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Plasma anthocyanins and their metabolites reduce *in vitro* migration of pancreatic cancer cells, PANC-1, in a FAK- and NF-kB dependent manner: Results from the ATTACH-study a randomized, controlled, crossover trial in healthy subjects

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ABSTRACT

Pancreatic cancer is primarily considered to be a metastatic disease with a low 5-year survival rate. We aimed to detect if plasma-isolated anthocyanins and their metabolites (PAMs) modulate pancreatic cancer cells migration and to describe molecular targets of PAMs in this process. Plasma metabolites were isolated by solid-phase extraction before and after a 28-days intervention trial involving 35 healthy subjects comparing effects of a daily anthocyanin-rich juice intake *vs.* placebo. Plasma extracts were used for migration and mechanistic *in vitro* studies as well as for metabolomic analysis. Pancreatic PANC-1 and AsPC-1 were used for migration studies in a Boyden chamber co-cultured with endothelial cells. Expression of adhesion molecules on cancer and endothelial cells were determined by flow cytometry and NF-kB (nuclear factor-kappa B) p65 and focal adhesion kinase activation were measured by immunoassays. UHPLC-MS/MS metabolomics was done in plasma and urine samples. Plasma extracts isolated after the intake of the anthocyanin-rich juice significantly reduced PANC-1 migration, but not AsPC-1 migration. In PANC-1, and to a lower extent in endothelial cells, plasma extracts after juice intake decreased the expression of ß1- and ß4-integrins and intercellular adhesion molecule-1. Pooled plasma from volunteers with the highest inhibition of PANC-1 migration (n = 10) induced a reduction of NF-kB-p65 and FAK-phosphorylation in cancer and in endothelial cells. Concerning metabolites, 14 were significantly altered by juice intervention and PANC-1 migration was inversely associated with the increase of o-coumaric acid and peonidin-3-galactoside. PAMs were associated with lower PANC-1 cell migration opening new strategies for metastatic pancreatic cancer treatment.

1. Introduction

Pancreatic cancer is predicted to become the second leading cause of cancer death within the next decade in Western countries. It is commonly associated with poor prognosis and low overall five-year survival rate (5–7%) due to the early metastatic potential of pancreatic cancer cells [1]. Indeed, more than 80% of the patients are suffering from metastases or unresectable tumors at time of diagnosis [1].

Abbreviations: CAM, Cell Adhesion Molecules; ACN, Anthocyanins; HUVEC, Human Umbilical Vein Endothelial Cells; TEER, Transepithelial electrical resistance; FAK, Focal Adhesion Kinase; ROS, Reactive Oxygen Species.

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Metastasis is a multistep process in which cell adhesion molecules (CAMs), such as integrins and selectins play pivotal roles [2]. Malignant cells from the primary tumor infiltrate the surrounding tissue and enter the circulation by blood vessel intravasation and extravasation into the target tissue [2,3]. Alterations in the expression of several CAMs are known to modulate the migratory and invasive potential of cancer cells [4].

Strategies to improve the health outcomes in pancreatic cancer are challenging. Not only primary prevention strategies associated with a healthy lifestyle are discussed, but also secondary prevention strategies. Epidemiological studies revealed a potential benefit of diets high in fruits and vegetables [5–7]. A group of bioactive phytochemicals responsible for 'anti-cancer' effects are anthocyanins (ACNs). ACNs could account for up to 80% of the total polyphenolic content in berries [8]. However, ACNs bioavailability is low, and large amounts of dietary ACNs are fermented by gut microbiota [9,10]. As a result, several phenolic acids and other metabolites are generated [10,11]. Whether parent ACNs or their metabolites are responsible for these 'anti-cancer' effects is not fully understood. Recently, a screening of polyphenol metabolites on HCT116 3D-spheroids revealed that the ACN-related metabolite, 3′,4′-dihydroxyphenyl-γ-valerolactone (3,4-DHPV), reduced cancer cells spheroid integrity [12] underlining the role of gut microbiota fermentation of ACNs. Furthermore, a blueberry extract decreased CAM expression in several cancer cell lines and inhibited migration of breast and prostatic cancer cell lines (MDA-MB-231 and PC-3 cells), respectively [13]. Nonetheless, only few *in vitro* studies reported changes in CAM expression in cancer and endothelial cells in response to ACNs.

Previously, we showed that 60 min after a single ACN dose, plasma extracts from volunteers inhibited migration of the pancreatic cancer cell line PANC-1 *in vitro* [14]. Furthermore, we observed a reduction of migration of the colon cancer cell line HT-29 after a daily ingestion of ACNs over 28 days [15]. Therefore, our aims were to investigate: 1) whether plasma metabolites, isolated after a 28-day intervention, would reduce migration of two pancreatic cancer cell lines (PANC-1 and AsPC-1); 2) whether expression of adhesion molecules on cancer and endothelial cells were influenced by plasma ACN metabolites; 3) which molecular mechanisms were involved; and 4) which metabolites in plasma and urine were altered during a long-term ACN intake and how they associate with the inhibitory effects on migration.

2. Materials and methods

All details on materials are given in the Supplementary Table 1.

2.1. Study design and subjects

Details on the ATTACH study (Anthocyanins Target Tumor cell Adhesion—Cancer vs. Endothelial Cell (HUVEC)) have been published previously [15]. Briefly, this was a randomised, double-blind, placebo-controlled, cross-over, 28-days intervention comparing the effects of an ACN-rich juice (J) (330 ml/day, 942 mg/L of ACNs and 2622 mg/L of total polyphenols) and an ACN-depleted (330 ml/day, 6.3 mg/L of ACNs and 115 mg/L of total polyphenols) placebo juice (P), with a 2-week wash-out and 1-week run-in period. Thirty-five young, healthy volunteers participated in the intervention. Sample size was calculated based on the results of our previous published migration study [14] with ß- and α-error of 0.8 and 0.05 and a drop-out rate of 20%. CONSORT flowchart diagram is shown in Supplementary Figure 1. Participants were randomly assigned (random-table). The study protocol was approved by the local ethic committee in Gießen (Germany) (registration number 13/10) and performed according to the guidelines laid down in the Declaration of Helsinki. Written informed consent was obtained from all participants and the trial is registered at DRKS (*Deutsche Register Klinischer Studien;* DRKS00014767). The present manuscript is reported following the CONSORT checklist Supplementary Table 2.

2.2. Blood and urine sample preparation

Blood and 24 h-urine samples were collected before (day 0) and at the end (day 28) of the two intervention periods. Blood was taken 6 h after beverages intake of day 28 into EDTA-tubes and centrifuged to separate plasma. Acidified urine samples were then stored at − 80 °C until assayed. Extraction of plasma metabolites for cell culture assays and plasma and urine sample preparation for metabolomics analysis are described in the Supplementary Data (S1 and S2).

2.3. Cell culture and functional assays

Details on cancer cell lines and endothelial cells are given in the Supplementary Data (S3 and S4).

2.4. Outcomes

2.4.1. Primary outcome

2.4.1.1. Cell migration of pancreatic cancer cells in-vitro. Tumor cell migration was assessed in a Boyden membrane chamber with the use of the CytoSelect 24-well Cell Migration Assay as described previously [14,15]. The 24-well feeder chamber (diameter of the chamber 6.5 mm; pore size 8 μm) tray was coated with 50 μL of 0.1% fibronectin and aspirated until dryness. Thereafter, HUVECs (5×10^4/ml) were seeded onto the fibronectin-coated inserts and allow to grow confluent. Two-days post-confluence, transepithelial electrical resistance (TEER) was determined before the experiments by using a Millicell® ERS volt-ohmmeter. A TEER value $\geq$ 250 Ω per cm^2 was used as an indicator for an intact endothelial layer suitable to be used for functional studies. Pancreatic cancer cells (1×10^5/ml) were seeded in Endothelial growth medium II supplemented with 2.5% fetal calf serum (FCS) containing diluted plasma extracts of the participants, whereas Endothelial growth medium II supplemented with 12.5% FCS was added to the lower basal chamber. The cells were incubated in the feeder tray for 36 h at 37 °C, and cells on the lower side were then detached from the membrane using a cell detachment solution and afterwards lysed with fluorescent dye-containing buffer. The extent of migration was assessed by the intensity of the fluorescence signal with a microplate fluorescence reader. The number of migrated cells was determined according to a calibration curve (0–7500 cells). The results are expressed as medians and IQR (25th-75th).

2.4.2. Secondary outcome

2.4.2.1. Analysis of CAMs by flow cytometry analysis. Basal and `CytoMIX´-stimulated expression of surface marker such as cell adhesion molecules (CAMs) and Vascular endothelial growth factor receptor (VEGF-R) were analyzed under pre-confluent (PANC-1 and AsPC-1) or post-confluent (HUVECs) conditions. Cells were washed twice with phosphate buffered saline (PBS) and detached with TrypLE™ Express-solution for cancer cells or accutase-solution (0.15 ml/cm^2) for endothelial cells. After the detachment, cells were centrifugated (220xg for 3 min) and supernatant was decanted. Pellet was washed twice with PBS, resuspended in cold 100 μL MACS Running Buffer (pH 7.2) for staining procedures. Results are expressed as means ± SD or median with IQR (25th-75th). More details about staining procedures are given in the Supplementary data (S5).

2.4.2.2. Cytokine quantification, detection of NF-kB p65, FAK (focal adhesion kinase) activation and reactive oxygen species (ROS) determination. Commercially available ELISAs were used to determine cytokines, activation of NF-kB p65 and FAK in the supernatants of cells and were done by the manufacturer's instructions. ROS determination and sample preparation have been described previously (14). The results are

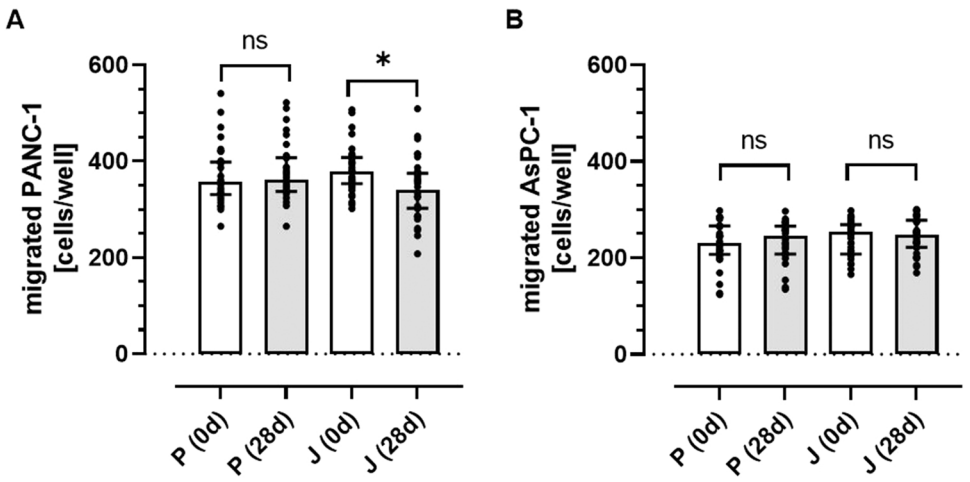

Fig. 1. Migration of PANC-1 and AsPC-1 in a Boyden chamber co-cultured with HUVECs representing endothelial cells *in vitro*. PANC-1 (A) and AsPC-1 (B) were exposed to plasma extracts from the placebo (P) and juice (J) intake before (0d) and after (28d) intervention. Migration across the endothelial layer into the lower chamber was measured after 36 h. Migration was measured fluorometrically in a Boyden chamber as described in Materials and Methods Section 2.4.1. Values are expressed as aligned dot blot with median and interquartile range (25th-75th). Significant differences were calculated with repeated measurements One-way ANOVA and values with * were different with *p-value* < 0.05 to corresponding controls (n = 34).

expressed as mean ± SD and more details are given in the Supplementary data (S6-S8).

2.4.3. Exploratory outcome

2.4.3.1. Targeted metabolomics UHPLC-MS/MS analysis. The UHPLC-MS/MS analysis has been explained previously [16]. Briefly, the analysis of plasma and urine was performed by using the 1290 Infinity UHPLC system coupled to a QTRAP 6500 mass spectrometer equipped with Ion Drive Turbo V ion source. Luna Omega Polar C18 column, 100 mm × 2.1 mm (i.d. 1.6 μm) with a porous polar C18 security guard cartridge were used to perform the chromatographic separation. More details are described in the Supplementary data (S9 and 10).

2.5. Statistical analysis

2.5.1. Cell culture data analyses

Data from the volunteers who completed all phases of the study were analyzed (n = 35). The outcome measures were prospectively designated as the differences in migration of PANC-1 and AsPC-1 *in vitro* (primary outcome) and mechanistic parameters (secondary outcome) of placebo and juice treatment before and after intervention. Before-treatment versus after-treatment data within groups were analyzed using a repeated measures one-way ANOVA with Šídák's post hoc test. The normality of continuous variables was assessed using Kolmogorov-Smirnov normality test. Asterisks are used in the figures to denote *p* values < 0.05, which were considered significant. GraphPad Prism 9 (Version 9.3.1.) was used for data analyses.

2.5.2. Metabolomics data analyses

Pre-processing of metabolomics data is described in the Supplementary Data (S10). To assess the effects of the juice intervention, we used a linear mixed model (LMM) including treatment (juice/placebo), age, and sex as fixed effects and subject as random effect. *P*-values were adjusted for multiple comparisons using the Benjamini-Hochberg false discovery rate (FDR) and results were integrated in a volcano plot. We selected as significant those metabolites with a $\log_2FC > |0.584|$ and an FDR-adjusted *p*-value < 0.05. Associations between plasma anthocyanin metabolites and migration experiments and adhesion molecules expression were tested using linear mixed models adjusted for age, sex and treatment as fixed effects, and subject as random effect. R statistical software version 4.1.3 was used for metabolomics statistical analyses.

3. Results & discussion

Thirty-five volunteers (female n = 27 and male n = 8), with a mean (± SD) age of 24.4 (± 2.3) years old, an initial body weight of 64 ± 18 kg and a BMI of 21.7 ± 2.6 kg/m^2, completed the protocol while one

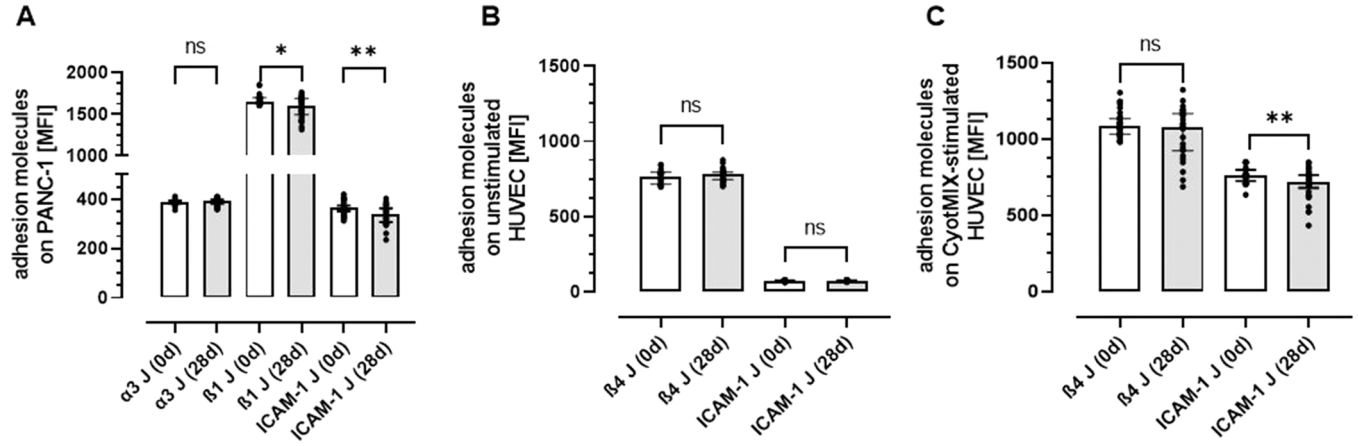

Fig. 2. Expression of CAMs on cancer cells and endothelial cells after incubation with plasma extracts from ACN-rich juice. Expression of adhesion molecules on PANC-1 (A) as well as on non-stimulated and CytoMIX-stimulated HUVECs (B and C) after incubation with plasma extracts from volunteers ingested the ACN-rich juice (J) over 28 days. CAMs were measured fluorometrically by flow cytometry as described in the Materials and Method Section 2.4.2. Values are expressed as median of main fluorescence intensity (MFI) with interquartile range (25th–75th). Significant differences were calculated with repeated measurements One-way ANOVA and values with * , * * and * ** were different with *p-value* < 0.05, *p-value* < 0.01 and *p-value* < 0.001 to corresponding controls (n = 34).

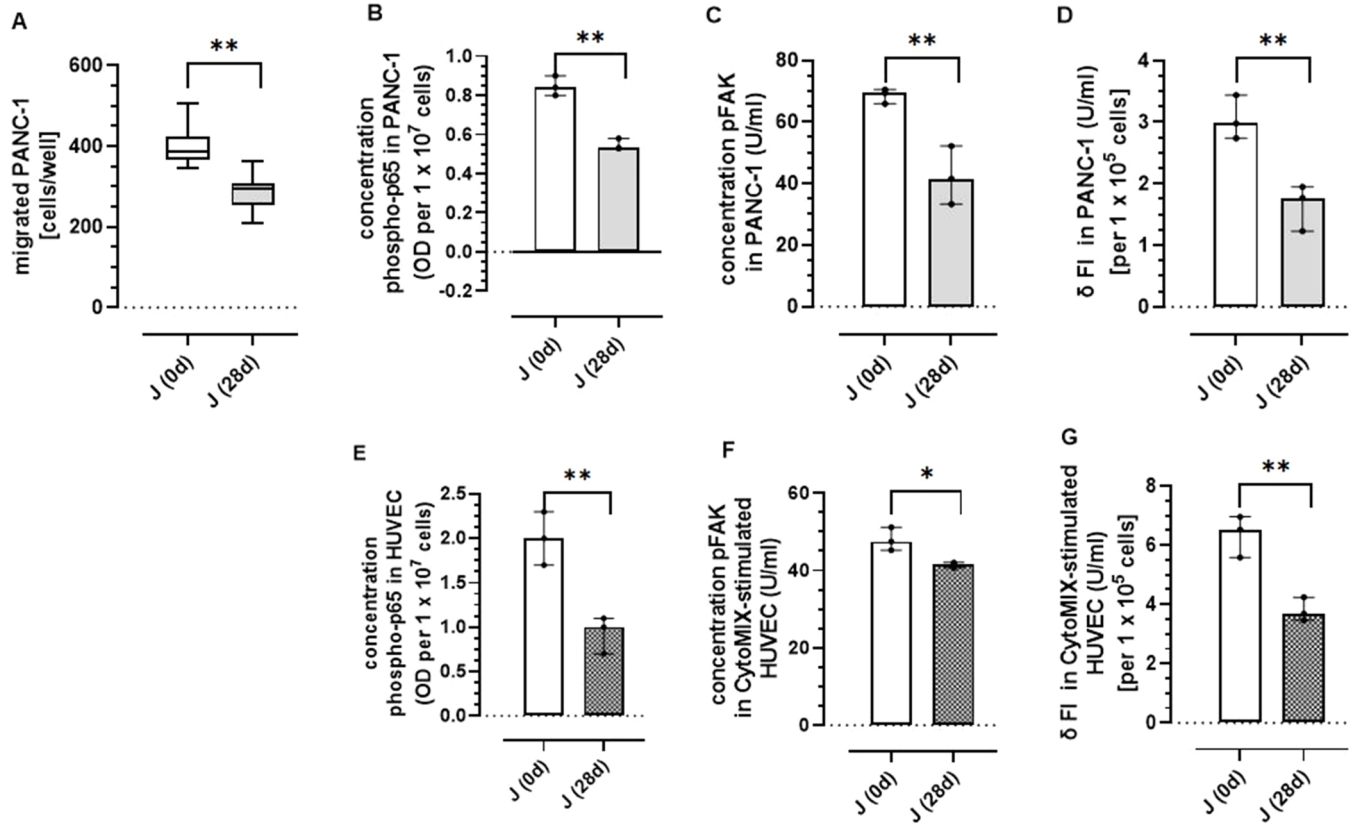

Fig. 3. Molecular Targets of plasma extracts previous and after juice intervention in PANC-1 and HUVECs. (A). PANC-1 migration across the endothelial layer into the lower chamber of co-culture model were measured after 36 h with pooled plasma metabolites of the 10 samples with the highest percentage of migration reduction). (B-G) PANC-1 and ĆytoMIX´-stimulated HUVECs were seeded onto 6-well plates and incubated with pooled PAMs for 36 h. Thereafter, cells were lysed and phosphorylation of p65 (B, E) and FAK (C, F) were measured colorimetrically by ELISA as described in the Materials and Method Section 2.4.2. (D, G) ROS generation was measured after 36-h exposure to PAMs from ACN-rich juice (J) before (d0) and after 28-d (d28) intervention. Thereafter, cells were washed with PBS and incubated with 5 μmol/L H_2DCFDA for 30 min at 37 °C. Fluorescence intensity was measured (Ex/Em 485/535) as described in Materials and Methods Section 2.4.2. Values are expressed as means with standard deviation. Significant differences were calculated with t-test and values with * * were different with *p-value* < 0.01 compared to corresponding controls (n = 3).

subject had an incomplete urine sample collection (blood n = 35; urine n = 34).

3.1. *Primary and secondary outcome: cancer cell migration, expression of adhesion molecules and mechanism*

As shown in Fig. 1, plasma extracts had different effects on cancer cell migration *in vitro* depending on the cancer cell line. In PANC-1, extracted-plasma metabolites after the ACN-rich juice reduced cell migration significantly in comparison to plasma extracts after placebo (Fig. 1A). On the other hand, no reduction was observed for the migration of AsPC-1 (Fig. 1B). As viability of PANC-1 and AsPC-1 was not affected by incubation with plasma extracts under the experimental conditions (data not shown), one reason for these differences could be the influence of plasma extracts on expression of surface molecules and secretion of cytokines that stimulate endothelial cells (Supplementary data S11 and S12). Briefly, in comparison to AsPC-1, PANC-1 expressed higher levels of α3-, α4-, ß1- and ß2-integrin, E-selectin, ICAM-1 and VEGF-R. However, only ß1-integrin and ICAM-1 expression were significantly reduced on PANC-1 by plasma metabolites extracted after juice intake (Fig. 2A). Furthermore, PANC-1 showed a higher secretion of cytokines, such as TNF-α, IL-1ß, and VEGF compared to AsPC-1, indicating a higher potential for activating CAMs on endothelial cells (Supplementary data S4). Higher expression of CAMs and cytokines secretion may be responsible for higher interaction with endothelial cells and thus migration of PANC-1 across the endothelial layer. To investigate the effect of plasma extracts after juice intake on CAMs of HUVECs, cells were stimulated for 36 h with a so-called ĆytoMIX´ (TNF-α, IL-1ß and VEGF), which were secreted by cancer cells. As shown in Fig. 2B and Fig. 2C, CytoMIX-stimulation resulted in increased expression of ß4-integrin and ICAM-1 in comparison to non-stimulated HUVECs, whereas plasma extracts after juice intake significantly reduced only CytoMIX-stimulated ICAM-1 expression.

Expression of CAMs on tumor cells and the expression of receptors on endothelial cells are important steps in the multistep-cascade of migration and metastasis [17]. It has previously been shown that ACNs were able to reduce cancer cell migration by inhibiting phosphorylation of signalling proteins [18,19]; however, we acknowledge that 311 mg/day of ACNs would be difficult to achieve within a diet. However, compared to other studies we used ACN and their metabolites isolated from plasma samples. Most of the previous studies examined the relation between ACN-rich diet and anti-cancer activities using food frequency questionnaires, or after extracting the ACN-related phenolic metabolites from a prepared stock solution directly used in cancer cells [20–24]. However, this result support studies showing that cancer chemoprevention combined with bioactive phytochemicals could be associated with less toxicity and improved efficacy [25–27].

To investigate the mechanisms behind the observed anti-migratory effects on PANC-1, we pooled the plasma extracts from the volunteers with the highest percentage of migration reduction (n = 10). NF-kB p65 and FAK phosphorylation as well as ROS generation were analyzed after incubation with this pool of extracted plasma samples. The redox-sensitive NF-kB and FAK pathways are associated with the regulation of adhesion molecules. As shown in Fig. 3A, incubation of cells with

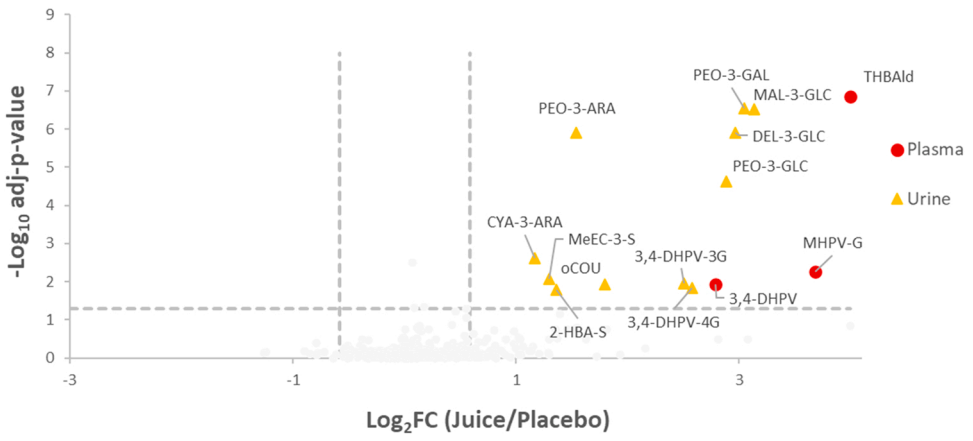

Fig. 4. Volcano plot of log_2FC vs log_{10} FDR-adjusted p-value, showing the metabolites that are significantly associated with ACNs intake in plasma, and urine. The FC value of each metabolite corresponds to the difference between log_2FCpost/pre during ACN-rich juice and log_2FCpost/pre during placebo intervention. To determine the effect of the ACN-rich juice, LMM was used with treatment (juice/placebo), sex and age as fixed factors and subject ID as random factor. *p*-values were calculated and adjusted using FDR (plasma n = 35 and urine n = 34). THBAld: 2,4,6-trihydroxybenzaldehyde; MHPV-G: 4′-hydroxy-3′-methoxyphenyl-γ-valerolactone glucuronide; 3,4-DHPV: 3′,4′-dihydroxyphenyl-γ-valerolactone; oCOU: o-coumaric acid; MeEC-3-S: 3′-methyl(epi)catechin sulphate; 2-HBA-S: 2-hydroxybenzoic acid sulphate; 3,4-DHPV-3 G: 3′,4′-dihydroxyphenyl-γ-valerolactone 3′-glucuronide; 3,4-DHPV-4 G: 3′,4′-dihydroxyphenyl-γ-valerolactone 4′-glucuronide; PEO-3-GAL: peonidin-3-galactoside; PEO-3-GLC: peonidin-3-glucoside; PEO-3-ARA: peonidin-3-arabinoside; CYA-3-ARA: cyanidin-3-arabinoside; MAL-3-GLC: Malvidin-3-glucoside; DEL-3-GLC: delphinidin-3-glucoside; PET-3-GLC: Petunidin-3-glucoside).

pooled plasma extracts after juice intervention over 36 h significantly reduced cell migration. Concomitantly, incubation with plasma extracts after juice intervention significantly blocked phosphorylation of p65 and FAK in PANC-1 and also in CytoMIX-stimulated HUVECs (Fig. 3B-C and E-F) and reduced ROS levels in PANC-1 and stimulated HUVECs (Fig. 3D and G). FAK and NF-kB p65 were associated with increased cancer cell migration and cancer metastasis through higher expression of α- and ß-integrins [28,29]. Therefore, reduction of intracellular cell signalling pathways by plasma ACN metabolites opens a new window concerning cancer preventive strategies.

3.2. *Exploratory outcomes: metabolomics analyses*

From plasma and urine metabolome, 14 out of 902 metabolites (379 in plasma and 523 in urine) were significantly associated with ACN-rich juice intake as shown in Fig. 4. Among these, 3 plasma metabolites resulting from ACN-gut microbiota metabolism were found to be increased: 2,4,6-trihydroxybenzaldehyde (THBAld), 4′-hydroxy-3′-methoxyphenyl-γ-valerolactone glucuronide (MHPV-G) and 3′,4′-dihydroxyphenyl-γ-valerolactone (3,4-DHPV). In urine, 11 metabolites increased after the ACN-rich juice intervention; from these, 6 metabolites were parent ACNs (peonidin-3-galactoside, peonidin-3-glucoside, peonidin-3-arabinoside, cyaniding-3-arabinoside, malvidin-3-glucoside and delphinidin-3-glucoside), while the other 5 metabolites were gut microbial phenolic metabolites (o-coumaric acid (oCOU), 3′-methyl-epicatechin sulphate (MeEC-3-S), 2-hydroxybenzoic acid-sulphate, 3,4-DHPV-3-glucuronide, and 3,4-DHPV-4-glucuronide). In particular, THBAld, 3,4-DHPV and MHPV-G had been shown to have anti-cancer activities, but at supra-physiological doses [22–24,30]. However, we did not observe any association between the increment of plasma THBAld, 3,4-DHPV and MHPV-G and the reduction in PANC-1 migration. Because ACNs can be rapidly absorbed in the stomach, reach the blood and disappear from the circulation within few hours [31,32], it was expected that parent ACNs were only significantly altered in urine and not in plasma. Associations between PANC-1 migration as well as CAM expression of PANC-1 and of stimulated HUVECs with plasma and urine metabolites altered by ACN-rich juice are shown in Supplementary Figure 6. The increment of peonidin and oCOU in urine were inversely associated with PANC-1 migration. Similarly, ICAM-1 expression in PANC-1 was inversely associated with the increase in urinary excretion of MeEC-3-S.

Limitations of our study are the use of extracted plasma from young and healthy volunteers on cancer cell lines *in vitro*. Due to the intense metabolization and fermentation of ACNs, which may vary according to age, sex, and lifestyle, we cannot ascertain that these anti-migratory effects would be observed in patients with cancer or if there would be any interaction with classical chemotherapeutics.

In conclusion, the study showed for the first time that ACN and metabolites isolated from plasma after a long-term ACN-rich juice intervention reduced the migration and expression of CAMs in PANC-1 cancer cells *in vitro* through activation of FAK- and NF-kB-pathways as well as the reduction of ROS. These results are promising and could open a window to investigate interactions of ACNs with classical cancer prevention strategies.

CRediT authorship contribution statement

HM, TM, RGD and CAL: Metabolome analyses. **IB, MF, SR and SK:** conceptualization of the study, migration studies and mechanistic analyses. TM, IB and SK performed the statistical analyses. HM, TM, and IB wrote the first draft of the manuscript. All the authors reviewed and approved the final version of the manuscript.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgment

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2022.114076.

References

[1] P.E. Guerrero, L. Miró, B.S. Wong, A. Massaguer, N. Martínez-Bosch, R. de Llorens, P. Navarro, K. Konstantopoulos, E. Llop, R. Peracaula, Knockdown of α2,3-sialyltransferases impairs pancreatic cancer cell migration, invasion and e-selectin-dependent adhesion, Int. J. Mol. Sci. 21 (2020) 1–24, https://doi.org/10.3390/ijms21176239.

[2] N. Makrilia, A. Kollias, L. Manolopoulos, K. Syrigos, Cell adhesion molecules: role and clinical significance in cancer, Cancer Invest 27 (2009) 1023–1037, https://doi.org/10.3109/07357900902769749.

[3] R. Zeeshan, Z. Mutahir, Cancer metastasis - Tricks of the trade, Bosn. J. Basic Med. Sci. 17 (2017) 172–182, https://doi.org/10.17305/bjbms.2017.1908.

[4] J. Cooper, F.G. Giancotti, Integrin signaling in cancer: mechanotransduction, stemness, epithelial plasticity, and therapeutic resistance, Cancer Cell 35 (2019) 347–367, https://doi.org/10.1016/j.ccell.2019.01.007.

[5] J.M. Chan, F. Wang, E.A. Holly, Vegetable and fruit intake and pancreatic cancer in a population-based case-control study in the San Francisco Bay Area, Cancer Epidemiol. Biomark. Prev. 14 (2005) 2093–2097, https://doi.org/10.1158/1055-9965.EPI-05-0226.

[6] N. Lunet, A. Lacerda-Vieira, H. Barros, Fruit and vegetables consumption and gastric cancer: a systematic review and meta-analysis of cohort studies, Nutr. Cancer 53 (2005) 1–10, https://doi.org/10.1207/s15327914nc5301_1.

[7] R.J. Jansen, D.P. Robinson, R.Z. Stolzenberg-Solomon, W.R. Bamlet, M. De Andrade, A.L. Oberg, K.G. Rabe, K.E. Anderson, J.E. Olson, R. Sinha, et al., Nutrients from fruit and vegetable consumption reduce the risk of pancreatic cancer, J. Gastrointest. Cancer 44 (2013) 152–161, https://doi.org/10.1007/s12029-012-9441-y.

[8] K. Maatta, K.-E.A, T.R, Phenolic compounds in berries of black, red, green, and white currants (Ribes sp.), Antioxid. Redox Signal (2001) *3*.

[9] X. Liu, D.A. Martin, J.C. Valdez, S. Sudakaran, F. Rey, B.W. Bolling, Aronia berry polyphenols have matrix-dependent effects on the gut microbiota, Food Chem. 359 (2021), 129831, https://doi.org/10.1016/j.foodchem.2021.129831.

[10] M.C. López De Las Hazas, J.I. Mosele, A. Macià, I.A. Ludwig, M.J. Motilva, Exploring the colonic metabolism of grape and strawberry anthocyanins and their in vitro apoptotic effects in HT-29 colon cancer cells, Antioxid. Redox Signal. 65 (2017) 6477–6487, https://doi.org/10.1021/acs.jafc.6b04096.

[11] J. Jokioja, B. Yang, K.M. Linderborg, Acylated anthocyanins: a review on their bioavailability and effects on postprandial carbohydrate metabolism and inflammation, Compr. Rev. Food Sci. Food Saf. 20 (2021) 5570–5615, https://doi.org/10.1111/1541-4337.12836.

[12] J. Rubert, P. Gatto, M. Pancher, V. Sidarovich, C. Curti, P. Mena, D. Del Rio, A. Quattrone, F. Mattivi, A Screening of Native (Poly)phenols and Gut-Related Metabolites on 3D HCT116 Spheroids Reveals Gut Health Benefits of a Flavan-3-ol Metabolite, Mol. Nutr. Food Res. 2101043 (2022) 1–10, https://doi.org/10.1002/mnfr.202101043.

[13] H. Lamdan, R.S. Garcia-Lazaro, N. Lorenzo, L.G. Caligiuri, D.F. Alonso, H.G. Farina, Anti-proliferative effects of a blueberry extract on a panel of tumor cell lines of different origin, Exp. Oncol. 42 (2020) 101–108, https://doi.org/10.32471/exp-oncology.2312-8852.vol-42-no-2.14766.

[14] S. Kuntz, C. Kunz, S. Rudloff, Inhibition of pancreatic cancer cell migration by plasma anthocyanins isolated from healthy volunteers receiving an anthocyanin-rich berry juice, Eur. J. Nutr. 56 (2017) 203–214, https://doi.org/10.1007/s00394-015-1070-3.

[15] I. Behrendt, I. Röder, F. Will, H. Mostafa, R. Gonzalez-Dominguez, T. Meroño, C. Andres-Lacueva, M. Fasshauer, S. Rudloff, S. Kuntz, Influence of Plasma-Isolated Anthocyanins and Their Metabolites on Cancer Cell Migration (HT-29 and Caco-2) In Vitro: Results of the ATTACH Study, *11*, 1341, Antioxidants 2022 Vol. 11 (2022) *1341*, https://doi.org/10.3390/ANTIOX11071341.

[16] R. González-Domínguez, O. Jáuregui, M.I. Queipo-Ortuño, C. Andrés-Lacueva, Characterization of the Human Exposome by a Comprehensive and Quantitative Large-Scale Multianalyte Metabolomics Platform, Anal. Chem. 92 (2020) 13767–13775, https://doi.org/10.1021/acs.analchem.0c02008.

[17] G. Zhou, D. Chiu, D. Qin, L. Niu, J. Cai, L. He, W. Huang, K. Xu, Detection and clinical significance of cd44v6 and integrin-β1 in pancreatic cancer patients using a triplex real-time RT-PCR assay, Appl. Biochem. Biotechnol. 167 (2012) 2257–2268, https://doi.org/10.1007/s12010-012-9752-2.

[18] W.C. Lim, H. Kim, Y.J. Kim, S.H. Park, J.H. Song, K.H. Lee, I.H. Lee, Y.K. Lee, K. A. So, K.C. Choi, et al., Delphinidin inhibits BDNF-induced migration and invasion in SKOV3 ovarian cancer cells, Bioorg. Med. Chem. Lett. 27 (2017) 5337–5343, https://doi.org/10.1016/j.bmcl.2017.09.024.

[19] C.C. Huang, C.H. Hung, T.W. Hung, Y.C. Lin, C.J. Wang, S.H. Kao, Dietary delphinidin inhibits human colorectal cancer metastasis associating with upregulation of miR-204-3p and suppression of the integrin/FAK axis, Sci. Rep. 9 (2019) 1–11, https://doi.org/10.1038/s41598-019-55505-z.

[20] R. Zamora-Ros, C. Not, E. Guinó, L. Luján-Barroso, R.M. García, S. Biondo, R. Salazar, V. Moreno, Association between habitual dietary flavonoid and lignan intake and colorectal cancer in a Spanish case-control study (the Bellvitge Colorectal Cancer Study), Cancer Causes Control 24 (2013) 549–557, https://doi.org/10.1007/s10552-012-9992-z.

[21] W. Kalt, J.E. McDonald, Y. Liu, S.A.E. Fillmore, Flavonoid metabolites in human urine during blueberry anthocyanin intake, J. Agric. Food Chem. 65 (2017) 1582–1591, https://doi.org/10.1021/acs.jafc.6b05455.

[22] S.C. Forester, Y.Y. Choy, A.L. Waterhouse, P.I. Oteiza, The anthocyanin metabolites gallic acid, 3-O-methylgallic acid, and 2,4,6-trihydroxybenzaldehyde decrease human colon cancer cell viability by regulating pro-oncogenic signals, Mol. Carcinog. 53 (2014) 432–439, https://doi.org/10.1002/mc.21974.

[23] R. Sankaranarayanan, C.K. Valiveti, D.R. Kumar, S. Van Slambrouck, S. S. Kesharwani, T. Seefeldt, J. Scaria, H. Tummala, G.J. Bhat, The flavonoid metabolite 2,4,6-trihydroxybenzoic acid is a CDK inhibitor and an anti-proliferative agent: a potential role in cancer prevention, Cancers (Basel). 11 (2019) 1–18, doi:10.3390/cancers11030427.

[24] P.R. Augusti, A. Quatrin, R. Mello, V.C. Bochi, E. Rodrigues, I.D. Prazeres, A. C. Macedo, S.C. Oliveira-Alves, T. Emanuelli, M.R. Bronze, et al., Antiproliferative effect of colonic fermented phenolic compounds from Jaboticaba (Myrciaria trunciflora) fruit peel in a 3d cell model of colorectal cancer, Molecules (2021) *26*, https://doi.org/10.3390/molecules26154469.

[25] S.K. Abraham, A. Eckhardt, R.G. Oli, H. Stopper, Analysis of in vitro chemoprevention of genotoxic damage by phytochemicals, as single agents or as combinations, Mutat. Res. - Genet. Toxicol. Environ. Mutagen. 744 (2012) 117–124, https://doi.org/10.1016/j.mrgentox.2012.01.011.

[26] J.A. Higgins, M. Zainol, K. Brown, G.D.D. Jones, Anthocyans as tertiary chemopreventive agents in bladder cancer: anti-oxidant mechanisms and interaction with mitomycin C, Mutagenesis 29 (2014) 227–235, https://doi.org/10.1093/mutage/geu009.

[27] X. Li, J. Xu, X. Tang, Y. Liu, X. Yu, Z. Wang, W. Liu, Anthocyanins inhibit trastuzumab-resistant breast cancer in vitro and in vivo, Mol. Med. Rep. 13 (2016) 4007–4013, https://doi.org/10.3892/mmr.2016.4990.

[28] S.K. Mitra, D.D. Schlaepfer, Integrin-regulated FAK-Src signaling in normal and cancer cells, Curr. Opin. Cell Biol. 18 (2006) 516–523, https://doi.org/10.1016/j.ceb.2006.08.011.

[29] M. Shen, Y.Z. Jiang, Y. Wei, B. Ell, X. Sheng, M. Esposito, J. Kang, X. Hang, H. Zheng, M. Rowicki, et al., Tinagl1 Suppresses Triple-Negative Breast Cancer Progression and Metastasis by Simultaneously Inhibiting Integrin/FAK and EGFR Signaling, e7, Cancer Cell 35 (2019) 64–80, https://doi.org/10.1016/j.ccell.2018.11.016.

[30] T. Grimm, A. Schäfer, P. Högger, Antioxidant activity and inhibition of matrix metalloproteinases by metabolites of maritime pine bark extract (Pycnogenol, Free Radic. Biol. Med. 36 (2004) 811–822, https://doi.org/10.1016/j.freeradbiomed.2003.12.017.

[31] I. Krga, D. Milenkovic, Anthocyanins: from sources and bioavailability to cardiovascular- health benefits and molecular mechanisms of action, J. Agric. Food Chem. 67 (2019) 1771–1783, https://doi.org/10.1021/acs.jafc.8b06737.

[32] Y. Wang, Q. Ni, J. Sun, M. Xu, J. Xie, J. Zhang, Y. Fang, G. Ning, Q. Wang, Paraneoplastic β cell dedifferentiation in nondiabetic patients with pancreatic cancer, J. Clin. Endocrinol. Metab. 105 (2020) E1489–E1503, https://doi.org/10.1210/clinem/dgz224.