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Characterization of extracellular vesicle miRNA identified in peripheral blood of chronic pancreatitis patients

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Abstract

Plasma-derived extracellular vesicles (EV) can serve as markers of cell damage/disease but can also have therapeutic utility depending on the nature of their cargo, such as miRNA. Currently, there are challenges and lack of innovations regarding early diagnosis and therapeutic options within different aspects of management of patients suffering from chronic pancreatitis (CP). Use of EV as biomarkers for pancreatic health and/or as adjuvant therapy would make a difference in management of these patients. The aim of this study was to characterize the miRNA cargo of EV purified from the plasma of CP patients and compared to those of healthy participants. EVs were isolated from plasma of 15 CP patients and 10 healthy controls. Nanoparticle tracking analysis was used to determine frequency and size, while NanoString technology was used to characterize the miRNA cargo. Relevant clinical parameters were correlated with EV miRNA cargo. ~ 30 miRNA species were identified to have significantly ($p < 0.05$) different expression in EV from individuals with CP compared to healthy individuals; ~ 40 miRNA were differentially expressed in EV from pre-diabetic versus non-diabetic CP patients. miR-579-3p, while exhibiting significantly lower (~ 16-fold) expression in CP compared to healthy and lower (~ 24-fold) in CP narcotic users compared to the non-users, is actually enriched (~ 32-fold) within EV in pre-diabetic CP patients compared to non-diabetic CP patients. A unique pattern was identified in female CP patients. These data support the prospect of using a plasma-derived EV cargo to assess pancreatic health and its therapeutic potential in CP patients.

Keywords Chronic pancreatitis · Extracellular vesicles · Total pancreatectomy · Auto-islet transplant · Exosome

Introduction

Chronic pancreatitis (CP) is a systemic inflammatory disease, characterized by irreversible morphologic changes that cause failure of the pancreas' exocrine and endocrine function [1]. The main objectives of treatment are pain management, nutrition, and the prevention of complications such as brittle type 3c diabetes mellitus (DM) or pancreatic cancer. Upon exhaustion of medical and endoscopic options, a range of surgical therapies are usually offered, including parenchymal preserving surgical procedures and total pancreatectomy (TP) with or without autologous islet cells

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transplantation (AIT) into the liver [2–5]. The success of a TPAIT is inherently dependent on three factors: the quality of islets in the diseased pancreas, their survival during isolation, and their environment within the transplanted organ [6, 7]. Presently, there are no tests to directly evaluate beta cell health and indirect measures, such as evaluating glycemic parameters (hemoglobin A1C, c peptide, continuous glucose monitoring), are often abnormal later in the disease process. Major factors in islet loss post-transplant include a combination of a non-specific innate immune response, pre-existing and transplant-induced cellular immune responses, and variations in hepatic cellular environments including vascular supply [8–11]. Though advances have been made in the last few years, there remain many challenges and a paucity of innovations regarding not only therapeutic options but also early diagnosis of complications. Thus, the goal of this investigation was to use novel approaches to identify the potential for extracellular vesicles (EV) to serve as biomarkers for diagnosis of complications.

Cells continuously secrete micro-vesicles, nano-vesicles, macromolecular complexes, and small molecules into the extracellular space. These EVs are found in abundance in body fluids, including blood, saliva, urine, and breast milk [12, 13]. EV contain nucleic acids, protein, and/or other biomolecules serving of carriers of this 'cargo.' As a mechanism of cell–cell communication, EVs deliver this cargo to cells in diverse locations in the body and thus can regulate many pathways including, but not limited to, angiogenesis, inflammation, and cell migration [14] and have been thus shown to induce tissue health and regeneration by delivering growth factors, proteins, miRNA, mRNA, non-coding RNA, and lipids [12, 15]. Depending on the nature of the cargo, EVs can play a role in immunomodulation of the microenvironment, making them a novel therapeutic option in many disease conditions [16]. Most relevant to this study, EVs have shown to be markers of cellular stress and are currently being used as biomarkers to predict and monitor the progression of various diseases [17, 18]. For these reasons, EVs represent a promising avenue for identifying and monitoring outcomes after TPAIT.

Characteristics of EVs in the plasma of patients with CP have not been described in the literature. It is plausible that the cargo characteristics of EVs could predict and monitor the progression of complications of CP, such as brittle type 3c DM and cancer. The identification of EV-specific biomarkers could drive patient-centered care and has the potential to be of extreme value in improving the outcomes and quality of life for patients with CP. Additionally, if the precise EVs with anti-inflammatory and angiogenesis potential could be identified and purified, they could be employed as therapeutics. The aim of this project was to characterize EVs purified from CP patients by their physical characteristics and miRNA content and to compare them to EVs purified from healthy participants. These data will inform the development of biomarkers as well as therapeutics for CP patients who undergo TPAIT. We hypothesize that plasma-derived EV miRNA content will reflect stages/severity of disease and thus islet health.

Methods

Patient enrollment

This was a collaborative study between the University of North Carolina at Chapel Hill Department of Surgery and the University of Miami Interdisciplinary Stem Cell Institute over a one-year period. After IRB approval, UNC personnel and UNC-affiliated clinics recruited two groups of study participants: group 1 patients had been diagnosed with CP, while in group 2, healthy volunteers served as a control. Group 1 patients had an existing diagnosis of CP based on clinical and laboratory parameters confirmed by both computerized tomography (CT) scan and magnetic resonance imaging (MRI) with pancreatic protocol. At the time of informed consent, subjects with acute pancreatitis, acute-on-chronic pancreatitis, or concurrent hepatocellular fibrosis were excluded. Additional exclusion criteria included patients without calcification and/or fibrosis on imaging, patients with pancreatic cancer, and patients in which cancer could not be ruled out by both imaging and negative CEA and CA19-9 levels. In Group 2, subjects were 18- to 60-year-old male or female volunteers with no past medical or surgical history. Potential subjects found to have a history of rheumatoid arthritis, Crohn's disease, immune-mediated colitis, fibromyalgia, polymyalgia rheumatica, giant cell arteritis, systemic lupus erythematosus, organ transplant rejection, and/or liver cirrhosis, history of lymphoma, leukemia, history of Hepatitis B Virus (HBV) infection Hepatitis C Virus (HCV), and/or Human Immunodeficiency Virus (HIV) on a questionnaire administered prior to informed consent were excluded. In both groups, individuals were required to be willing and able to provide up to 50 ml of blood, able to communicate verbally and in writing in English, and willing and able to provide informed oral and written consent. The UNC CP and autologous islet cell transplant program evaluated 180 patients for CP. Forty-seven patients were operated on, including 19 who underwent TPAIT. 15 CP patients and 10 controls were enrolled. Demography of participants is shown in Table 1. From these individuals, a volume of 40–50 ml of blood was collected in four 10 ml tubes with EDTA anticoagulant.

Table 1 Demographics and characteristics

	Cases $n=15$	Control $n=10$
Age (years), mean (SD), patients	44.3 (13.1)	33.4 (7.8)
Male, *n* (%)	9 (60)	3 (30)
Disease duration (months), median (IQR)	29 (18–86)	NA (Not applicable)
# of Endoscopic procedures, median (IQR)	2 (1–3)	NA
Surgeries, *n* (%)		NA
Prior surgery	4 (26.7)	
Future surgery	7 (46.7)	
Hgb A1c (%), median (IQR)	5.6 (5.2–7.0)	NA
C-peptide (ng/ml), median (IQR)	2.5 (1.5–5.1)	NA
Insulin, *n* (%)	3 (20.0)	NA
Diabetes status, *n* (%)		
Nondiabetic	9 (60)	10(100)
Prediabetic	2 (13.3)	0
Diabetic	4 (26.7)	0
Narcotic, *n* (%)	5 (33)	0

Extracellular vesicle preparation

Collected peripheral blood was centrifuged at 2000 *g* for 30 min for plasma separation resulting in an average of 25mls of plasma per participant from which EVs were isolated. Plasma was removed and diluted two times with saline. Diluted plasma was then centrifuged at 20,000 *g* for 30 min to remove large apoptotic bodies prior to ultracentrifugation. Ultracentrifugation (Beckman Coulter) was performed at 100,000 *g* for 120 min using a Ti70 rotor to pellet the remaining EVs. EV pellets were resuspended in 2–3 ml of saline, filtered with a 0.22uM syringe filter, and frozen at -80 °C. EVs are classified primarily based on their size, whereby exosomes are ~ 30–120 nm diameter, and microvesicles (MV) are 100–1000 nm diameter [19–21]. As current isolation methods do not allow for separation of exosomes (30–120 nm) and small MV (100–120 nm), this study interrogated these populations together and are refer to as EV throughout.

Nanoparticle tracking analysis

Nanoparticle tracking analysis was performed on the final EV products using the ZetaView QUATT instrument (Particle Metrix) and ZetaView (version 8.05) software. Mean concentrations and mode size were determined from 10 videos taken of one sample analyzed at a 1:1000 dilution with filtered PBS with a 488 nm laser, pump speed 30, and camera shutter of 100. Each measurement obtained from the 10 videos was internally quality controlled by the instrument, with videos removed for failing quality control.

Transmission electron microscopy analysis of EV

Copper carbon formvar grids were glow discharged immediately prior to loading with the sample. CP and healthy EV samples were processed undiluted. The grid was floated on 10 µL sample drop for 5 min, washed two times with water by floating on the drop of water for 30 s, and negatively stained with 2% uranyl acetate by floating on the drop of stain for 30 s. The grid was blot dried with Whatman paper and imaged with a Jeol-1230 electron microscope.

miRNA analysis

In order to interrogate the miRNA cargo of these EVs, we employed NanoString technology and the nCounter Human miRNA Panel v2 allowing for simultaneous evaluation of 800 miRNAs [22]. RNA was isolated from EVs which were disrupted with 10% TritonX. exoRNeasy Kit Part II (QIAcube) and miRNA easy Kit were used for total mRNA isolation as per the manufacturer's protocol. The quantity and quality of the mRNA in each sample was determined (A260/A280 and A260/A230), by NanoDrop ND1000 (NanoDrop Technologies, Waltham, MA, USA). 100 ng of total EV mRNA was used for human NanoString nCounter miRNA microarray assay (Nanostring Technologies, Seattle, WA, USA) according to the manufacturer's instructions. These miRNAs were hybridized to probes at 65 °C for 30 h. Hybridized probes were extended and quantified using the nCounter Prep Station and Digital Analyzer. The nCounter-generated relative fluorescent intensities were analyzed using nSolver 4.0 software according to the manufacturer's instructions (Nanostring Technologies, Seattle, WA, USA). Associated data are held in NCBI GEO GSE173514.

Statistical analysis

Data from each sample were normalized using built-in positive controls to control for technique in hybridization, housekeeping genes to account for varying sample degradation, and built-in negative controls to account for background signal. Samples were grouped according to their parameters. For each miRNA, fold changes between groups were calculated using the mean of the normalized samples and the significance was calculated using an unpaired t-test. *P* values adjusted for multiple comparisons were determined.

Results

Isolation and characterization of EVs

We determined the concentration and size distribution of nanoparticles isolated from plasma of patients with CP and healthy individuals using Nanoparticle tracking analysis (NTA) and the Particle MetrixTM platform in the UNC-CH Nanomedicine Core (Fig. 1). These data demonstrate that equivalent concentrations of EVs were isolated from the plasma of CP and healthy participants (Fig. 1A). In addition, the size of the EVs isolated corresponding to exosomes and small EVs in both cohorts (Fig. 1B), with the median size of the EV population being significantly higher in plasma from CP patients (Fig. 1C). We utilized transmission electron microscopy to further demonstrate presence of EV and further characterize the EV (Fig. 1D) in patient and healthy

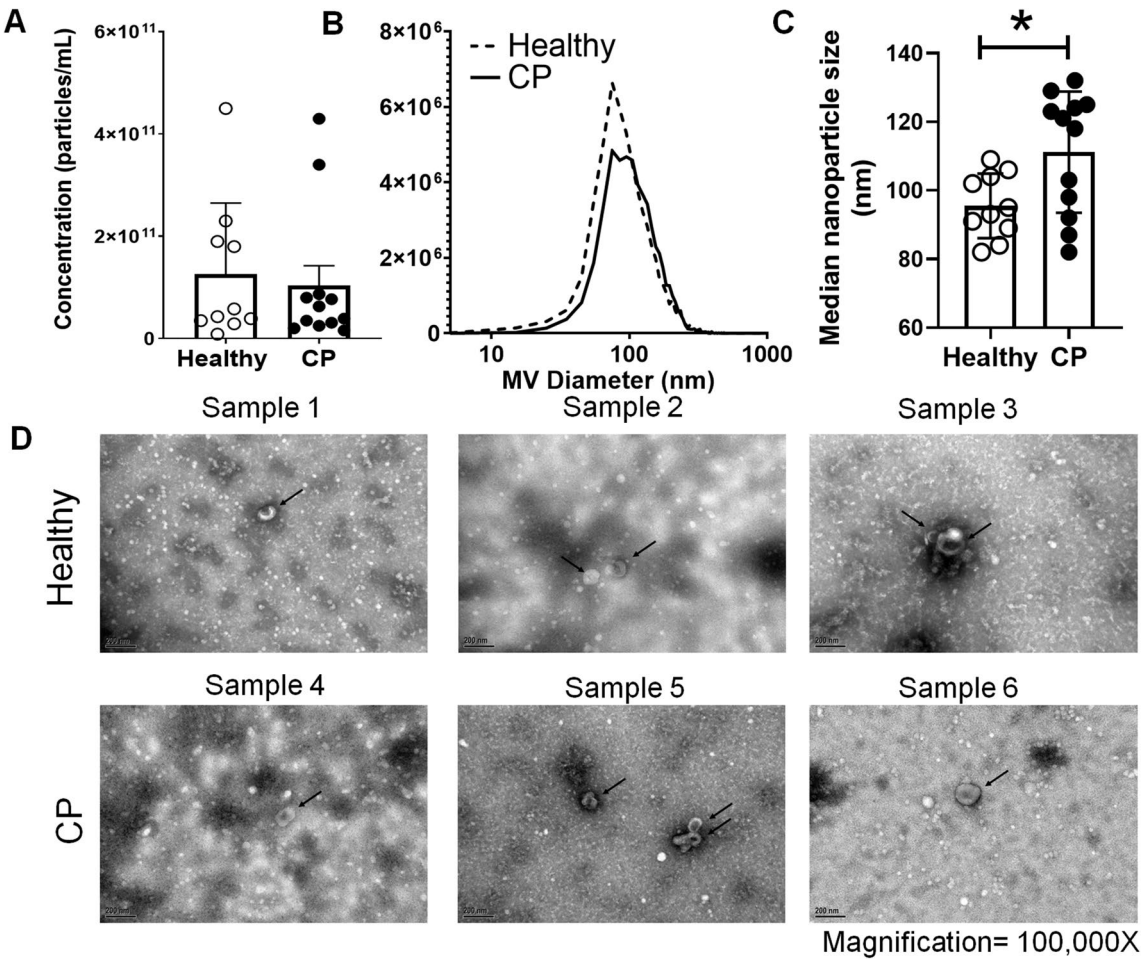

Fig. 1 Physical differences in extracellular vesicles (EVs) from chronic pancreatitis (CP) patients compared to healthy EVs. CP patients recruited into our program were subjected to **A**–**C** Nanoparticle tracking analysis to determine size and concentration distribution (data are shown as $*p < 0.05$ by Mann–Whitney test; data ± SEM), and **D** transmission electron microscopy to determine presence of EV. EV are highlighted by arrows

samples. Electron microscopy revealed single EV in all the healthy samples and CP samples, and some diversity in higher-order EV aggregates in CP samples, likely accounting for the increased size observed by NTA.

Plasma-derived EV miRNA profiling of CP patients and healthy subjects

In order to qualitatively and quantitative compare and contrast the cargo of EVs isolated from CP vs. healthy participants, mRNA was purified (with resultant equivalent purity between sample groups (Supplemental Fig. 1)) and the miRNA profiles were elucidated using Nanostring technology. Figure 2 shows the heat map of the per-row normalized expression levels of selected miRNAs differentially expressed in CP and healthy subjects' plasma-derived EV. As shown in the scatter plot (Fig. 3A), ordered by statistical significance, we identified various miRNAs that were differentially expressed in the plasma EV of CP patient samples relative to those of healthy control subjects. Figure 3B identifies ~ 30 miRNA which are significantly differentially expressed ($p < 0.05$) in EV from those with CP compared to health. As an example, we identified that miR-579-3p exhibits significantly *lower* (~ 16-fold) expression in EV from CP compared to healthy individuals (Fig. 3C). These data suggest that there are significant differences between the miRNA cargo of CP and healthy vesiculomes. We therefore examined this patient cohort for significant EV miRNA cargo differences in other clinically relevant comparisons.

Plasma-derived EV miRNA profiles within CP patients stratify with narcotic usage and diabetic status

While there were significant differences in the miRNA cargo of plasma EV from CP patient samples relative to healthy control subjects, we also evaluated whether specific miRNA profiles correlated with clinical correlates of CP disease. Firstly, there is a clinical need to be able to differentiate between patients who are suffering pain from increasing pancreatic fibrosis and are therefore able to successfully wean off narcotic usage after pancreatectomy *versus* those with stable disease but have established central narcotic desensitization leading to increased and continued narcotic usage even after pancreatectomy.

We observed that ~ 30 miRNAs were differentially expressed in EV isolated from CP patients requiring narcotics compared to those EV from CP patients not requiring narcotics (Fig. 4A) as well as those EVs isolated from healthy participants (Fig. 4B). Interestingly, only ~ 13 of the miRNAs are significantly different when comparing the cargo of EV from non-narcotic CP patients to the cargo of EV from healthy subjects (Fig. 4C).

For diabetic status, it is would be clinically relevant to differentiate between pre-diabetic patients who have actual islet-damage *versus* those who are experiencing islet stress that is simply increasing blood sugars. When the diabetic state of the CP patients was taken into consideration, we determined that there were ~ 40 miRNAs, 90% of which were upregulated, which were differentially expressed in

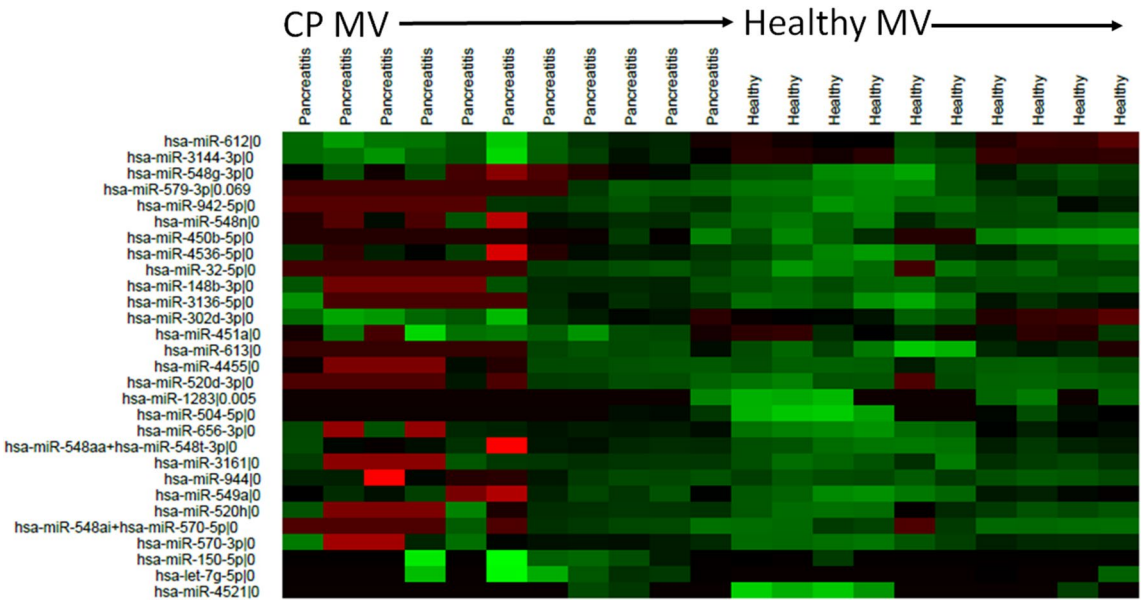

Fig. 2 miRNA payload differences in extracellular vesicles (EVs) from chronic pancreatitis (CP) patients compared to healthy EVs. CP patients recruited into our program were subjected to NanoString miRNA analysis using the nCounter Human miRNA Panel v2, allowing for simultaneous evaluation of 800 miRNAs to determine miRNA payload

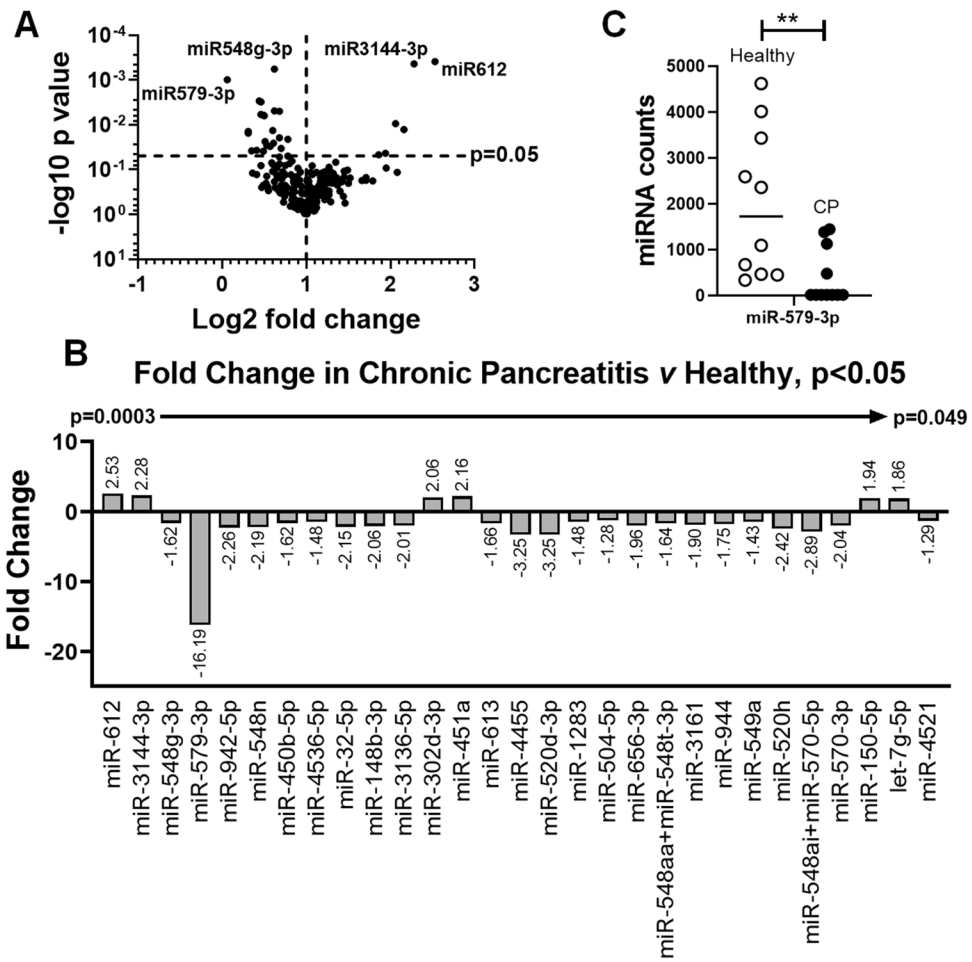

Fig. 3 Chronic pancreatitis (CP) induces altered extracellular vesiculars (EVs) miRNA profiles. CP patients recruited into our program were subjected to NanoString miRNA analysis using the nCounter Human miRNA Panel v2, allowing for simultaneous evaluation of 800 miRNAs to determine miRNA payload; **A** log2-transformed differential fold change in immune gene expression, with associated *p*-value significance (using Welch's T Test), after data normalization to housekeeping and internal control genes by nSolver v3.0; **B** significantly different ($p < 0.05$) miRNA identities within CP versus healthy EVs; **C** demonstrates log2 fold change of miR-579-3p miRNA within CP versus healthy EVs (data are shown as $^{**}p < 0.005$ by Mann–Whitney test; data ± SEM)

EV isolated from CP patients who were pre-diabetic compared to non-diabetic CP patients (Fig. 4D). For example, miR-579-3p was expressed at significantly lower (~ 16-fold) levels in EV from CP compared to healthy individuals (Fig. 3C). In addition, the use of narcotics in CP was associated with even lower expression of miR-579-3p (~ 24-fold) when compared to CP patients who did not use narcotics (Fig. 4A). Yet, miR-579-3p is actually enriched (~ 32-fold) within EV isolated from pre-diabetic CP patients compared to EV from non-diabetic CP patients (Fig. 4D). Taken together, these data suggest that there are overlapping and unique miRNA profiles of plasma EVs that may be of utility in indicating different levels of severity of CP as well as health/disease of the pancreas within CP patients.

Plasma-derived EV miRNA profiles within CP patients undergoing pancreatic surgery

As various miRNA were enriched in the plasma EV of CP patients with differing CP severity, we hypothesized that specific EV miRNA profiles also existed within CP participants who underwent pancreatic surgery. Mechanistically, there is also a need to determine the immune repercussions of previous pancreatic surgery, for example partial pancreatectomy, and determine biomarkers that differ in CP patients with no previous surgery versus those that with previous surgeries. Indeed, the miRNA profile was significantly different within EV isolated from CP patients who had partial pancreatectomy when compared to EVs from healthy individuals (Fig. 5A) as well as EVs from CP patients without any surgery (Fig. 5B).

Plasma-derived EV miRNA profiles within CP patients segregate with sex

Rates of CP are more common in males, whereby sex-related differences in the rates of induction and severity of CP have been observed. Mechanistically, there is a need to elucidate the mechanisms of differing CP etiologies between males and females. As such, we evaluated whether sex affected the miRNA cargo of EV. Here, we found that there were 11 EV miRNAs that had differential expression in men and women (Fig. 6A). These differences were not observed when comparing miRNA profiles within EV from healthy females

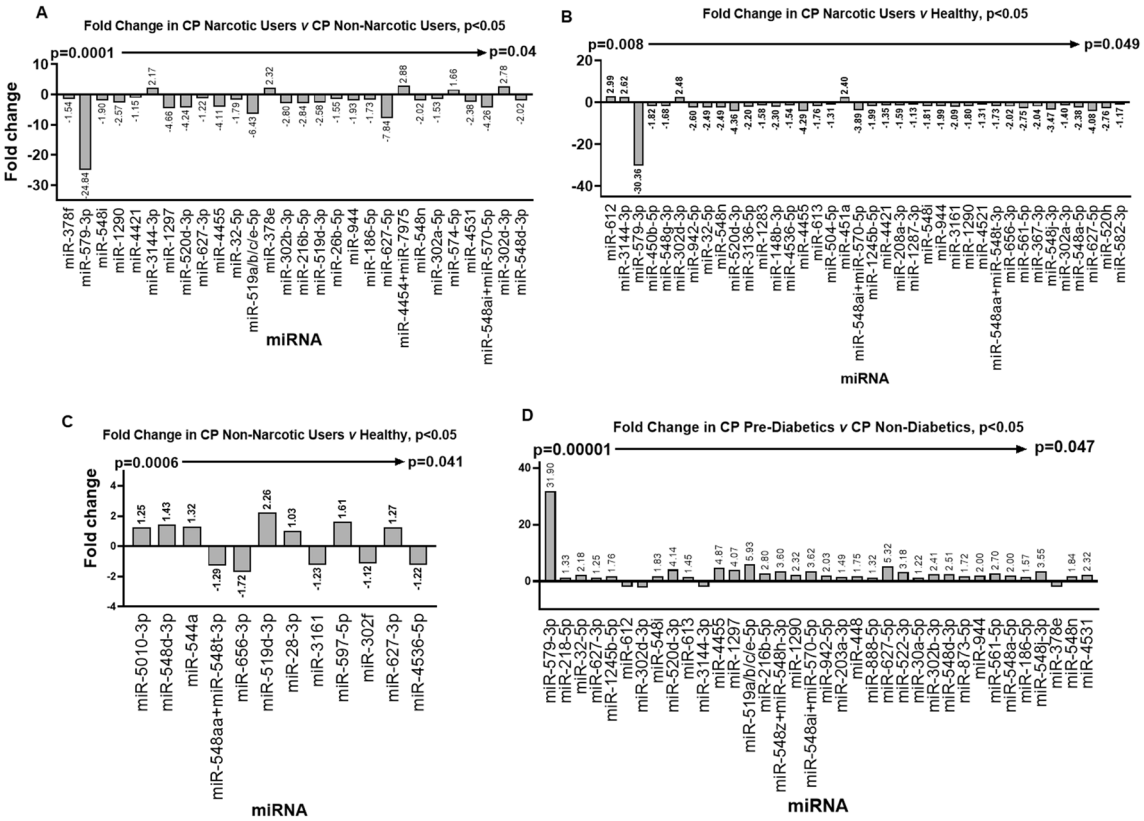

Fig. 4 Severity and diabetic state in chronic pancreatitis (CP) induces altered extracellular vesicular (EVs) miRNA profiles. NanoString miRNA analysis was used to determine significantly different ($p < 0.05$) EV miRNA identities within **A** narcotic-using CP versus non-narcotic-using CP patients, **B** narcotic-using CP versus healthy individuals, **C** non-narcotic-using CP versus healthy individuals and **D** pre-diabetic CP patients vs non-diabetic CP patients

to those of healthy males (Fig. 6B). With that said, there were three EV miRNAs that did demonstrate significant differences in expression when comparing those from healthy males and females (Fig. 6B). Furthermore, when comparing females with CP to healthy females, ~ 30 EV miRNAs were observed to be significantly altered (Fig. 6B). Specifically, we observed that miR-579-3p is only significantly altered within female CP patients, with a 37-fold decrease when compared to healthy female subjects. These differences were not observed when comparing miRNA profiles within EV from males with CP to those from healthy males (Fig. 6C–D). Notably, while there was overlap between the identity of these miRNAs and those which were associated with disease severity (Fig. 6E), there was no significant difference in etiology of pancreatitis related to gender, and hence, difference in EV was not related to etiology.

Discussion

The development of novel diagnostic markers and therapeutic options in the field of CP at a cellular level is slow [23, 24]. As our program is heavily involved in surgical management of CP, specifically utilizing TPAIT, our aim was to explore plasma markers with the potential to help us identify islet health/disease with the long-term goal of being able to predict the health of islets prior to harvest for TPAIT and thus improving the likelihood of success upon transplantation. Such markers have excellent potential for improving outcomes of autologous and allogeneic islet cell transplant.

There are limited data that describe the EV molecular signature ("vesiculome") which can indicate beta cell stress within CP patients in comparison to healthy individuals. We and other groups have tested and discussed the presence and use of EV in pancreatic disease and other conditions [25, 26]. For instance, Bonjoch et al. demonstrated that in acute pancreatitis, the level of circulating MVs is significantly increased, whereby these MVs contribute to symptoms of disease [27]. In addition, others have demonstrated that the concentration of EV in bile samples discriminates between

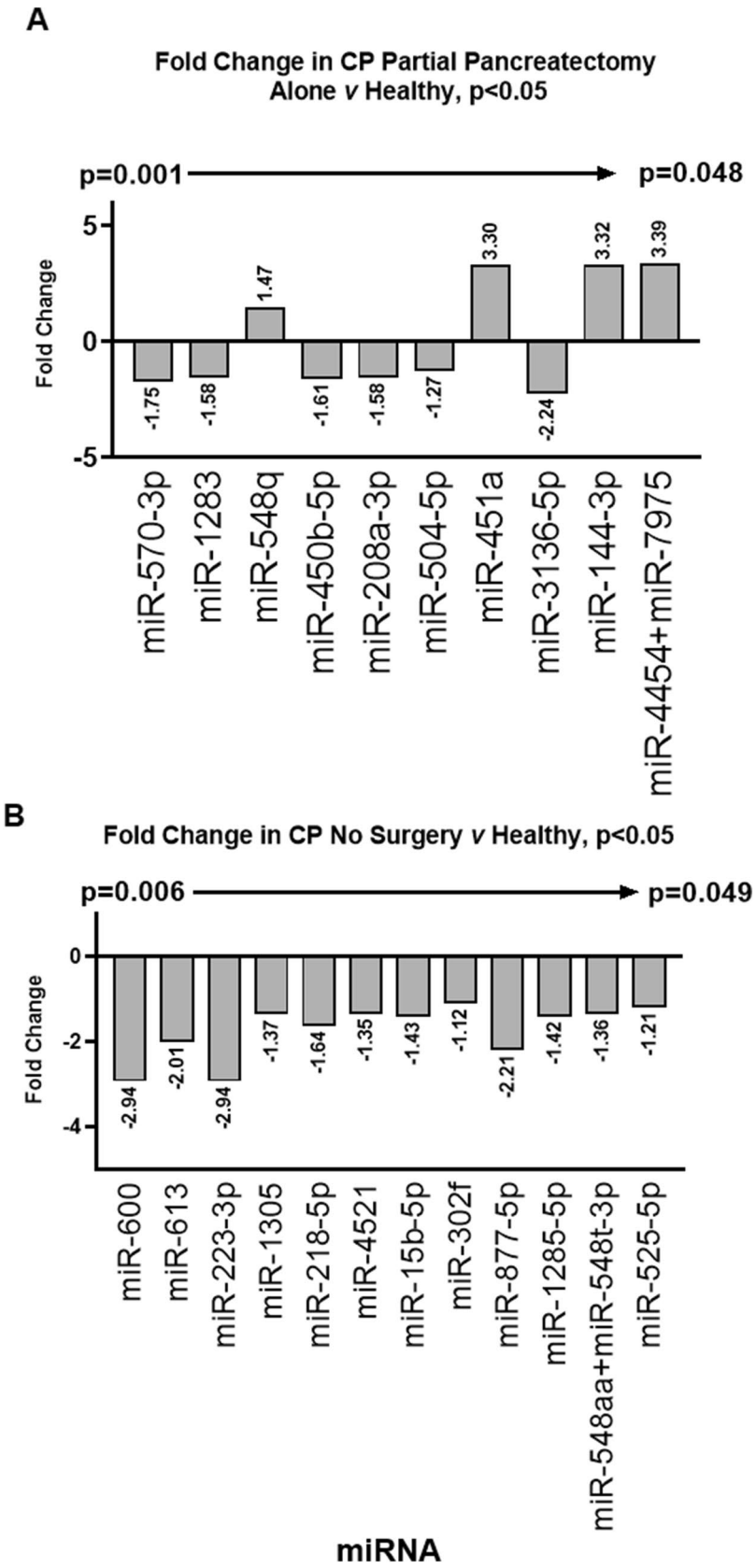

Fig. 5 Surgical status in chronic pancreatitis (CP) induces altered extracellular vesicular (EVs) miRNA profiles. NanoString miRNA analysis was used to determine significantly different ($p < 0.05$) EV miRNA identities within **A** CP patients who underwent partial pancreatectomy alone versus healthy individuals, and **B** non-surgical CP patients versus healthy individuals

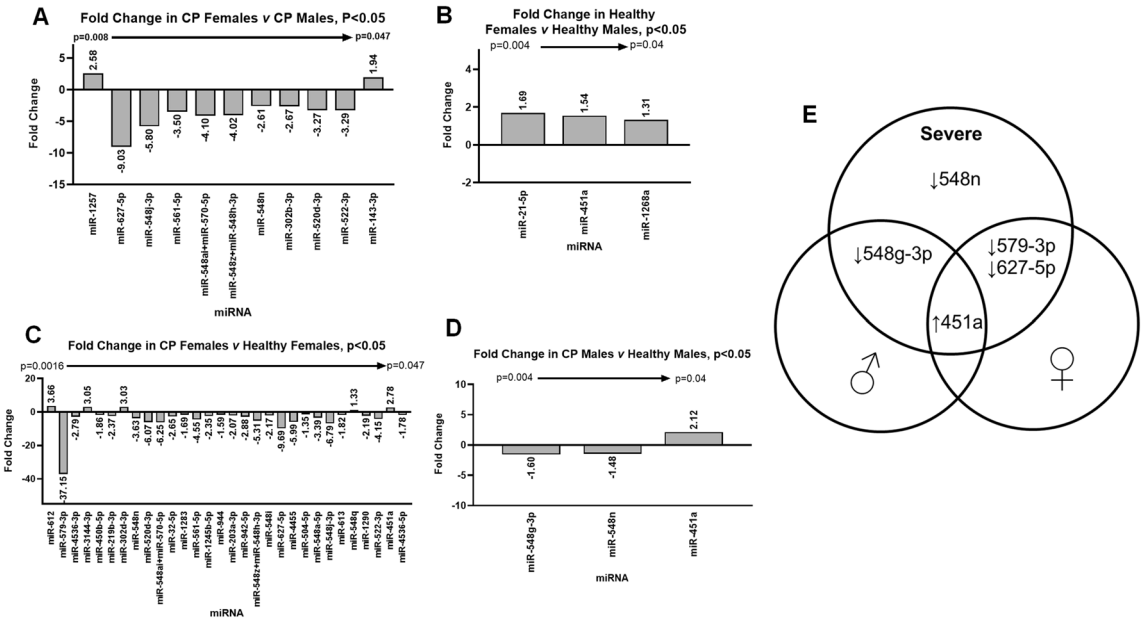

Fig. 6 Sex of chronic pancreatitis (CP) patients dictates altered extracellular vesicular (EVs) miRNA profiles. NanoString miRNA analysis was used to determine significantly different ($p < 0.05$) EV miRNA identities within **A** female CP patients versus male CP patients, **B** female healthy individuals versus male healthy individuals, **C** female CP patients versus female healthy individuals, and **D** male CP patients versus male healthy individuals. Panel **E** demonstrates the overlap between severity and sex on miRNA identity in EVs

patients with malignant vs. nonmalignant common bile duct stenosis with 100% accuracy [28]. Therefore, we completed an initial study with a small cohort of CP patients and healthy controls, and an unbiased "screen" of EV-associated miRNA identities using a next-generation barcoded multiplex gene expression assay.

We purified EV from CP and healthy patients and demonstrated the presence of EV using NTA and electron microscopy. Using our unbiased approach, from a panel of 800 human miRNA, we have revealed EV miRNA "fingerprints" that correlate with CP and other clinical characteristics, such as severe CP patients requiring narcotics compared to those CP patients not taking narcotics, and those which are pre-diabetic versus those that are not. We have also delineated clear differences in EV miRNA in female vs. male CP patients. The clinical relevance of these comparisons is highlighted in the relevant Results sections. Specifically, when comparing CP to healthy subjects, we have identified EV miRNAs previously implicated in pancreatic cancer lesions (e.g., miR-3144 and miR-548g-3p), glucose sensitivity, and inflammatory responses (e.g., miR-612 and miR-let7). We have also identified novel miRNAs not previously associated with CP as well as miRNA which correlates strongly with CP severity and diabetes in CP patients. Intriguingly, we have also identified a sex bias for EV miRNA content (e.g., miR1257 and 627-5p), both of which correlate with pancreatic cancer, diabetes, and glucose sensitivity [29, 30]. Though not attributed in current sample size, differing etiologies of CP between males and females may well be driving these EV changes, which will be a focus of future studies.

The literature demonstrates miR-579-3p to be significantly dysregulated in patients with pancreatic cancer and promotes an anti-inflammatory state [31–33]. This was the most significant miRNA noted within different groups. miR-379-5p is predicted to target the transcription factor Activating transcription factor 3 (ATF3), well known to regulate inflammatory and metabolic responses under conditions of stress and/or infection [34]. ATF3 is highly responsive to estrogen; this coupled with the fact that females often have recurrent pancreatitis episodes superimposed on CP, we speculate that miR-579-3p may represent a novel regulatory element normally countering estrogen-amplified pancreatic inflammation. Lack of miR-579-3p as we observed in female CP patients may represent an undescribed mechanism of CP maintenance and represents an avenue of future adjunctive MV therapy.

EVs have become a novel adjuvant therapeutic option for a range of degenerative and chronic orthopedic, musculoskeletal, and skin disorders [16, 21, 35–40]. As such, we hypothesize that EVs from healthy individuals have the potential to promote an anti-inflammatory microenvironment and encourage angiogenesis (citation) and thus have promise as an adjunctive therapy during islet cell transplantation. Further studies encouraged by the data generated

here will evaluate whether EVs can reduce the host inflammatory responses as well as attenuate the response of the transplanted islets, leading to improved survival following transplantation. Though not specifically identified, our data provide indicators that lay a foundation for this mechanistic research.

The strength of the study is that it is the first to analyze in detail the EV miRNA profile of patients with CP using an unbiased evaluation of miRNA cargo from an array of 800 miRNA simultaneously in the same clinical sample. This resulted in the identification of miR-579-3p as a novel microRNA biomarker associated with CP. These 'vesiculome' patterns have the potential to pave a way to provide clinical guidance in the timing of surgical therapies like TPAIT, early indication of islet graft dysfunction or failure. Our data also provide a launching pad for examining EV as adjunctive therapeutics. Together these data pave the way to translate these biomarkers and potential therapies into validation studies and then from bench to bedside in the future.

The limitations of study lie in the small sample size; however, we chose NanoString for the value in unbiased miRNA analysis, which is cost-prohibitive to perform on a large number of patients. We applied rigorous statistical analysis to ensure we had rigor and reproducibility. Indeed, even in such a small cohort, we have demonstrated significant differences in certain miRNA identities. These were hypothesis-generating experiments, and we are collecting samples form larger CP cohorts to use more cost-effective qtPCR to verify predictive value and understand biological mechanism. There could be some overlap when subgroup analyses between patients were performed, but it cannot be argued that the categories selected were broad and relevant differentiators. These were all patients who were presented for surgical evaluation and some of them did undergo surgery. It would have been advantageous to collect tissue sample for the analysis, but cost constraints would have further reduced the plasma sample size. Taken together, as this was our basic aim, we have generated an initial exploratory plasma miRNA 'vesiculome' during CP, and we will utilize this information to validate changes in pancreatic health in CP patients.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11010-021-04248-5.

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Author contributions Conception or design of the work: CSD, AK, RM, SMW. Data collection: MAB, MLW, CM, XB, BMW. Data analysis and interpretation: CSD, AK, RM, SMW, CM, MLW, LGC. Drafting the article: RM, CSD. Critical revision of the article: CSD, TB, SMW, AK, RM, CM.

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Data availability At request.

Declarations

Conflict of interest AK discloses a relationship with AssureImmune Cord Blood Bank and Aceso Therapeutic that includes equity.

Ethical approval This study has been approved by the Institutional Review Board at the University of North Carolina.

Consent to participate Informed consent was obtained for each participant.

Consent for publication All participants consented to publication.

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