution to 300 μL prior to measurement. Autofluorescence and fluorescence of the affinity reagents in the absence of EVs were also determined, and 0.1% Triton X100 lysis was used as a control to disrupt EV signals. Isolated small EVs were coupled to 4 μm aldehyde/sulphate latex beads (Life Technologies), and blocked with 1% bovine serum albumin in PBS for 2 h followed by 100 mM glycine. For Supplementary Fig. 5A, instrument settings and gates were set as shown in Supplementary Fig. 1A. For Supplementary Fig. 5B instrument settings and gates were set as described earlier [6,30,32] using Megamix beads (BioCytex) and 1 μm Silica Beads Fluo-Green (Kisker) as shown in Supplementary Fig. 1B. Data were analyzed using FlowJo software (Treestar).

2.7. Mass spectrometry of EV preparations

EV pellets were re-suspended in 10 μL water and proteins were extracted using repeated freeze-thaw cycles followed by miniaturized tryptic digestion as previously described [33]. Peptide digests were analyzed using a Bruker Maxis II Q-TOF (Bremen, Germany) with CaptiveSpray nanoBooster ionization source. Peptides were separated online using a 25 cm Waters Peptide BEH C18 nanoACQUITY 1.7 μm particle size UPLC column on the Dionex Ultimate 3000 Nano LC System (Sunnyvale, CA, USA). Trapping was performed on an Acclaim™ PepMap100™ C18 NanoTrap column (5 μm, 100 Å, 100 μm × 20 mm, Thermo Fisher). Data were evaluated with Funrich [34] and Scaffold 4.5.3 (http://www.proteomesoftware.com), using Mascot (Matrix Science, London, UK; version Mascot 2.5) and X!Tandem (The GPM, thegpm.org; version 2007.01.01.1) search engines. Protein identification criteria against Swissprot (2015_08) database was in accordance to strict criteria of Molecular and Cellular Proteomics guidelines. Briefly, 7 ppm peptide mass tolerance, 0.05 Da fragment mass tolerance and 2 missed cleavages were allowed and the following modifications were searched: carbamidomethylation as fixed modification, while deamidation (NQ), oxidation (M) and pyro-carbamidomethylation (N-term C) as variable modifications.

2.8. Protein and lipid determination of EV preparations

Protein content of EVs was determined using the Micro BCA Protein Assay Reagent Kit (Thermo Fisher), and lipid content was determined with the sulfophosphovanilin reaction as described earlier [6].

2.9. Statistical analysis

Data were analyzed with GraphPad Prism v.4 using unpaired *t*-test for parameters with normal distribution and Mann Whitney test for parameters with non-normal distribution. P values lower than 0.05 were considered statistically significant.

2.10. Data availability

MS datasets are available from the publication date on EVpedia

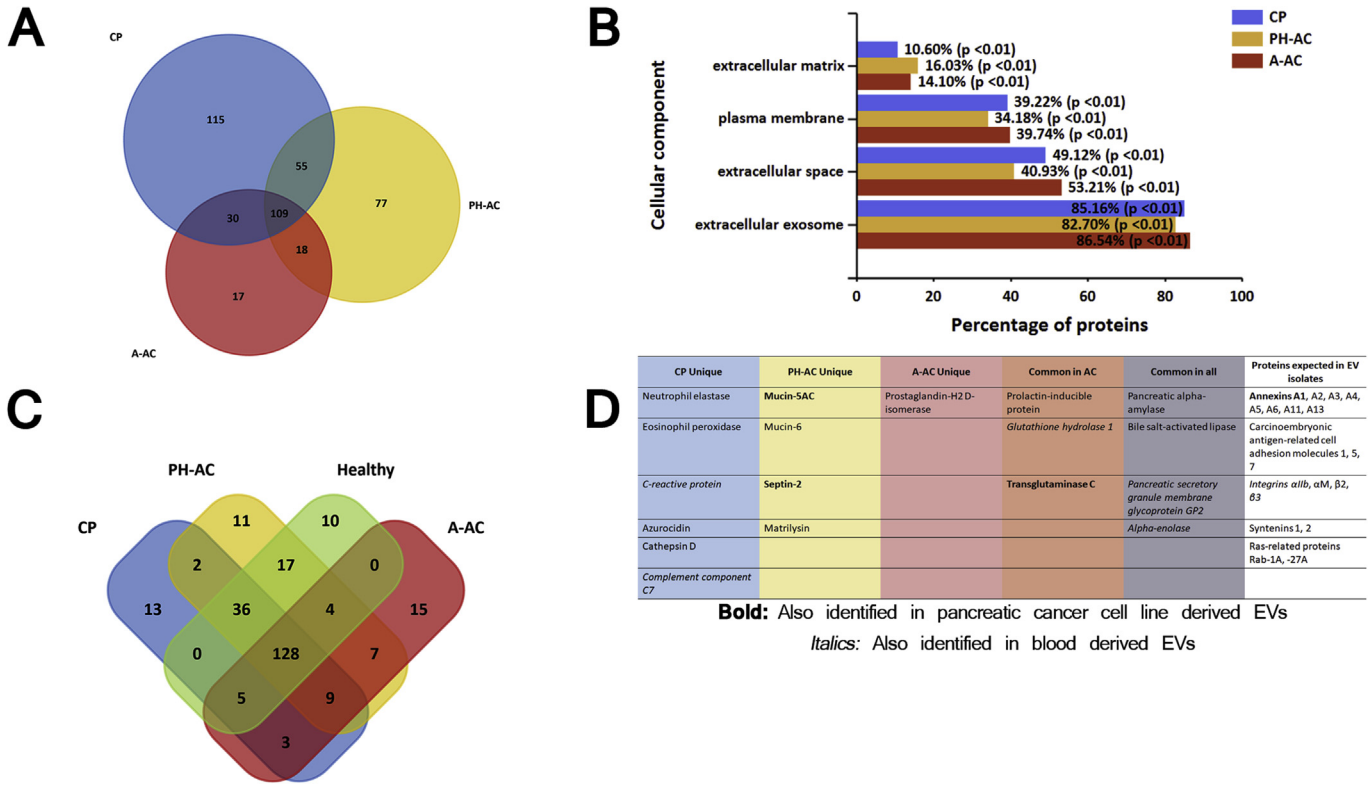


Fig. 2. Mass spectrometry of large extracellular vesicles in pancreatic juice and blood plasma.
Panel A shows shared and unique proteins found in large extracellular vesicles from pancreatic juice samples of patients with chronic pancreatitis (CP), and adenocarcinomas of the pancreatic head (PH-AC) or hepatopancreatic ampulla (A-AC). Panel B shows cellular component analysis of proteins found in large extracellular vesicles from pancreatic juices of each patient group. Panel C shows shared and unique proteins in large extracellular vesicles from blood plasma of the above patient groups as well as healthy donors. Panel D shows some of the unique and common proteins as well as those expected to be present in extracellular vesicle isolates were found in large extracellular vesicles in pancreatic juices of the patient groups.

(http://www.evpedia.info/), Vesiclepedia (http://microvesicles.org/), and Exocarta (http://www.exocarta.org/). All other data generated or analyzed during this study are included in this published article (and its Supplementary Information files).

3. Results

3.1. Human pancreatic juice samples contain extracellular vesicles

Human PJ samples were obtained by intraoperative punctures of patients with CP, PH-AC, or A-AC. PJ-derived small, intermediate, and large EVs were analyzed by TEM showing characteristic phospholipid bilayer-enclosed EV structures in all pellets (Fig. 1A–C). Furthermore, EV pellets of conditioned media of Capan-1 cells showed similar structures (Supplementary Fig. 2). Protein to lipid ratios of PJ EVs were found within the expected ranges for typical EV preparations with values of 0.41 ± 0.09, 0.30 ± 0.06, and 0.37 ± 0.07 (Mean ± SEM) for large, intermediate, and small EVs respectively (Supplementary Fig. 3).

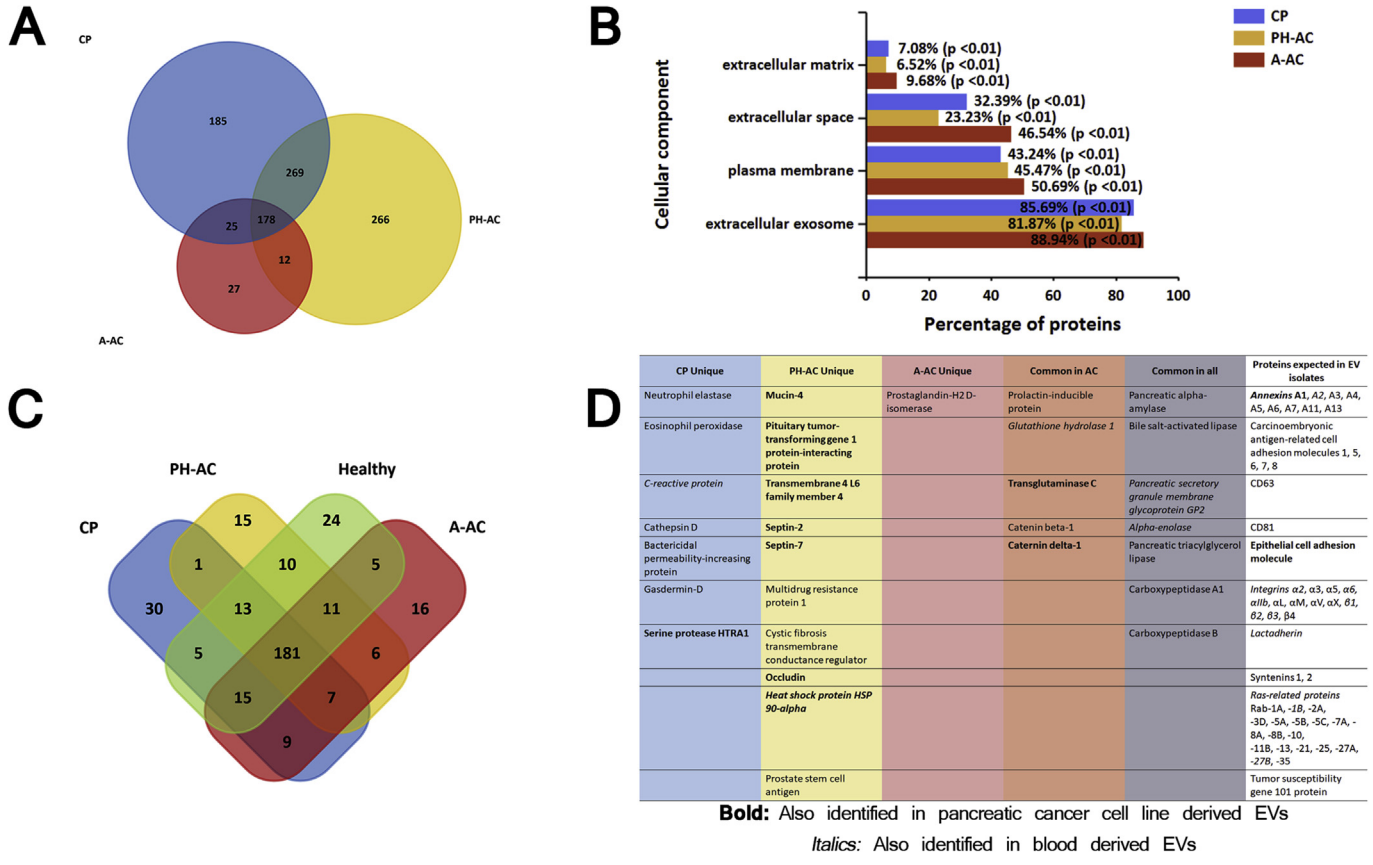
3.2. Size and concentration of EVs in human pancreatic juice samples

PJ samples from CP (n = 6), PH-AC (n = 6), and A-AC (n = 5) patients were submitted to TRPS-based size and concentration determination. Fig. 1D shows representative particle size distributions. PJs had EVs with diameter sizes of 1646.0 ± 41.5 nm, 189.8 ± 11.41 nm, and 125.4 ± 3.8 nm (Mean ± SEM) for large, intermediate, and small EVs respectively. While the diameter sizes of PJ and PC cell line derived EVs were similar, PJ had higher concentrations of small and intermediate but not large EVs (Fig. 1D). TRPS showed that small EVs were more abundant in PJ samples than intermediate and large ones across all patient groups with mean concentrations of $6.1 \pm 2.2 \times 10^6$, $1.7 \pm 0.5 \times 10^{10}$, and $2.9 \pm 0.6 \times 10^{10}$ particles/mL (Mean ± SEM) for large, intermediate, and small EVs respectively (Fig. 1E). However, patient groups did not significantly differ in concentrations of small and intermediate sized EVs, but patients with A-AC had significantly lower amounts of large EVs as compared to patients with CP with mean concentrations of $2.0 \pm 0.9 \times 10^6$ particles/mL compared to of $1.1 \pm 0.6 \times 10^7$ particles/mL (Mean ± SEM) (Fig. 1F).

3.3. Mass spectrometry of EVs

Mass spectrometry of EV pellets from PJ, conditioned media of PC cell lines, and blood plasma samples identified high number of proteins with at least two peptides in EV fractions isolated from each source (Supplementary Table 4). MS data from PJ-derived EVs showed relative enrichment with proteins expressed in pancreas as compared to blood or bile fluid (Supplementary Fig. 4).

Next, we compared the identified proteins in EV pellets among patients with CP, PH-AC, and A-AC (n = 4 in each group). Fig. 2A and



CP Unique	PH-AC Unique	A-AC Unique	Common in AC	Common in all	Proteins expected in EV isolates
Neutrophil elastase	**Mucin-4**	Prostaglandin-H2 D-isomerase	Prolactin-inducible protein	Pancreatic alpha-amylase	***Annexins*** **A1**, *A2*, A3, A4, A5, A6, A7, A11, A13
Eosinophil peroxidase	**Pituitary tumor-transforming gene 1 protein-interacting protein**		*Glutathione hydrolase 1*	Bile salt-activated lipase	Carcinoembryonic antigen-related cell adhesion molecules 1, 5, 6, 7, 8
C-reactive protein	**Transmembrane 4 L6 family member 4**		**Transglutaminase C**	*Pancreatic secretory granule membrane glycoprotein GP2*	CD63
Cathepsin D	**Septin-2**		Catenin beta-1	*Alpha-enolase*	CD81
Bactericidal permeability-increasing protein	**Septin-7**		**Caternin delta-1**	Pancreatic triacylglycerol lipase	**Epithelial cell adhesion molecule**
Gasdermin-D	Multidrug resistance protein 1			Carboxypeptidase A1	*Integrins α2*, α3, α5, *α6, αIIb*, αL, αM, αV, αX, *β1, β2, β3*, β4
Serine protease HTRA1	Cystic fibrosis transmembrane conductance regulator			Carboxypeptidase B	*Lactadherin*
	Occludin				Syntenins 1, 2
	Heat shock protein HSP 90-alpha				*Ras-related proteins* Rab-1A, *-1B*, -2A, -3D, -5A, -5B, -5C, -7A, -8A, -8B, -10, -11B, -13, -21, -25, -27A, *-27B*, -35
	Prostate stem cell antigen				Tumor susceptibility gene 101 protein

Bold: Also identified in pancreatic cancer cell line derived EVs

Italics: Also identified in blood derived EVs

Fig. 3. Mass spectrometry of intermediate extracellular vesicles in pancreatic juice and blood plasma.
Panel A shows shared and unique proteins found in intermediate extracellular vesicles from pancreatic juice samples of patients with chronic pancreatitis (CP), and adenocarcinomas of the pancreatic head (PH-AC) or hepatopancreatic ampulla (A-AC). Panel B shows cellular component analysis of proteins found in intermediate extracellular vesicles from pancreatic juices of each patient group. Panel C shows shared and unique proteins in intermediate extracellular vesicles from blood plasma of the above patient groups as well as healthy donors. Panel D shows some of the unique and common proteins as well as those expected to be present in extracellular vesicle isolates were found in intermediate extracellular vesicles in pancreatic juices of the patient groups.

C shows Venn diagrams of unique and shared proteins in pellets of large EVs from PJ (A) and blood plasma (C) for each disease group. Fig. 2B shows cellular component Gene Ontology (GO) annotation of proteins identified in large EVs from PJ with most proteins belonging to the "extracellular exosome" GO category. Fig. 2D shows candidate markers found in PJs including those i) detected uniquely in each patient group, ii) commonly in both adenocarcinomas, iii) in all large EVs, and iv) considered typical proteins expected in EV isolates [35]. Bold letters show proteins also identified in EVs of PC cell lines. Italics show proteins found in blood plasma as well. Figs. 3 and 4 show results obtained with intermediate and small EVs pellets, respectively.

3.4. Flow cytometric detection of the candidate pancreatic cancer proteins associated with EVs

We used flow cytometry for detection of candidate markers in EVs isolated from PJs and conditioned media of PC cell lines. Supplementary Fig. 5A shows PJ EVs stained with fluorescent antibodies to known EV proteins as well as proteins identified by MS of PJ EV isolates. PJ EVs showed positivity for CD63, CD81, CD107a (LAMP-1), MUC5AC, CFTR, and TO-PRO3 DNA binding dye. Supplementary Fig. 5B shows detection of MUC1, MUC4, MUC5AC, CFTR, and MDR1, as well as annexin V on EVs isolated from PC cell lines. While some differences were observed in staining intensities, all markers were present in EV fractions secreted by the tested PC cell lines. Supplementary Fig. 5C demonstrates PJ EVs immunocaptured using magnetic beads showing positivity for the membrane tetraspanin CD63.

4. Discussion

PC remains a malignancy with an outstandingly high mortality rate and EVs have been suggested to represent a promising source of circulating markers for tumors like PC. In this study and earlier reports from our group [25,26], we have shown for the first time that EVs are present in human PJ samples. This finding allowed for a novel approach whereby EVs were directly analyzed from PJs of cancer patients.

The presence of EVs in the enzyme-rich milieu of PJ is somewhat unexpected. Multiple evidence for the existence of EVs in PJ samples are presented in this study. These include i) electron microphotographs (Fig. 1A–C), ii) particle detection in typical EV size ranges by TRPS (Fig. 1D–F), iii) enrichment of proteins in EVs isolates corresponding to the "extracellular exosome" GO category including typical proteins expected in EV isolates [35] (Figs. 2–4, iv) detection of EV markers (CD63, CD81 and LAMP-1) on PJ EVs by flow cytometry (Supplementary Fig. 5A) and v) demonstration of the presence of CD63 positive EVs captured by immunoaffinity on magnetic beads (Supplementary Fig. 5C).

This study differed from previous ones where EV biomarkers were searched either from conditioned media of PC cells or from the circulation, where the vast majority of EVs are of non-tumor origin [19–22]. Another original aspect of this study was analysis

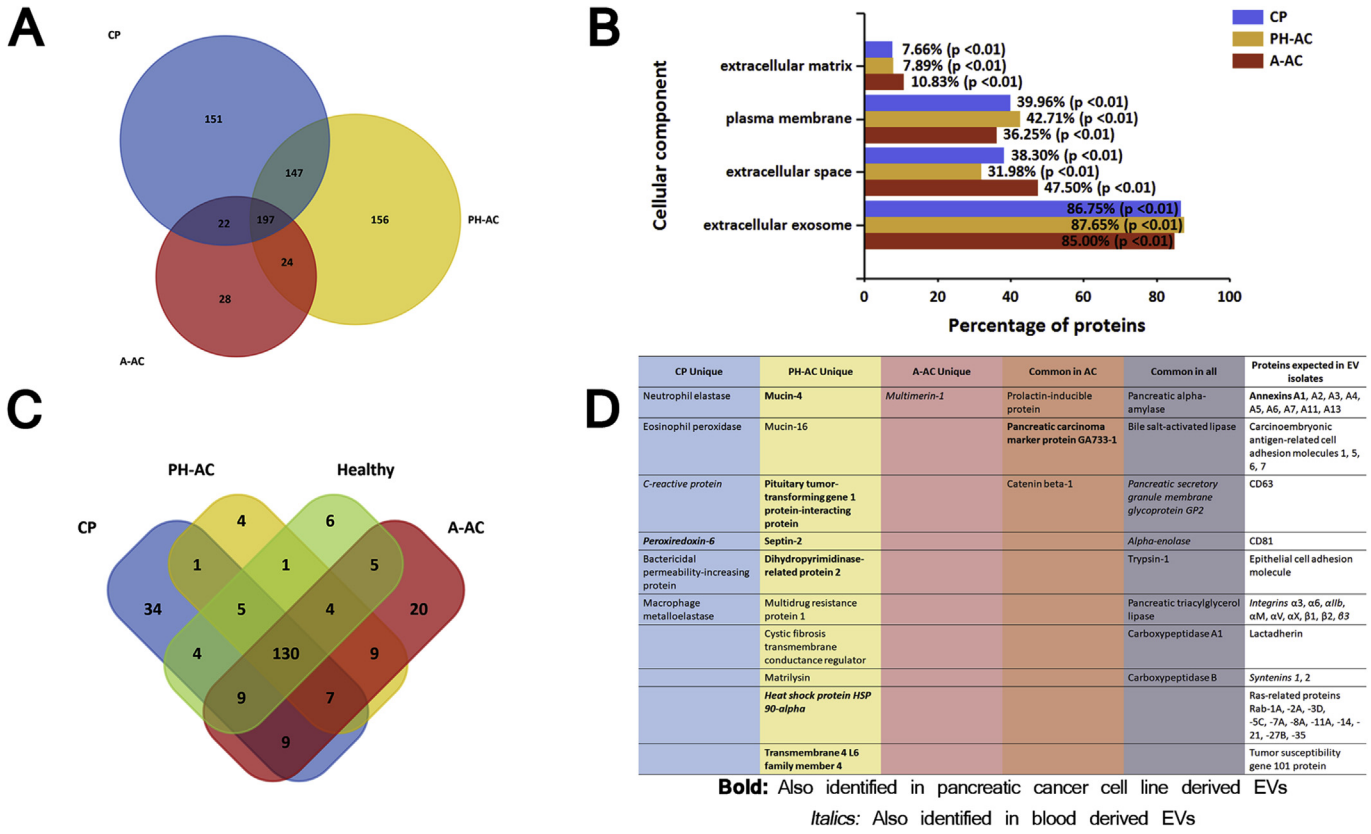


Fig. 4. Mass spectrometry of small extracellular vesicles in pancreatic juice and blood plasma.
Panel A shows shared and unique proteins found in small extracellular vesicles from pancreatic juice samples of patients with chronic pancreatitis (CP), and adenocarcinomas of the pancreatic head (PH-AC) or hepatopancreatic ampulla (A-AC). Panel B shows cellular component analysis of proteins found in small extracellular vesicles from pancreatic juices of each patient group. Panel C shows shared and unique proteins in small extracellular vesicles from blood plasma of the above patient groups as well as healthy donors. Panel D shows some of the unique and common proteins as well as those expected to be present in extracellular vesicle isolates were found in small extracellular vesicles in pancreatic juices of the patient groups.

of separate size-based EV fractions inspired by previous works from our laboratory and others showing that size-based EV fractions differ in nucleic acid [10], protein [5], and lipid properties [6].

Our proteomic analysis revealed that 41.8%, 48.4% and 50.1% of the identified proteins were unique to the different size-based EV fractions of patients with CP, PH-AC and A-AC, respectively. These data confirm that a specific EV fraction may carry markers not present in other fractions. More importantly, we found unique proteins for each patient group in the EV fractions. These included Mucin-4, Mucin-5AC, Mucin-6, and Mucin-16, detected exclusively in PJ EVs of PH-AC patients in accordance with the long known association of mucins with PC [36]. Other characteristic PH-AC proteins included HSP 90 alpha, cystic fibrosis transmembrane conductance regulator (CFTR) and multidrug resistance protein 1 (MDR1). MDR1 was shown earlier in association with prostate cancer-derived EVs [37]. Furthermore, CFTR has been found in association with EVs [38] and it has also been implicated in the pathomechanism of PC with mutations increasing the risk for this malignancy [39]. Importantly, we validated the presence of some of these proteins by flow cytometry on PC cell line-derived and PJ-derived EVs.

While this and other studies [20–22,24–26,40] provide some molecular candidates for diagnosis of PC, there is need for further validation with greater number of patient samples before EV diagnostics become a reality in the clinic. Nevertheless, in this study we provide evidence that PJ is a biofluid in which EVs are present and can be isolated to serve as potential sources of biomarkers for PC.

Author contributions

XO, ÁS, LT, EIB designed experiments. XO, ÁS, MB, MR, KP, BS, ZS, KST, KV, LT performed experiments; XO, LT analyzed data. ÁK performed TEM analysis. XO, LT, and KV carried out MS analysis. XO, ÁS, ÉP, KV, LH, LT, ZW, and EIB wrote and edited the paper.

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Conflicts of interest

XO completed this research while at Semmelweis University but has been employed at AstraZeneca since September 2016. All other authors declare no conflicts of interests.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bbrc.2018.03.107

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.bbrc.2018.03.107

References

[1] M. Ilic, I. Ilic, Epidemiology of pancreatic cancer, World J. Gastroenterol. 22 (2016) 9694–9705.
[2] A. Vincent, J. Herman, R. Schulick, et al., Pancreatic cancer, Lancet (London, Ed.) 378 (2011) 607–620.
[3] B. György, T.G. Szabó, M. Pásztói, et al., Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles, Cell. Mol. Life Sci. 68 (2011) 2667–2688.
[4] C. Théry, Exosomes: secreted vesicles and intercellular communications, F1000 Biol. Rep. 3 (2011).
[5] J. Kowal, G. Arras, M. Colombo, et al., Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes, Proc. Natl. Acad. Sci. U.S.A. 113 (2016) E968–E977.
[6] X. Osteikoetxea, A. Balogh, K. Szabó-Taylor, et al., Improved characterization of EV preparations based on protein to lipid ratio and lipid properties, PLoS One 10 (2015) e0121184.
[7] V.R. Minciacchi, S. You, C. Spinelli, et al., Large oncosomes contain distinct protein cargo and represent a separate functional class of tumor-derived extracellular vesicles, Oncotarget 6 (2015) 11327–11341.
[8] K. Al-Nedawi, B. Meehan, J. Micallef, et al., Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells, Nat. Cell Biol. 10 (2008) 619–624.
[9] H. Valadi, K. Ekström, A. Bossios, et al., Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells, Nat. Cell Biol. 9 (2007) 654–659.
[10] R. Crescitelli, C. Lasser, T.G. Szabo, et al., Distinct RNA profiles in subpopulations of extracellular vesicles: apoptotic bodies, microvesicles and exosomes, J. Extracell. Vesicles 2 (2013).
[11] E. Lázaro-Ibáñez, A. Sanz-Garcia, T. Visakorpi, et al., Different gDNA content in the subpopulations of prostate cancer extracellular vesicles: apoptotic bodies, microvesicles, and exosomes, Prostate 74 (2014) 1379–1390.
[12] A. Llorente, T. Skotland, T. Sylvänne, et al., Molecular lipidomics of exosomes released by PC-3 prostate cancer cells, Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1831 (2013) 1302–1309.
[13] K. Szabo-Taylor, B. Ryan, X. Osteikoetxea, et al., Oxidative and other post-translational modifications in extracellular vesicle biology, Semin. Cell Dev. Biol. 40 (2015) 8–16.
[14] E.I. Buzás, B. Gyorgy, G. Nagy, et al., Emerging role of extracellular vesicles in inflammatory diseases, Nat. Rev. Rheumatol. 10 (2014) 356–364.
[15] H. Peinado, M. Aleckovic, S. Lavotshkin, et al., Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET, Nat. Med. 18 (2012) 883–891.
[16] A. Zomer, C. Maynard, F.J. Verweij, et al., Vivo imaging reveals extracellular vesicle-mediated phenocopying of metastatic behavior, Cell 161 (2015) 1046–1057.
[17] X. Yu, L. Deng, D. Wang, et al., Mechanism of TNF-α autocrine effects in hypoxic cardiomyocytes: initiated by hypoxia inducible factor 1α, presented by exosomes, J. Mol. Cell. Cardiol. 53 (2012) 848–857.
[18] X. Osteikoetxea, A. Nemeth, B.W. Sodar, et al., Extracellular vesicles in cardiovascular diseases, are they Jedi or Sith? J. Physiol. 594 (2016) 2881–2894.
[19] J. Skog, T. Würdinger, S. van Rijn, et al., Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers, Nat. Cell Biol. 10 (2008) 1470–1476.
[20] C. Thery, Cancer: diagnosis by extracellular vesicles, Nature 523 (2015) 161–162.
[21] X. Lai, M. Wang, S.D. McElyea, et al., A microRNA signature in circulating exosomes is superior to exosomal glypican-1 levels for diagnosing pancreatic cancer, Canc. Lett. 393 (2017) 86–93.
[22] K. Liang, F. Liu, J. Fan, et al., Nanoplasmonic quantification of tumour-derived extracellular vesicles in plasma microsamples for diagnosis and treatment monitoring, Nat. Biomed. Eng. 1 (2017) 0021.
[23] A.R. Beaudoin, G. Grondin, A. Vachereau, et al., Detection and characterization of microvesicles in the acinar lumen and in juice of unstimulated rat pancreas, J. Histochem. Cytochem. : official journal of the Histochemistry Society 34 (1986) 1079–1084.
[24] J. Zheng, J.M. Hernandez, A. Doussot, et al., Extracellular matrix proteins and carcinoembryonic antigen-related cell adhesion molecules characterize pancreatic duct fluid exosomes in patients with pancreatic cancer, HPB (Oxford): the official journal of the International Hepato Pancreato Biliary Association (2018 Jan 12) pii: S1365-182X(17)31197-8.
[25] A. Szucs, X. Osteikoetxea Vélez, L. Harsanyi, et al., Detection and characterization of extracellular vesicles in patients with pancreatic cancer, Pancreatology, 15 S119.
[26] X. Osteikoetxea, K. Vékey, Á. Szucs, et al., Detection and characterization of extracellular vesicles in pancreatic juice and blood of patients with pancreatic cancer, in: The Fifth International Meeting of ISEV, ISEV2016, Co-Action Publishing, Rotterdam, The Netherlands, 2016, pp. 10.3402/jev.v3405.31552.
[27] S.B. Edge, D.R. Byrd, C.C. Compton, et al., AJCC Cancer Staging Manual, Springer, New York, 2010.
[28] S.B. Edge, C.C. Compton, The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM, Ann. Surg Oncol. 17 (2010) 1471–1474.
[29] K.Y. Bilimoria, D.J. Bentrem, C.Y. Ko, et al., Validation of the 6th edition AJCC pancreatic cancer staging system: report from the National cancer database, Cancer 110 (2007) 738–744.
[30] X. Osteikoetxea, B. Sódar, A. Németh, et al., Differential detergent sensitivity of extracellular vesicle subpopulations, Org. Biomol. Chem. 13 (2015)

9775–9782.
[31] R. Vogel, F.A.W. Coumans, R.G. Maltesen, et al., A standardized method to determine the concentration of extracellular vesicles using tunable resistive pulse sensing, J. Extracell. Vesicles 5 (2016).
[32] B.W. Sodar, A. Kittel, K. Paloczi, et al., Low-density lipoprotein mimics blood plasma-derived exosomes and microvesicles during isolation and detection, Sci. Rep. 6 (2016) 24316.
[33] L. Turiák, P. Misják, T.G. Szabó, et al., Proteomic characterization of thymocyte-derived microvesicles and apoptotic bodies in BALB/c mice, J. Proteonomics 74 (2011) 2025–2033.
[34] M. Pathan, S. Keerthikumar, C.-S. Ang, et al., FunRich: an open access standalone functional enrichment and interaction network analysis tool, Proteomics 15 (2015) 2597–2601.
[35] J. Lötvall, A.F. Hill, F. Hochberg, et al., Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the International Society for Extracellular Vesicles, J. Extracell. Vesicles 3 (2014), https://doi.org/10.3402/jev.v3403.26913.
[36] D. Moschovis, G. Bamias, I. Delladetsima, Mucins in neoplasms of pancreas, ampulla of Vater and biliary system, World J. Gastrointest. Oncol. 8 (2016) 725–734.
[37] C. Corcoran, S. Rani, K. O'Brien, et al., Docetaxel-resistance in prostate cancer: evaluating associated phenotypic changes and potential for resistance transfer via exosomes, PLoS One 7 (2012) e50999.
[38] C. Vituret, K. Gallay, M.P. Confort, et al., Transfer of the cystic fibrosis transmembrane conductance regulator to human cystic fibrosis cells mediated by extracellular vesicles, Hum. Gene Ther. 27 (2016) 166–183.
[39] R.R. McWilliams, G.M. Petersen, K.G. Rabe, et al., Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations and risk for pancreatic adenocarcinoma, Cancer 116 (2010) 203–209.
[40] S. Heiler, Z. Wang, M. Zöller, Pancreatic cancer stem cell markers and exosomes - the incentive push, World J. Gastroenterol. 22 (2016) 5971–6007.