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Protective role of oleic acid against palmitic acid-induced pancreatic fibrosis

Sonia Frendi[1], Anaïs Chassac[3], Killian Veron[1], Jérôme Raffenne[4], Rémy Nicolle[2], Miguel Albuquerque[1,3], Valérie Paradis[1,3], Anne Couvelard[1,3], Jérôme Cros[1,3] and Vinciane Rebours[1,5*]

Abstract

Background Obesity has been associated with several pancreatic disorders and is an important risk factor for pancreatic cancer. Nevertheless, the role of lipids in the early steps of carcinogenesis is unknown. Although we previously identified two types of pancreatic fatty infiltration with different lipid compositions that were associated with precancerous lesions and fibrosis, their mechanisms of action have not been clarified.

Methods We hypothesized that saturated palmitic acid and mono-unsaturated oleic acid (OA and PA) could play diverse roles in the activation of pancreatic stellate cells (PSCs) during the genesis of pancreatic fibrosis and the promotion of precancerous lesions.

This study explored the lipotoxic effect of OA and PA on PSCs and exocrine pancreatic tissue (acinar cells). We also explored PA-induced pyroptosis in PSCs. A three-dimensional culture system of organotypic slices from human pancreatic tissues was used as well as a two-dimensional culture of hTERT immortalized PSCs.

Results The results show that PA could induce the secretion of collagens and inflammatory cytokines (IL18) in PSCs ($p < 0.05$). We defined a standardized protocol of precision-cut pancreas slices cultured from human non-tumoral pancreatic tissue ($n = 9$). Both OA and PA are involved in the initiation of acinar cell transformation into ductal cells. OA was found to have a protective effect against PA-induced fibrosis ($p < 0.05$).

Conclusion These results highlight the antagonistic roles of oleic and palmitic acids in the initiation of pancreatic fibrosis and show that palmitic acid has a profibrotic role.

Introduction

Pancreatic adenocarcinoma (PDAC) is the fourth cause of cancer mortality in western countries and is expected to be the second leading cause of cancer death in the United States by 2030 [1].

Unlike other cancers such as lung or uterine cancer which are mainly caused by exposure to the single agents, tobacco and papillomavirus, respectively, PDAC may be associated with several risk factors, both genetic and environmental. Several risk factors have been identified such as family history and genetic factors, age, tobacco, pancreatic inflammation, diabetes mellitus and obesity [2, 3].

The role of obesity in the initiation and development of PDAC is still poorly understood. However, certain

*Correspondence:
Vinciane Rebours
vinciane.rebours@aphp.fr
[1] Paris-Cité University, Inflammation Research Center (CRI), INSERM, U1149, F-75018 Paris, France
[2] Paris-Cité University, Inflammation Research Center (CRI), INSERM, U1149,, CNRS, ERL 8252, F-75018 Paris, France
[3] Pathology Department, Bichat and Beaujon Hospitals, AP-HP, FHU MOSAIC, Paris-Cité University, Paris, France
[4] JR-Analytics (Jr-Analytics.Fr), 33370 Pompignac, France
[5] Pancreatology and Digestive Oncology Department - DMU Digest, Beaujon Hospital, AP-HP, INSERM, UMR 1149, Paris-Cité University, Clichy, France

pathways that induce a chronic inflammatory state have been proposed, for example factors derived from ectopic fat deposition, oxidative stress, alterations in adipokine secretion, insulin resistance and hyperinsulinemia. These pathways could create a favorable microenvironment for early PDAC carcinogenesis [4, 5].

PDAC involves various changes in exocrine cells known as precancerous lesions, such as acinar to ductal metaplasia (ADM) and pancreatic intraepithelial neoplasia (PanIN) [6, 7]. It is also characterized by the strong desmoplastic reaction of healthy tissue in response to tumor cells. These changes mainly affect the connective tissue and are characterized by the recruitment and activation of fibroblasts associated with the accumulation of the extracellular matrix (ECM) [8, 9].

During pathological processes, the regeneration and repair of the pancreas is managed by several cell types: acinar cells, duct cells, immune cells and pancreatic stellate cells (PSC) [10]. PSC play a major role in this process. They can be activated and change their phenotype to become "myofibroblast-like" cells at the origin of a remodeling of the ECM. Several soluble factors can regulate their activation, such as IL-1, IL-6, PDGF or TGFB… Activated PSCs promote cell proliferation, the production of collagen and cytokines/chemokines (IL8, MCP-1), the synthesis of adhesion molecules, remodeling of the ECM by synthesis of metalloproteases and their inhibitors and regulation of angiogenesis. They are essential in the tissue changes linked to pancreatic inflammation and cancer [11].

During obesity, the dietary fat overload increases the risk of pancreatic diseases. Several studies have shown that different species of dietary fatty acids (FA) contribute differently to cellular lipotoxicity. The study by Ben-Dror et Birk showed that saturated dietary fat (palmitic acid, PA) induces pancreatic acinar lipotoxicity and cellular stress while monounsaturated fatty acids (oleic acid, OA) play a protective role against palmitic acid cytotoxicity. These FAs compose the main structure of most lipid species [12].

In a previous study, we identified two types of pancreatic fatty infiltration (intra and extralobular) with different lipid compositions that were associated with obesity and preneoplastic lesions (PanINs). We also showed that acinar cells had different phenotypes depending on the presence of and proximity to the intralobular fatty infiltration in obese patients. Several lipid metabolic pathways, oxidative stress and inflammatory pathways were found to be upregulated in acinar tissue during intralobular fatty infiltration. In addition, early acinar transformations called acinar nodules were identified in association with obesity suggesting that they are the first reversible precancerous pancreatic lesions to occur in these patients. On the other hand, there were more PanINs in obese patients and they were positively correlated to the two types of fatty infiltration scores as well as to fibrosis.

Our previous study suggest that the lipid composition of pancreatic fatty infiltration plays a major role in pancreatic oncogenesis and fibrogenesis [13].

We hypothesized that palmitic and oleic acids play different roles in pancreatic stellate cell activation and could play a role in the processes of pancreatic fibrosis and oncogenesis.

We investigated the lipotoxic effects of palmitic acid (PA) and oleic acid (OA), saturated and mono-unsaturated acids respectively, either alone or in combination, on pancreatic stellate cells (PSC) and pancreatic exocrine tissue using complementary experimental models: 2D cell culture and precision-cut pancreas slice cultures from human non-tumoral pancreatic tissue. We assessed their roles in PSC activation and in the initiation of acinar transdifferentiation in the pancreatic parenchyma.

Material and methods

Cell culture

hTERT Immortalized human pancreatic stellate cell lines (PS1) were obtained from the BARTS Cancer Institute (Cancer Research UK – Centre of excellence, University of London) and cultured by modifying the Rosendahl et al. protocol [14].

Briefly, monolayer cultures were maintained in DMEM/Hams Nutrient Mix F12 supplemented with 10% of DMEM 1X, 10% of fetal calf serum, penicillin–streptomycin (1%), amphotericin B (1%) and L-glutamine (2mM). All the medium compounds were obtained from Gibco® ThermoFisher, Life Technologies. Cells were grown in 5% CO2 at 37∘C. Cells were subject to the different fatty acid treatments (oleic acid, OA and palmitic acid, PA) for 48h.

Precision-cut pancreatic slice cultures

Pancreatic specimens were retrieved from the surgical department of Beaujon Hospital (Clichy, France) as fresh as possible after resection to minimize ischemia and preserve cell viability (maximum delay $\leq$ 3 h). The clinical characteristics and indications for surgery are summarized in supplemental Table 1. All patients provided written consent to participate to the study. This study was approved by the Institutional Review Board (IRB-00006477) of our institution. Patients who received antitumoral treatments (radiation or chemotherapy) before surgery were excluded from the study.

Preparation of precision cut pancreatic slices (PCPS) was performed by modifying the protocols by Rebours et al. and Hammoutene et al. [15, 16]. Briefly, 8 mm cylindrical core samples were taken by a pathologist from the

most distal nontumoral tissue. All steps up to incubation were performed on ice (at 4 °C) and the core samples were submerged in a specific slicing buffer during the slice preparation procedure. A Leica vibrating-blade microtome (VT1200S, Leica Biosystems, Germany) was used to obtain 250 µm thick slices, under aseptic conditions, with the following settings: amplitude 3mm; speed 0.5 mm/s.

A tissue sample was included in a 5% low-gelling-temperature agarose bloc (Sigma-Aldrich) then mounted to the metal tray filled with an ice-cold slicing buffer composed of Hank's balanced salt solution (HBSS, Gibco®, Life Technologies, USA) supplemented with 5mM of glucose, 50µg/mL of Pen-Strep and amphotericin B (1%) (Gibco ®, ThermoFisher).

Control slices were obtained at time point 0. The first and last slices were immediately fixed in formalin overnight and embedded in paraffin for pathological analysis. Three other slices were also immediately frozen in liquid nitrogen for RNA investigations. Subsequent slices were kept in a new freshly prepared ice-cold slicing buffer until the slicing process was completed. Each slice was placed in 8 µm PET-tissue culture inserts (ThinCert™, Greiner bio-one) in 6 wells plates containing 2 mL of culture medium to maintain the slice in an air–liquid interface and avoid tissue hypoxia. The culture medium was composed of DMEM/Hams Nutrient Mix F12 supplemented with 5% of fetal calf serum, HEPES (1%), Glutamax (1mM), dexamethasone (0.1µM), glucose (11mM, (Sigma-Aldrich)), insulin (100µg/mL), trypsin inhibitor (10µg/mL), penicillin–streptomycin (50µg/mL) and amphotericin B (1%). All medium compounds were obtained from Gibco® ThermoFisher, Life Technologies. After 2 h of pre-incubation, slices were cultured in 5% CO2 at 37°C under continuous orbital agitation (70 rpm) for 24h in culture medium and in culture medium supplemented with OA or PA (final concentration of 60µM). After 24 h of culture, PCLS were washed in cold phosphate buffer saline and immediately frozen in liquid nitrogen and stored at -80 °C until processing (for RNA experiments) or fixed in 10% formalin and embedded in paraffin for pathological analyses (Fig. 4 A).

Fatty acids preparation

Both PS1 and PCPS were cultured with fatty acids (oleic acid, OA (monounsaturated FA) compared to palmitic acid, PA (saturated FA)) for 48h (PS1) or 24h (Slices). Fatty acid-supplemented medium was prepared according to a slightly modified Spector protocol [17]. Briefly, Palmitic acid (PA, P9767, Sigma-Aldrich®) and Oleic acid (OA, O7501, Sigma-Aldrich®) were dissolved in a 1:1 ethanol and water ratio, then gently mixed at 57°C until they were completely dissolved. The control untreated cells/slices received the same vehicle solution without the FAs. PA or/and OA were added to the cells for 48h (PS1) or 24h (slices) at different doses. The FA concentrations were adjusted according to the cell toxicity.

Cell viability/Cytotoxicity analyzes

FA cytotoxicity was determined in both PS1 and slice cultures using a PrestoBlue™ cell viability reagent (Invitrogen™, ThermoFisher scientific) with a Multisky scan and Fluroscan (SkanIt Software for Microsoft version 6.0.2.3, TheromoFischer Scientific).

PS1 were cultured in 96-well plate overnight at a density of 8000 cells/well and were subject to different concentrations of OA or PA. After 48 h of exposure to FAs, cells were washed with Dulbeco's phosphate-buffered saline (DPBS, Gibco® ThermoFisher scientific) then incubated with PrestoBlue™ cell viability reagent (Invitrogen™, ThermoFisher scientific, USA) according to the manufacturer's instructions. The inhibitory concentration at 50% (IC50) of each FA was determined using GraphPad Prism version 10.0.0 for Windows, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com.

For slices culture, the viability was assessed by incubating the PrestoBlue™ cell viability reagent for 6 h than 100µL of the supernatant was collected in a 96-well plate to be analyzed according to manufacturer's instructions.

Fat accumulation assessment in PS1

Cells were grown on glass cell culture chamber slides (Lab-tekII Chamber Slide System, Nunc, Thermo Fisher) and were subject to different FA concentrations (IC25 and IC50 doses) for 48h. After removal of medium, chambers were removed then the cell slides were chemically fixed in isopropyl alcohol at 60% (1min) then incubated for 10min in oil red O solution followed by a short incubation in isopropyl alcohol at 60% for 30 s. The nuclei were stained with Mayer's Hemalun for 1min, then the glass slides were rinsed in water twice. Cells were immediately photographed and analyzed by light microscopy (Leica DMRB, Germany).

Oil red O staining was quantified by a semi-quantitative algorithm using HALO® software (v2.0.1061; 2016; Indica Labs, Inc. https://www.indicalab.com/halo/).

Immunohistochemistry (IHC) staining for pancreatic slices

After 24h of culture, the slices were fixed in formalin overnight then imbedded in paraffin. Immunostaining was performed after dewaxing and rehydrating the slides. Antigen retrieval was obtained by pretreatment at a high temperature at pH9 in TRIS Buffer. PBS was substituted for the primary antibody and used as a negative control. The slides were immunolabelled with monoclonal

or polyclonal antibodies against the cytoplasmic acinar marker B-cell lymphoma 10 (BCL10, Santa Cruz Biotechnology, 1:300, sc-5273) co-immuno-stained with the nuclear ductal marker SRY-box transcription factor 9 (Sox9, Millipore, 1:600, ab5535), against the ductal marker cytokeratin 7 (CK7; Abcam, 1:400, ab125212), against alpha smooth muscle actine (αSMA); Dako1:600, M851) to assess the activation of PSC**.**

RNA isolation in PS1 and pancreatic slices

Total RNA was isolated from cultured cells using RNeasy mini kit (Qiagen, France) according to the manufacturer's protocol. Total RNA was extracted from frozen slices after disruption and tissue lysis using Trizol™ reagent protocol (Invitrogen, ThermoFisher Scientific). RNA yield and quality were determined by UV absorption on a NanoDrop One spectrophotometer (ThermoScientific®).

RNA sequencing in PS1

Approximately 200 ng of total RNA from each sample of PS1 cells was used for RNA Sequencing. RNA library preparation was performed according to the manufacturer's instructions (KAPA mRNA HyperPrep Kit from Roche). Final samples pooled library prep were sequenced on ILLUMINA Novaseq 6000 with SP-200 cartridge (2 × 800Millions of 100 bases reads), corresponding to 2 × 33Millions of reads per sample after demultiplexing. This work was performed with equipment and services from the iGenSeq core facility (Genotyping and sequencing), at ICM (Paris, France).

Quantitative real-time PCR (qRT-PCR) analysis in PS1 and pancreatic slices

RNA was reverse-transcribed into cDNA using a high capacity cDNA Synthesis Quantitect reverse transcription Kit (Qiagen, France) according to the manufacturer's instructions. Realtime PCR was performed to assess gene expression in a Bio-Rad QPCR Machine using TAQMAN UNIVERSAL Master MIX II NO UNG (Qiagen, France). Each sample was amplified in triplicate, and the expression of GAPDH (Hs02786624), PPiA (Hs04194521), HPRT1 (Hs02800695) and 18S (Hs03003631) was used as an internal control for every PCR assay. TaqMan® probes were: ACTA2 (Hs00426835), Amy1A,B (Hs00420710); COL1A1 (Hs00164004), COL1A2 (Hs01028956); IL1B (Hs01555410); IL6 (Hs00174131); IL18 (Hs01038788); SPINK1 (Hs01004508); PTF1A (Hs00603586); SOX9 (Hs00165814).

Secretome analysis of PS1 cultures

PS1 cells were grown with the FA treatments for 48h in serum free medium then the medium was collected for each condition. The conditioned media were centrifuged and total secreted proteins were estimated using the BCA Protein Assay Kit (BioRad, France) by the Brad-Ford and Lowry methods. Protein quality was estimated using SDS gel. Samples were analyzed at the Jacques Monod Institute platform using the LC–MS/MS method (see supplemental methods).

Statistical analysis

Experiments were performed at least three times and quantitative data are expressed as means ± SEM or medians with ICR.

Data were evaluated with a 2-tailed, unpaired Student's t test or compared by one-way analysis of variance using GraphPad Prism version 10.0.0 for Windows, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com. A value of $p < 0.05$ was considered to be statistically significant.

Results

Differential fatty acids toxicity on PS1 cell lines

Analysis of the cytotoxicity of the fatty acids (OA and PA) showed different degrees of lipotoxicity on PS1 cell lines. The toxicity of PA was higher on PS1 cell lines (IC50 = 95.8µM) than OA (IC50 = 171.2µM) (Fig. 1A). The isobologram analysis of the combination of OA with PA showed an antagonistic effect between the two fatty acids (Fig. 1B) with a probable protective effect of OA against PA. Because PA was shown to induce cell death by pyroptosis in pancreatic acinar cells [12], we evaluated pyroptosis markers in PS1 treated with FA. By RNA sequencing, we found no significant effects of the FA on the expression of pyroptosis markers. Nevertheless, PA tended to increase

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Fig. 1 Fatty acids uptake and safety in pancreatic stellate cell lines. **A** Inhibitory concentration of OA and PA in pancreatic stellate cell lines (PS1). PA displays a higher toxicity than OA. **B** Isobologram analysis of the ratio of OA and PA shows an antagonistic effect between OA and PA. **C** Representative microscopic images of Oil red O stainings on PS1 cells treated with different doses of fatty acids. **D** Quantification of Oil red O stainings on PS1 cells treated with different doses of fatty acids using Halo showing a dose-dependent accumulation of fatty acids in PS1 cells. **E** Perilipin 1 and 2 mRNA expression showing the dose-dependent lipid droplet formation especially with Plin2 (an early indicator of lipid droplet formation). Data are expressed as mean ± SEM. ***p < 0,0005; **p < 0,005; *p < 0,05. Only significant comparisons (Mann Whitney unpaired t-test) were shown. *OA* oleic acid, *PA* Palmitic acid, *IC50* inhibitory concentration at 50%

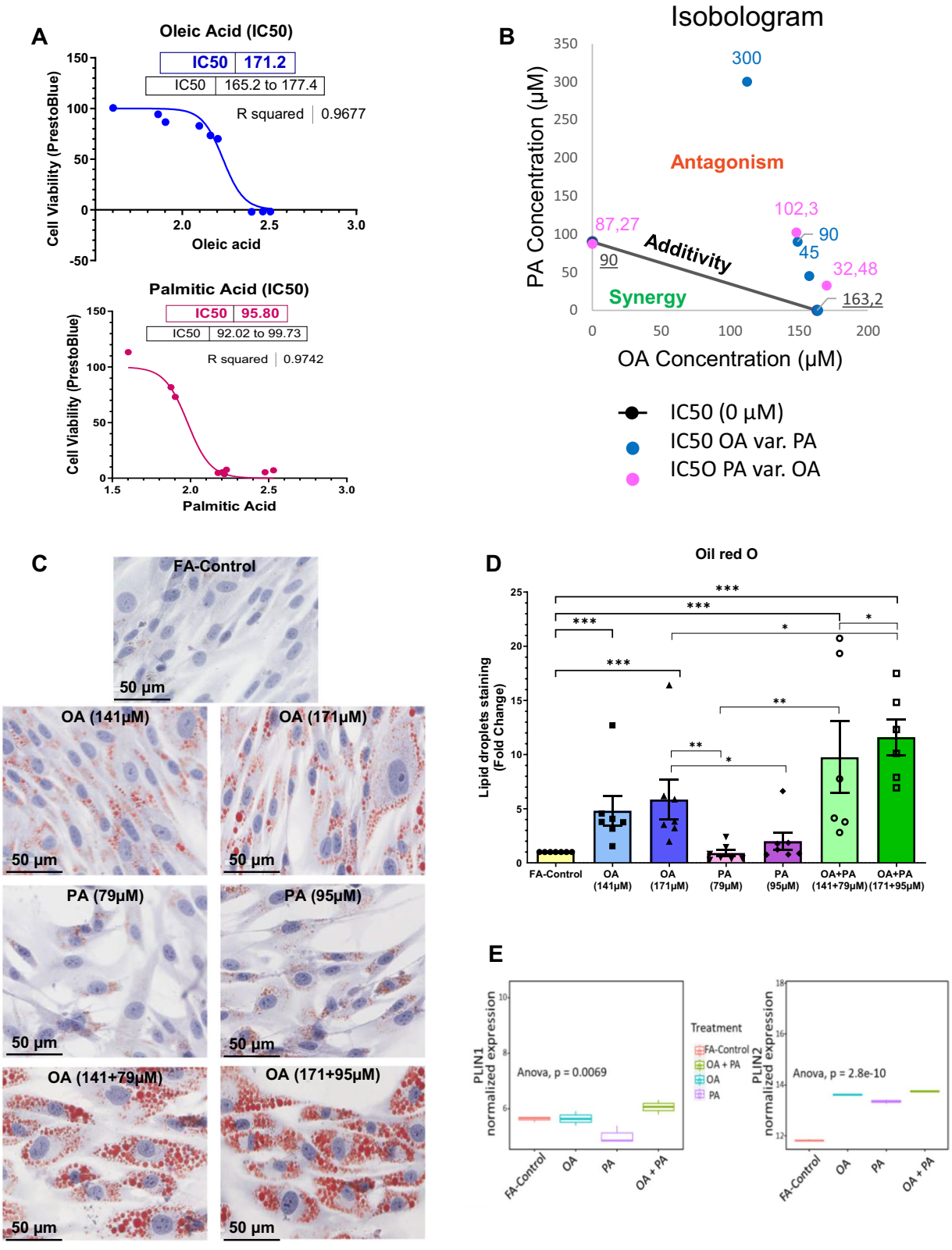

Fig. 1 (See legend on previous page.)

NLRP3 and GSDMD expression in PS1 (supplemental Fig. 1A, B). No effect was observed in CASP1 protein expression (supplemental Fig. 1C). Apoptosis was also analyzed using CASP3 protein but no significant results were found (supp. Figure 1D).

Fat overload in PS1 treated by fatty acids

PS1 cell lines were treated with OA (141µM or 171µM), PA (79µM or 95µM) and with fatty acid (FA) combination (OA + PA). Fat accumulation in PS1 cells increased according to FAs dose-treatments (Fig. 1C, D). Fat accumulation was greater with OA than with PA. It was proportional to the amount of FA added and increased when the two fatty acids were combined (Fig. 1D). Perilipin 1 and 2 (Plin1, Plin2), genes involved in the regulation of lipid droplet formation, were also assessed at a transcriptomic level. Plin2 was the most relevant marker in accordance with the findings in oil-red-O staining (Fig. 1E).

The consequences of fatty acids on the PS1 transcriptome and secretome

We analyzed the transcriptome and secretome of PS1 cell lines treated with OA (141µM), PA (79µM) and the FA combination (OA + PA).

Treatment with OA induced an enrichment in proliferation pathways in the transcriptome of PS1 cells compared to control conditions. The same effect was observed when PS1 cells were treated with PA (Table 1, Fig. 2A). On the other hand, the treatment of PS1 cells with a combination of OA + PA resulted in a decrease in these proliferation pathways, showing the antagonistic effects of OA and PA. In addition, OA + PA increased the interleukin-6 (IL-6) pathway (Table 1, Fig. 2A). 4E eukaryotic translation initiation factor (EIF4E) was also increased in PA-treated PS1 (supplemental Fig. 1E).

After treatment with FAs, PS1 cells displayed a decrease in the epithelial-to-mesenchymal transition (EMT) compared to the control conditions (Table 1, Fig. 2A). A decrease in collagen degradation was also observed in the transcriptome (Fig. 2B) and the secretome (Fig. 2C) of PS1 cells treated with OA,

Table 1 Pathways enriched in PS1 cells treated with FA (transcriptomic analysis)

		Untreated vs OA		Untreated vs PA		Untreated vs OA+PA	
	Pathway	NES	Adj. pVal	NES	Adj. pVal	NES	Adj. pVal
Proliferation pathways	HALLMARK_E2F_TARGETS	2.40	5.28E−08	3.23	2.50E−49	− 2.63	5.04E−11
	HALLMARK_G2M_CHECKPOINT	2.19	1.50E−05	3.10	2.50E−49	− 2.72	8.53E−13
	REACTOME_G2_M_CHECKPOINTS	1.96	6.47E−02	2.78	2.06E−22	− 1.69	1.93E−01
	REACTOME_DNA_REPLICATION	2.01	4.17E−02	2.73	7.73E−20	− 1.89	7.03E−02
Fatty acid metabolism	HALLMARK_FATTY_ACID_METABOLISM	− 1.34	1.24E−01	− 1.37	4.68E−02	2.28	1.78E−05
	HALLMARK_ADIPOGENESIS	1.01	4.42E−01	− 1.46	1.75E−02	2.22	2.39E−05
	KEGG_FATTY_ACID_METABOLISM	− 1.11	4.91E−01	− 1.08	5.34E−01	2.26	3.53E−02
	REACTOME_FATTY_ACID_METABOLISM	− 1.18	3.75E−01	− 1.37	1.63E−01	2.37	1.11E−04
	GO_UNSATURATED_FATTY_ACID_METABOLIC_PROCESS	− 1.52	1.62E−01	− 1.54	4.99E−02	2.08	3.63E−02
EMT	HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	− 1.63	1.23E−02	− 1.80	6.60E−05	− 1.44	4.64E−02
	REACTOME_DEGRADATION_OF_THE_EXTRACELLULAR_MATRIX	− 2.09	9.22E−04	− 2.04	4.15E−07	− 1.46	3.43E−01
	REACTOME_COLLAGEN_DEGRADATION	− 2.04	1.17E−02	− 1.91	6.51E−04	− 1.79	2.77E−01
IL6 pathway	HALLMARK_IL6_JAK_STAT3_SIGNALING	− 1.34	1.54E−01	− 1.85	4.18E−04	1.87	3.12E−02

FA control: Fatty acid control, OA: Oleic acid, PA: Palmitic acid, OA+PA: Combination of oleic and palmitic acids

PA and OA + PA compared to controls. These results showed that OA and PA can reduce the collagen degradation in PS1 cells.

OA and PA roles in activation of PS1

Based on the observation that PS1 cell lines treated with OA and/or PA induced a decrease in collagen degradation pathways, we hypothesized that these FA could play a role in PS1 cell activation and transformation to fibroblast-like cells. We first analyzed the protein expression of several fibroblast markers such as alpha-smooth muscle actin (αSMA) and collagens. Western blot analysis of αSMA expression showed a tendency to increase with OA and PA treatment alone associated with a significant decrease with the combination of OA + PA (Fig. 3A). The protein expression of COL1A1 (Fig. 3B) was only increased in PS1 cells with PA; it decreased when PA was combined with OA (Fig. 3B). PA also increased the secretion of other COL1A types (II and III) (Fig. 3C) in opposition to OA which decreased this effect. We confirmed the functional antagonistic role of OA and PA (in addition to their toxicity) on PS1 cell activation, i.e. the expression and secretion of αSMA and collagens.

We also found that treatment with PA induced expression of the proinflammatory cytokine IL18 associated with its decreased expression with the combination of OA + PA (Fig. 3D). Interestingly, the combination of OA + PA increased RNA expression of IL1B (Fig. 3E).

Based on these results we explored the role of fatty acids on the other cellular components of the pancreas especially on the development of fibrosis using a 3D organotypic culture model of human pancreatic slices (Fig. 4A).

OA and PA toxicity on precision cut pancreas slices

We first tested the viability of pancreas slices after 24h of FA treatment (60 µM) or with the vehicle (ETOH, FA-Control). OA and PA alone were not found to affect the viability of the slices (Fig. 4B) and showed no toxicity on the pathological examination (Fig. 4C). However, there was significant toxicity in slices treated with a combination of OA + PA (Fig. 4B, C).

OA and PA induce acinar cell transformation

The expression of acinar (amylase, AMY1), ductal (Sox9) and fibroblast markers (COL1A1/2 and ACTA2) was assessed on slices at time point 0 (T0) and after 24 h of culture with or without fatty acids.

OA and PA induced a decrease in AMY1 compared to FA-controls (vehicle) (Fig. 5A). No effects were observed with OA + PA treatment.

Interestingly, OA and PA treatment alone did not affect ductal marker (SOX9) expression and their combination significantly increased SOX9 expression compared to OA or PA-treated slices in IHC analysis (Fig. 5B) suggesting that they played a role in the transdifferentiation of acinar cells to ductal cells.

Because we found that OA and PA stimulated PS1 cell line activation, we explored the fibroblastic markers COL1A1, COL1A2 and αSMA (herein ACTA2) mRNA expression in slices treated with FA. As expected, OA but not PA treatment decreased COL1A1 (Fig. 5C) and COL1A2 (Fig. 5D) expression. There was no effect on ACTA2 mRNA expression (Fig. 5E).

Discussion

This study confirmed the antagonistic cytotoxic effects of 2 fatty acids, oleic and palmitate acid, on PSCs. The cytotoxicity of PA was greater than that of OA which played a protective role against PA. Similar results were observed in recent studies in other cell types such as hepatocytes. These studies showed that PA could induce programmed cell death due to endoplasmic reticulum stress and the pyroptosis process [18, 19]. We did not find PA-induced pyroptosis in PSCs.

We also found that PA and more importantly, OA induced fat droplet accumulation in PSCs. This overload was more important when both molecules were combined suggesting that the mono-unsaturated fatty acid OA improves the PA overload in PSC. In the literature, lipidomic analyses have shown that the main components of pancreatic lipids are PA and OA, supporting the hypothesis that both molecules play a role in fat

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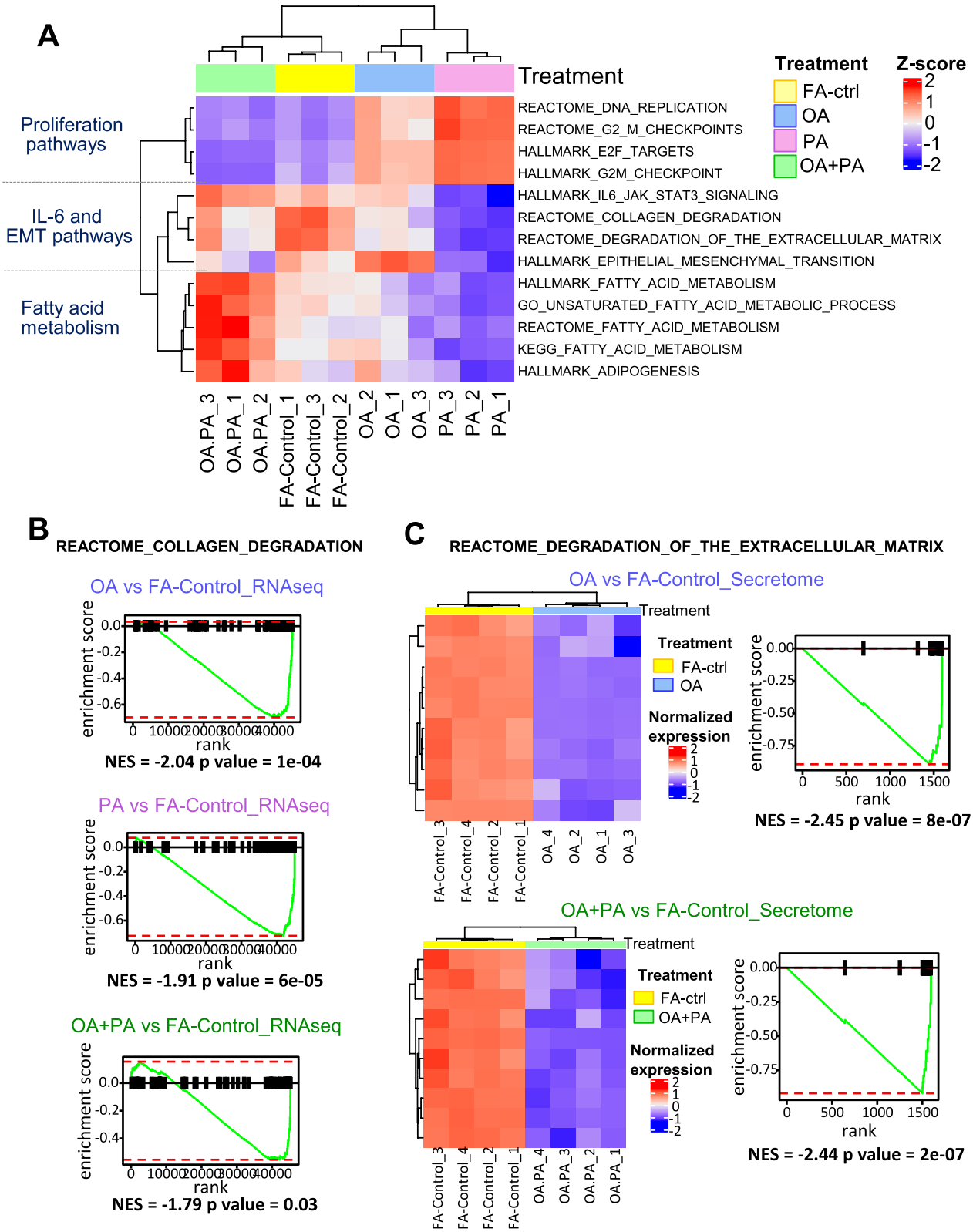
Fig. 2 Transcriptomic and secretome modifications in PS1 cells treated with fatty acids. **A** Gene set variation analysis (GSVA) of the pathways enriched in pancreatic cell lines (PS1) treated with different doses of fatty acids. Both OA and PA enrich proliferation pathways in opposition to the effect of their combination, thus confirming their antagonistic interaction. **B** Leading edge of the transcriptome (RNAseq) of PS1 cells treated with OA, PA and OA + PA shows the decrease in the collagen degradation pathway compared to untreated conditions. **C** GSVA heat maps and leading edges of the secretome of PS1 cells shows the decrease in the ECM degradation pathway compared to untreated conditions. *OA* oleic acid, *PA* Palmitic acid, *FA-ctrl* fatty acid control (untreated condition), *ECM* extracellular matrix, *RNAseq* RNA sequencing



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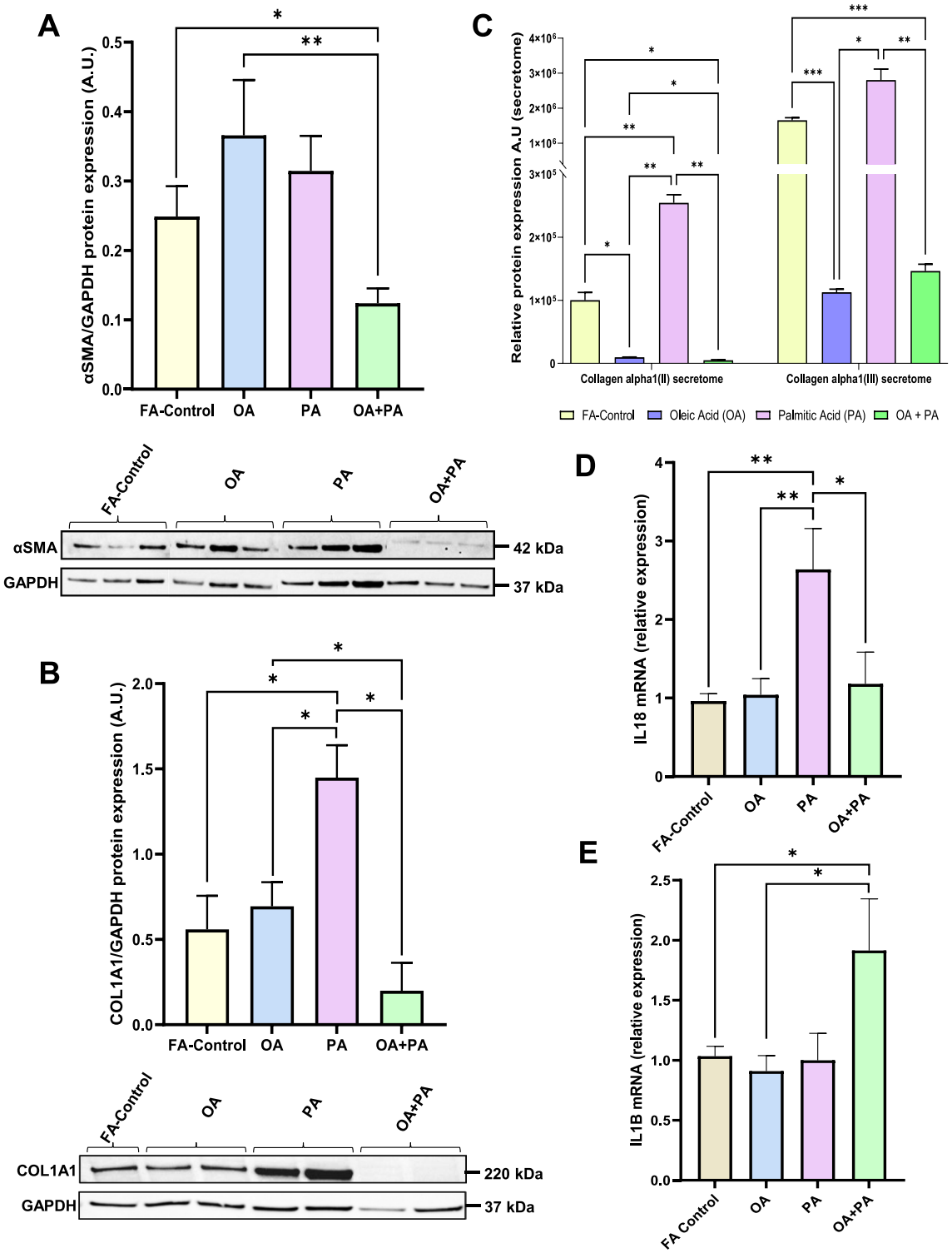

Fig. 3 Role of OA and PA in the activation of PS1 cells. **A** There is a tendency for alpha-smooth muscle actin (αSMA) to increase in PS1 treated with PA and OA alone on immunoblot analysis while its expression is reduced with the combination. **B** COL1A1 expression in PS1 cells. **C** COL1AII and COL1AIII secretion is increased in PS1 with PA treatment and reduced by OA treatment alone in secretome analysis. The combination of OA + PA reduces the expression of fibroblast markers confirming their antagonistic effect. **D**, **E** IL-18 RNA expression is increased with PA treatment and IL-1β expression is decreased in PS1 cells treated with both fatty acids in qPCR analysis. Data are expressed as mean ± SEM.**p < 0,005; *p < 0,05. Only significant comparisons (Mann Whitney unpaired t-test) were shown. *OA* oleic acid, *PA* Palmitic acid, *FA-ctrl* fatty acid control (untreated condition)

absorption and cell toxicity and that OA could play a role in PA absorption [20].

The use of lipid droplet staining would not be an accurate method to analyze PSC activation because treatment with fatty acids resulted in fat accumulation in PSCs due to fat uptake. Thus, we used other methods to assess PSC activation and fibrosis, in particular an analysis of the expression of fibroblast markers such as collagens.

One study in primary rat PSCs showed that OA but not PA could maintain PSC activation by promoting their proliferation and migration. PA induced endoplasmic reticulum (ER) stress as shown by the elevated Xbp1 splicing and CHOP levels [21]. An evaluation of fatty acid-induced ER stress, especially PA, on PSCs in vitro in human cell lines and ex vivo using pancreatic organotypic culture tissues would be important to evaluate whether these fatty acids have the same effect on our models.

Our study showed that OA and PA increased the proliferation pathways in PSCs compared to control conditions while on the other hand their combination (OA + PA) led to a decrease in proliferation pathways confirming their antagonistic roles. A decrease in collagen degradation was also observed in the transcriptome and the secretome of PSCs treated with OA, PA and OA + PA compared to control conditions. We also found that OA and PA reduced the collagen degradation in PSCs suggesting that OA and PA could play a role in the activation of PSCs and their transformation into fibroblast-like cells. A recent in vitro and in vivo study on rat PSCs showed that their activation could be mediated by pyroptosis after treatment with lipopolysaccharides [22].

Interestingly, PA but not OA stimulated the production of fibroblast markers COL1A1 and COL1A2/3 in PSCs, with no impact on the expression of alpha-smooth muscle actin (αSMA), suggesting that OA plays a protective role against the development of fibrosis. The functional antagonistic role of OA and PA (in addition to their toxicity) was confirmed in the activation of PSCs by the reduction in αSMA expression and the production of collagens with OA + PA. We also found that PA induced the expression of proinflammatory cytokines such as IL18. These results confirm that PA but not OA can induce PSCs activation. Assel JM et al., showed that PSCs acquire a pro-inflammatory phenotype when they are activated. Activated PSCs can secrete proteins that stimulate the proliferation and migration of pancreatic tumoral cells in an eIF4E (4E eukaryotic translation initiation factor)-dependent manner. Our results showed that PA increased eIF4E expression and could induce the same effects suggesting its possible role in pancreatic carcinogenesis [23].

The first studies using ex vivo organotypic slices characterized the physiology of exocrine and endocrine cells in mouse and human tissues [24–26].

The use of ex vivo culture tissue has been recognized to be a valued model for translational research. It is one of the most appropriate models to perform several analyses, such as drug-testing and intercellular dialogues investigations, because of the combination of the epithelial cells with the microenvironment compartment (extracellular matrix compounds, immune and stromal cells) and the use of a supportive medium to all the cellular compounds [26–29].

This study is the first one to investigate the role of FAs in the activation of PSCs during pancreatic parenchymal transformation and fibrosis using an ex vivo 3D model of precision cut pancreatic slices from non-tumoral human tissue.

The PCPS model can be used to analyze the transformation of pancreatic parenchyma in response to OA and PA.

One limitation of this model was the limited duration of cultures, making impossible to analyze the long-term consequences of FAs. Significant fibrotic changes could not be evaluated during this short period. Thus, we used qRT-PCR to detect early events and transformations.

We highlight that OA and PA induce a decrease in acinar markers in acinar cells, compared to FA-controls and that the OA + PA combination increases the expression of the nuclear ductal marker SOX9. This suggests that OA and PA could induce early acinar transformations i.e. the loss of the acinar phenotype with a possible acquisition of a ductal-like phenotype, which is an early step of pancreatic carcinogenesis.

This is similar to our previous study in a 3D model which showed that acinar cells could change to ductal phenotype under oxidative stress conditions associated

(See figure on next page.)

Fig. 4 Fatty acids safety on PCPS. **A** Protocol flowchart and analytical readouts of PCPS generated from fresh human non-tumoral pancreatic tissue. **B** Tissue viability was preserved in OA and PA doses used on PCPS after 24 h of culture, matching the levels of untreated conditions. **C** Evaluation of the tissue on HES slides at baseline and after 24 h of culture (scale at 50µm). Data are expressed as mean ± SEM.**p < 0,005; *p < 0,05. Only significant comparisons (Mann Whitney unpaired t-test) were shown. PB-Control, PrestoBlue control (medium with PrestoBlue without any tissue slice); Untreated-24h, PCPC after 24h of culture with medium; *OA* oleic acid, *PA* Palmitic acid, *PCPS* precision cut pancreas slices, *FFPE* formalin-fixed paraffin embedded, *HES* hematoxylin–eosin-safran-staining

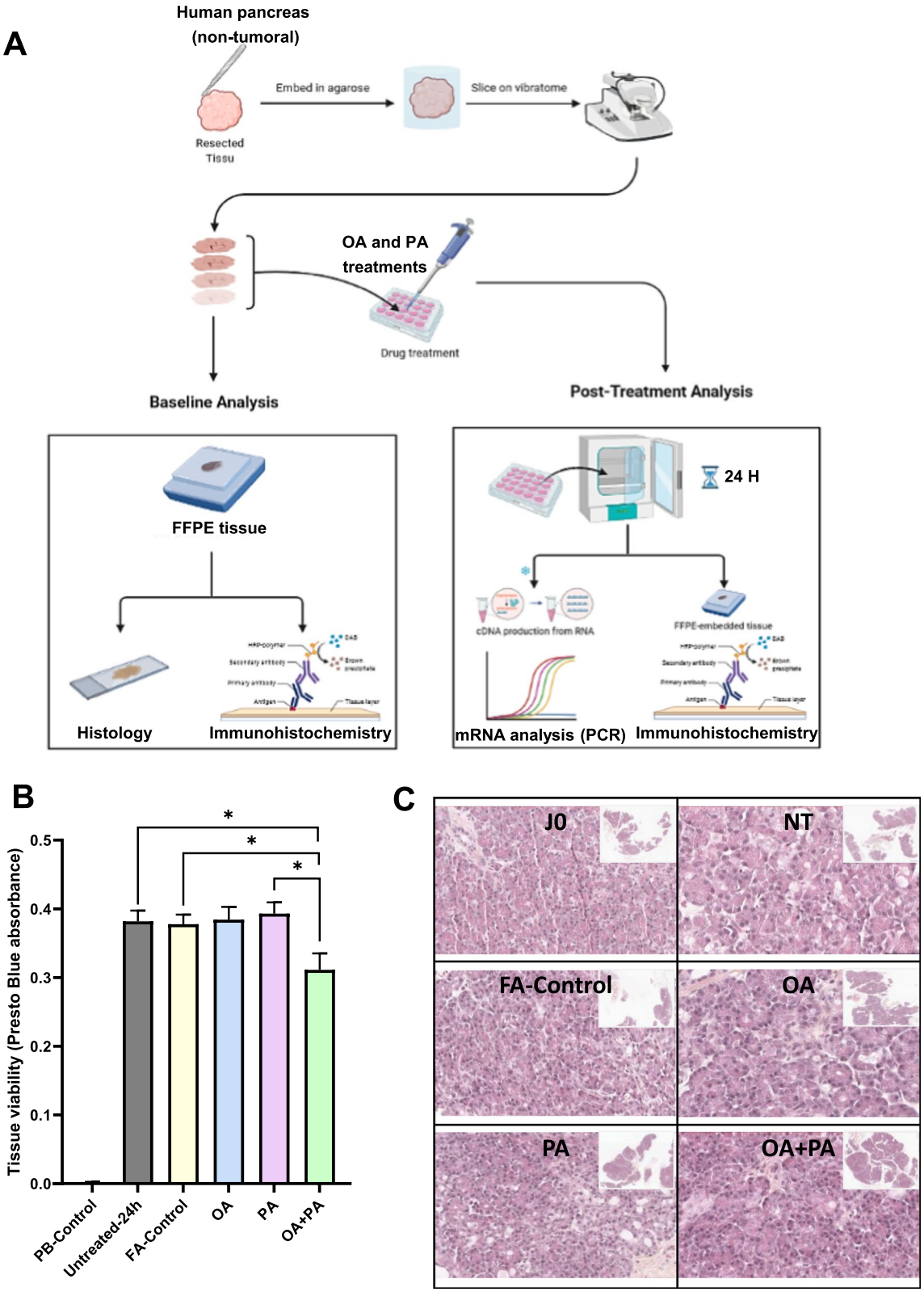

Fig. 4 (See legend on previous page.)

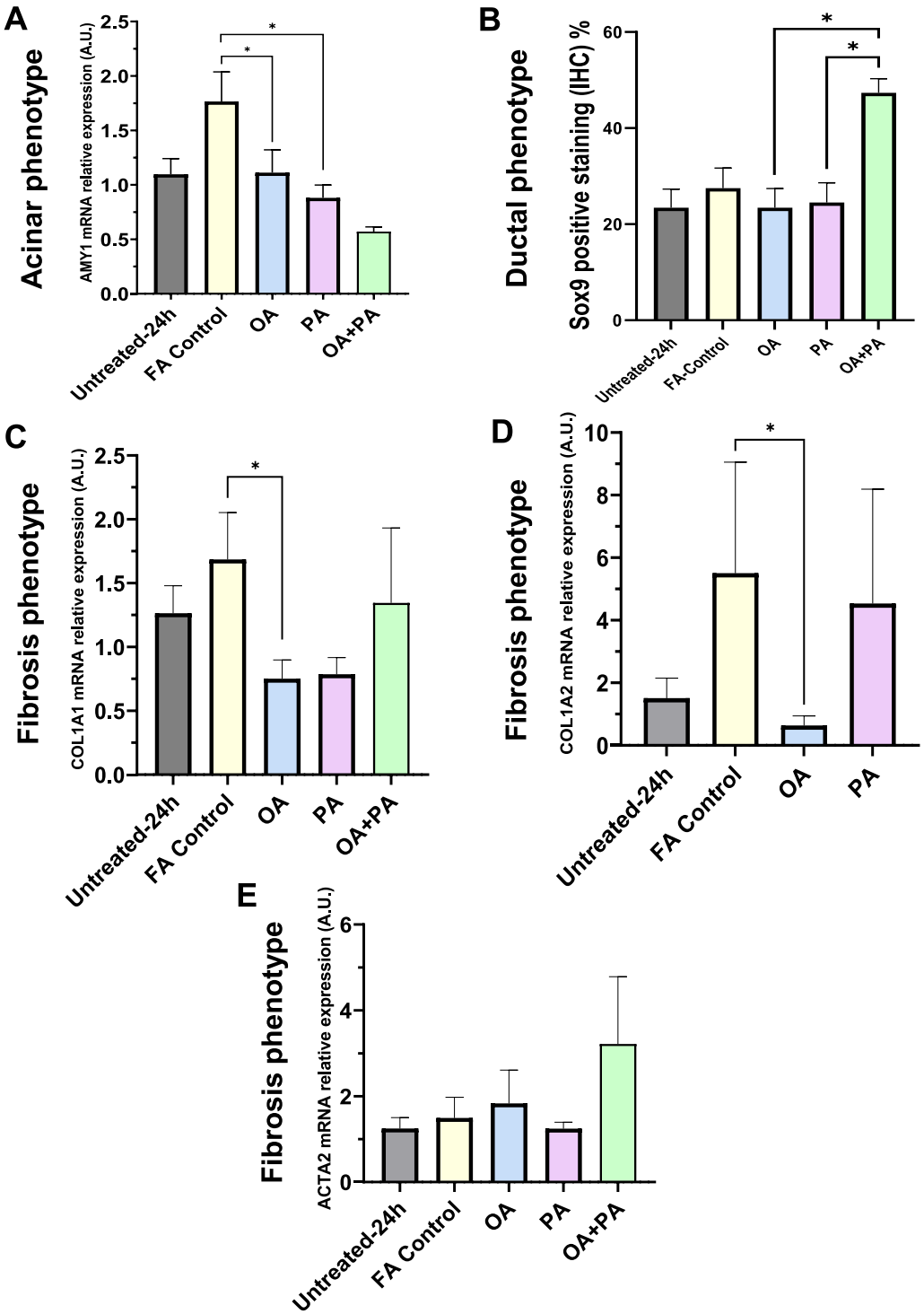

Fig. 5 Fatty acids affect acinar phenotype and fibrosis in PCPS. **A** AMY1 (acinar marker) mRNA expression in PCPS was reduced on qPCR analysis after culture with OA and PA alone. **B** Quantification of ductal marker SOX9 immunostaining in PCPS at 24h of culture and after culture with fatty acids shows increased expression in PCPS treated with the combination of OA + PA. **C**–**E** qPCR analyses of fibroblastic markers: COLA1 (**C**) COL1A2 (**D**) and ACTA2 (**E**) in PCPS treated with fatty acids. Results are similar to the effects observed in PS1 cell lines alone (OA reduces the RNA expression of collagens in pancreatic slices). Data are expressed as mean ± SEM..***p < 0,0005;**p < 0,005; *p < 0,05. Only significant comparisons (Mann Whitney unpaired t-test) were shown. *OA* oleic acid, *PA* Palmitic acid, *FA-ctrl* fatty acid control (vehicle condition), *Untreated-24h* PCPC after 24h of culture with medium, *PCPS* precision cut pancreas slices, *HES* AMY1, amylase

with the activation of PSCs [15]. Fat co-culture conditions were found to increase this phenomenon.

We also found that OA played a protective role against fibrosis in this model by reducing COL1A1/2 expression.

We provide additional evidence that specific lipid species, in this case OA and PA, play different roles in the initiation of cell transformations in obesity. Some fatty acids (such as PA) could induce cellular stress and inflammation leading to the initiation of precancerous lesions and others (such as OA) could play an antagonistic and protective role especially in the process of pancreatic fibrosis. Our results provide new insight into the potential in situ role of fatty acids in the preneoplastic and fibrogenesis process in the pancreas in obese patients.

Abbreviations

ADM	Acinar-to-ductal metaplasia
BCL10	B-cell lymphoma 10
BMI	Body mass index
CK7	Cytokeratin 7
FA	Fatty acid
FFPE	Formalin-fixed paraffin-embedded
GSEA	Gene set enrichment analysis
GSVA	Gene Set Variation Analysis
HES	Hematoxylin-eosin-safran-stained
IHC	Immunohistochemistry
IPMN	Intraductal papillary mucinous neoplasia
OA	Oleic acid
PA	Palmitic acid
PanIN	Pancreatic intra epithelial neoplasia
PBS	Phosphate buffered saline
PCPS	Precision cut pancreatic slices
PDAC	Pancreatic adenocarcinoma
PSC	Pancreatic stellate cells
RNA-seq	RNA sequencing
Sox9	SRY-Box transcription factor 9
αSMA	Alpha smooth muscle actin

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-025-06313-9.

Supplementary Material 1. Figure S1. Role of OA and PA on PS1 pyroptosis and proliferation. Exploration of pyroptotic cell death in PS1 treated with FA using several effectors and markers such as NLRP3 mRNA and protein expression (A), GSDMD mRNA expression in qPCR analysis (B), and CASP1 protein quantification in Western blot analysis (C). Exploration of the cell death via apoptosis using CASP3 protein expression in PS1 treated with FA (D) shows no effect of the FA on this apoptosis marker in PS1 cells. (E) 4E eukaryotic translation initiation factor (EIF4E) RNA expression was increased in PS1 after culture with PA. Data are expressed as mean ± SEM.**p< 0,005; *p< 0,05. Only significant comparisons (Mann Whitney unpaired t-test) were shown. OA, oleic acid; PA, Palmitic acid; FA-ctrl, fatty acid control (vehicle condition).

Supplementary Material 2. Table S1: Patient characteristics (PCPS,

Supplementary Material 3. Table S2: Gene count table of PS1 cells treated with FA (transcriptomic analysis).

Supplementary Material 4. Table S3: Protein count table of PS1 cells treated with FA (Secretome analysis).

Supplementary Material 5.

Author contributions

Sonia Frendi: Study design and concept, data acquisition, data analysis, manuscript drafting; Rémy Nicolle, Anaïs Chassac, Miguel Albuquerque, Jérôme Raffenne: data analysis and manuscript revision; Valérie Paradis: Critical revision; Jérôme Cros, Anne Couvelard, Vinciane Rebours: Study design and concept, data analysis, manuscript drafting and critical revision.

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Data availability

RNA sequencing and secretome analyses: Raw count data are available in supplemental data.

Declarations

Competing interests

There are no conflicts of interest to declare.

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