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SPARC Matricellular Protein is Enriched in the Bioactive Fraction of the Human Vascular Pericyte Secretome

Elisa Avolio, PhD¹; * Giuseppe Mangialardi, MD PhD¹; * Sadie C Slater, PhD¹; * Valeria Vincenza Alvino, PhD¹; Yue Gu, MSc¹; William Cathery, MSc¹; Antonio P Beltrami, MD PhD²; Rajesh Katare, PhD³; Kate Heesom, PhD¹; Massimo Caputo, MD¹; Paolo Madeddu, MD¹

* Authors equally contributing to the work

¹ Bristol Medical School, Translational Health Sciences, University of Bristol, Bristol, United Kingdom; ² University of Udine, Udine, Italy; ³ Department of Physiology-HeartOtago, Otago School of Medical Sciences, University of Otago, Dunedin, New Zealand.

Corresponding author

Paolo Madeddu, MD
Professor of Experimental Cardiovascular Medicine
Bristol Medical School: Translational Health Sciences,
University of Bristol,
Upper Maudlin Street. BS28HW, Bristol, United Kingdom
Email: mdprm@bristol.ac.uk
Tel and fax: +44 (0) 117 34 23904

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Abstract

Aims: To ascertain if human pericytes produce SPARC (acronym for Secreted Protein Acidic and Cysteine Rich), a matricellular protein implicated in the regulation of cell proliferation, migration, and cell-matrix interactions; clarify if SPARC expression in cardiac pericytes is modulated by hypoxia; and determine the functional consequences of SPARC silencing.

Results: Starting from the recognition that the conditioned media (CM) of human pericytes promote proliferation and migration of cardiac stromal cells, we screened candidate mediators by mass-spectrometry analysis. Of the fourteen high-confidence proteins (<1% FDR) identified in the bioactive fractions of the pericyte CM, SPARC emerged as the top-scored matricellular protein. SPARC expression was validated using ELISA and found to be upregulated by hypoxia/starvation in pericytes that express PDGFR α . This subfraction is acknowledged to play a key role in extracellular matrix remodelling. Studies in patients with acute myocardial infarction showed that peripheral blood SPARC correlates with the levels of Creatine kinase Mb, a marker of cardiac damage. Immunohistochemistry analyses of infarcted hearts revealed SPARC is expressed in vascular and interstitial cells. Silencing of SPARC reduced the pericyte ability to secrete collagen1a1, without inhibiting the effects of CM on cardiac and endothelial cells. These data indicate that SPARC is enriched in the bioactive fraction of the pericyte CM, is induced by hypoxia and ischemia, and is essential for pericyte ability to produce collagen.

Innovation: This study newly indicates that pericytes are a source of the matricellular protein SPARC.

Conclusion: Modulation of SPARC production by pericytes may have potential implications for post-infarct healing.

Introduction

Previous work has shown that pericyte-like cells from the adventitia of large arteries and veins (alias adventitial vein pericytes, APCs) have a therapeutic potential for the treatment of myocardial and peripheral ischemia.(4,7,15) In a murine model of MI, intra-myocardial transplantation of APCs promoted reparative angiogenesis, enhanced the number of cycling cardiomyocytes while decreasing apoptosis, and recruited stromal cells to the infarct border zone.(15) We confirmed the angiogenic activity of APCs in a swine model of reperfused MI.(2) Mechanistic investigation uncovered that at least part of the APC healing properties can be attributed to the secretion of miR-132, which acts as a paracrine activator of cardiac healing through inhibition of Ras-GTPase activating protein and methyl-CpG-binding protein 2.(15) Moreover, miR-532-5p regulates the secretion of angiopoietin-1 by APCs through inhibition of the transcriptional repressor BACH1.(21) Another study showed that brain pericytes release a plethora of growth factors and cytokines and bioactive vesicles.(9) Further investigation is warranted to unveil the paracrine properties of pericytes.

In the present study, we aimed to characterize the functional effects of the APC secretome on cardiomyocytes and cardiac stromal cells (CSCs). We performed a purification of the bioactive component using size exclusion liquid chromatography, followed by mass-spectrometry (MS) analysis of the chromatogram fractions. Results indicate the enrichment of the matricellular protein Secreted Protein Acidic and Rich in Cysteine protein (SPARC) in the bioactive fraction of the APC secretome. Immunoreactive SPARC was increased in peripheral blood of patients with acute MI and localized at vascular level in the infarcted zone. Moreover, cultured human cardiac pericytes express SPARC, with protein being upregulated under hypoxia and starvation in the pericyte fraction that express the PDGFR α .

Results

Functional effects of the APC secretome on cardiomyocytes

In our previous study in a murine model of MI, intra-myocardial transplantation of APCs enhanced the number of cycling cardiomyocytes, as assessed by Ki67 staining and *in vivo* labelling with 5-ethynyl-2'-deoxyuridine, a thymidine analogue that is incorporated into neo-synthesized DNA during the S-phase of the cell cycle.(4) To confirm this effect was mediated by secreted factors, here we assessed the ability of the APC-CM (n=6 cell lines) to stimulate cardiomyocyte proliferation. To this aim, we applied the APC-CM to iCell cardiac progenitor cells (CPCs) and derived cardiomyocytes. These cells have been used in a range of applications spanning disease research, drug discovery, toxicity testing, and regenerative medicine.(16,17) We verified the classical antigenic phenotype of iCell CPCs and cardiomyocytes (**Supplementary Figure I**) and the spontaneous beating properties of differentiated cardiomyocytes (video available on request).

Importantly, stimulation with the APC-CM augmented the number of mitotic figures in iCell CPCs (EdU: 1.2-fold and Aurora B: 1.3-fold increases compared with exposure to unconditioned medium (UCM) ($P<0.01$ for both comparisons, **Figure 1**). We also observed a proliferative stimulation of cardiomyocytes (EdU: 1.3-fold and Aurora B: 1.5-fold increases compared to UCM, $P<0.01$ and $P<0.01$, respectively, **Figure 2**). In particular, the number of mitotic figures corresponding to Anaphase and Telophase was increased by 1.7-fold in the APC-CM group compared with the UCM group ($P<0.01$ vs. UCM, **Figure 2**).

Our previously published studies showed that APC transplantation exerted pro-survival effects on cardiomyocytes,(15) and that APC-CM protected adult rat cardiomyocytes from apoptosis *in vitro*.(4) Here, we confirmed an anti-apoptotic effect of the APC-CM on human cardiomyocytes exposed to low oxygen (**Figure 3**).

Altogether, this data indicates that the proliferative and pro-survival action of APCs on cardiac cells is mediated by a paracrine crosstalk between the two cell populations.

Functional effects of the APC secretome on cardiac stromal cells.

In previous *in vivo* studies, injected APCs were able to recruit CSCs to the peri-infarct zone.(4,15) Here, we performed *in vitro* studies where CSCs were exposed to the APC secretome in a transwell migration assay. To determine if the APC-CM activates directed and/or undirected motility, i.e., chemotaxis - chemokinesis, we compared cell migration following application of the CM to the lower compartment or both compartments of the assay (**Figure 4A**). In both cases, the APC-CM markedly stimulated the *in vitro* migration of human CSCs (n=6 cell lines, $P<0.01$ vs. UCM). Moreover, we showed that the APC-CM stimulated HUVEC migration (**Figure 4B**) but was ineffective in promoting lung fibroblast migration (**Figure 4C**), thus suggesting a cell-specific attractive action. The pro-migratory effect on CSCs was also verified in a scratch assay, where cell proliferation was not operative because of the addition of the mitosis inhibitor mitomycin C (**Figure 4 D&E**). This data indicates that the APC-CM contains factors able to enhance both spontaneous motility and directional migration.

A candidate approach to identify bioactive components

Next, we combined analytic biochemistry and pharmacological inhibition methods to unveil the candidate mechanisms. For this screening, we focused on the migration of human CSCs as a functional assay. Following purification using Amicon® filters and differential ultracentrifugation to separate microvesicles and exosomes, the active pro-

migratory component was retained in the soluble fraction of the APC-CM (n=3 cell lines, data not shown). Additionally, the stimulation of CSC migration was abrogated by pre-incubation of APC-CM with proteinase K but not by heat (120°C) or RNase A, suggesting the bioactive component is a protein resistant to heat-induced unfolding (**Supplementary Figure IIA&B**). Fractionation experiments additionally indicated that the molecular mass of the pro-migratory protein(s) is > 30 kDa (**Supplementary Figure IIC**). Moreover, a kinase assay showed the APC-CM induced ERK1/2 activation in CSCs (**Supplementary Figure IID**).

We next used blocking antibodies to inhibit growth factors and chemokines, previously identified in the APC-CM.(4) Pre-incubation with antibodies against SDF-1 or SCF was unable to prevent the induction of CSC migration. Likewise, a cocktail of blocking antibodies against HGF, FGF, VEGFA, and TIE2 failed to inhibit the APC-CM pro-migratory activity (data not shown).

Altogether, these data suggest that one or more proteins are responsible for the promigratory activity of the APC-CM, possibly through activation of ERK1/2. The blocking approach indicates that the bioactive elements do not belong to the class of traditional growth factors associated with pericytes.

Unbiased approach to identify bioactive components

We next adopted a discovery strategy using proteomics. We separated the APC-CM components by size exclusion liquid chromatography, followed by liquid chromatography mass-spectrometry (LCMS) analysis of the chromatogram fractions (peaks A and B) that exerted a pro-migratory effect on CSCs (**Figure 5** and **Supplementary Figure III**).

The raw spectral data was searched against the Uniprot Human database to generate a list of proteins identified in both the active fractions at <1% false discovery rate (FDR). From this list, we next excluded proteins detected in the UCM or in the non-migratory fractions of the APC-CM. Fourteen human proteins emerged as potential candidates (**Supplementary Table I** and **Supplementary Figure III**). In addition, **Supplementary Figure IV** shows the annotated MS spectra and Ion Series information for proteins that were only identified by 1 or 2 peptides.

We were particularly interested in two top-scored matricellular proteins from the list of candidates. In the first case, identified peptides matched to both the full-length Secreted Protein Acidic and Rich in Cysteine protein (SPARC, MW = 43 kDa) and the related 'highly similar' SPARC protein (B4DRV4/SPARCL1, MW = 62 kDa). The other identified protein was Transforming Growth Factor Beta Induced (TGFB1, 68 kDa). All of them are secreted factors that bind to collagens and control cell adhesion to extracellular matrix proteins. Using ELISA, we confirmed the APC-CM expresses both SPARC and TGFB1 (**Figure 6A**).

STRING network analysis identified SPARC and TGFB1 are associated with other proteins with a PPI enrichment p-value = 1.51×10^{-14} (**Figure 6B-D**). Such an enrichment indicates that the proteins are biologically connected, as a group. Gene Ontology (GO) of biological process recognized an association of the network with extracellular structure organization (GO:0043062, FDR 1.04×10^{-15}), extracellular matrix organization (GO:0030198, FDR 2.77×10^{-14}), and positive regulation of endothelial cell migration (GO:0010595, FDR 6.68×10^{-9}), whereas the top molecular function was collagen binding (GO:0005518, FDR 3.71×10^{-8}). No further enrichment was obtained by pooling all the 14 hits of the list in the STRING analysis. Altogether, these data suggest bioactive components that influence CSC motility may also contribute to the modulation of processes related to cellular and extracellular matrix remodelling and angiogenesis.

SPARC expression by human cardiac pericytes

Matricellular proteins are modulated by hypoxia and redox signalling through diverse mechanisms, which are often cell or tissue specific and context dependent. Previous studies showed SPARC expression is upregulated under hypoxic stress in glioma cells and bone marrow mesenchymal cells.(14,20) However, we could not find any previous reference on SPARC regulation by hypoxia in cardiac cells. As shown in **Figure 7A**, hypoxia and growth factor depletion (HYPO-GFD) induced mRNA levels of SPARC in human cardiac pericytes; however, protein levels (assessed by ELISA) did not differ in cardiac pericytes conditioned media collected under basal conditions or HYPO-GFD.

We next evaluated SPARC expression using immunocytochemistry analysis of human cardiac pericytes stained with antibodies against the PDGF receptor- α (PDGFR α) or PDGF receptor- β (PDGFR β) (**Figure 7B**). The two receptors reportedly identify distinct populations that have, respectively, profibrotic and proangiogenic activities.(5,13) Experiments on three human pericyte lines confirmed PDGFR β was highly expressed, while PDGFR α was scarcely present under basal conditions, but increased from 7 to 21% of total cells after HYPO-GFD (**Figure 7C-D**). As shown in **Figure 7E**, PDGFR β positive cardiac pericytes expressed SPARC with higher frequency compared with PDGFR α positive cardiac pericytes under basal conditions (88 vs. 55%, respectively $p=0.05$). This difference was abrogated by HYPO-GFD, due to a reduction of SPARC in PDGFR β positive cardiac pericytes (from 88 to 77%, $p=0.009$) and an increase in PDGFR α positive cardiac pericytes (from 55 to 87%, $p=0.04$). Because of these reciprocal changes, the frequency of SPARC in the total population was unchanged before and after HYPO-GFD (78 ± 2 and $80\pm 2\%$, respectively; $p=0.50$). **Figure 7F** shows SPARC staining of human coronary endothelial cells, which were used as a control being a known source of the ECM protein.(8) These data illustrate a previously unforeseen heterogeneity in SPARC expression by cardiac pericyte subpopulations.

SPARC expression in peripheral blood and heart following acute MI

We next assessed if the expression of SPARC is modulated following acute MI. Patients were classified as having small, medium, and large infarcts according to the levels of CKMB. Clinical data of the cohort subgroups are provided in **Supplementary Table II**. SPARC was increased in peripheral blood of patients with large STEMI (samples collected in the first 12 hrs after symptom manifestation) as compared with controls, considering age, sex, BMI, and major comorbidities as covariates (**Figure 8A**). Interestingly, 5 patients in the small MI group and 4 in both the medium and large MI groups, for a total of 13 out of 30 patients (43%), had SPARC values within the range of controls, suggesting they were unable to upregulate the ECM-associated protein after the acute ischemic event.

Moreover, SPARC expression was assessed in the human and swine heart. Fluorescent and DAB immunostaining of hearts explanted from patients with ischemic heart failure demonstrated the expression of SPARC in vascular and interstitial cells of the infarcted area and remote zone. SPARC staining was also seen in the vasculature of donor hearts not used for transplantation (**Figure 8B-C**). Moreover, we observed an upregulation of SPARC in the heart of swine that underwent reperfused MI compared with normal swine hearts (**Figure 8D**).

Cause-effect relationship or association

Altogether, the above data indicate that SPARC is produced by pericytes and induced by hypoxia and starvation *in vitro* and by ischemia *in vivo*. To verify if SPARC is responsible

for the functional effects of the pericyte CM, we repeated the assays using the CM of APCs that were preliminarily silenced for SPARC. The effective silencing was confirmed measuring SPARC levels in CM by ELISA (**Supplementary Figure V A**). Silencing of SPARC did not affect the APC intrinsic function, including viability and proliferation (**Supplementary Figure V B-D**), but it significantly reduced the production of Collagen 1 α 1 (**Supplementary Figure V E**). Silencing of SPARC did not influence the migratory, proliferative, and anti-apoptotic effects of the CM on CSCs (**Supplementary Figure VI A-D**). Likewise, the silencing did not influence the effects of CM on EC survival and capacity to form network on Matrigel (**Supplementary Figure VI E-G**). Therefore, we conclude that SPARC is enriched in the bioactive fraction of the pericyte CM, is induced by hypoxia and ischemia, and is essential for pericyte ability to produce collagen.

Discussion

SPARC belongs to a class of matricellular proteins that also comprises tenascin, osteopontin, bone sialoprotein, thrombospondins, and galectins. They are expressed at high levels during development, but typically only appear in postnatal tissue repair, where their amounts increase remarkably.(12) SPARC-related proteins encompass others six members, B4DRV4/SPARCL1, SPOCK1, SPOCK2, SPOCK3, SMOC1, and SMOC2, which share three similar domains (a follistatin-like domain, a kazal-like segment, and a EF-hand/calcium-binding domain).(10)

SPARC and B4DRV4/SPARCL1 share 61% aa in those regions and both exceed the 30 kDa cut-off that, in our study, identified the pro-migratory component of the APC-CM. Moreover, these rich in cysteine proteins often undergo epigenetic changes, which confer them with higher MW and resistance to heating denaturation. The other identified factor, TGFBI, is a 68 kDa matricellular protein and a member of the fasciclin domain containing protein family that also includes periostin. Once secreted, TGFBI interacts with other extracellular matrix proteins participating in collagen organization and cross-linking; it also influences cell migration and proliferation in cancer.

Validation by ELISA confirmed the presence of immunoreactive SPARC and TGFBI in the APC-CM with a concentration ~ 60 ng/ml. The two proteins were also detected in the CM of pericytes isolated from myocardial tissue leftovers of cardiac surgery, suggesting they are secreted by pericytes resident in different human tissues.

Starting from these observations, we focused on the modulation of SPARC expression in pericytes. Interestingly, SPARC was induced by hypoxia and starvation in the cardiac pericyte fraction that expresses the receptor PDGFR α and may represent a transitional phenotype between pericytes and myofibroblasts. Moreover, SPARC was expressed in the infarcted and chronically ischemic heart and increased in the peripheral circulation in parallel with the gold standard biomarker of myocardial damage.

These data suggest that induction SPARC release from pericytes could represent a homeostatic response to myocardial ischemia. Importantly, analysis of the functional annotation of SPARC indicates this protein plays key roles in pericyte-driven processes necessary to cardiovascular healing, including fibrillar collagen production, extracellular matrix remodelling and angiogenesis.(6) Additional support to this concept is provided by gene deletion studies. In pericytes, SPARC inhibits latent TGF- β activity and can interfere with the binding of PDGF and EGF receptors to their respective ligands.(1) Expression of SPARC is associated with pericyte migration, an effect dependent upon the TGF- β receptor endoglin and integrin α v; whereas *SPARC*-deficient pericytes exhibited increased basal TGF- β 1 activity and decreased migration, an effect blocked by inhibiting TGF- β 1.(18) *Sparc*-null mice show decreased pericyte-associated vessels in an orthotopic model of pancreatic cancer.(18) In the infarcted heart, SPARC expression is key for the rapid formation of a collagenous scar.(22) *Sparc*-null mice reportedly fail to form a strong infarct scar and frequently die because of cardiac rupture compared with wild-type mice;(6,19) this adverse outcome being prevented by adenovirus-mediated forced expression of *SPARC*.(19) The benefit of SPARC gene therapy by adenoviral delivery was also evident in wild-type animals, which showed a stronger scar and improved cardiac function post-MI.(19)

Intriguingly, the recombinant version of SPARC could not reproduce the functional effects of the bioactive CM fractions, neither silencing abolished these effects *in vitro*. However, silencing significantly reduced the ability of pericytes to produce collagen. It is therefore possible is that SPARC induction in pericytes is relevant for modulation of the extracellular matrix during tissue healing.

Innovation

We discovered the human pericytes secretome contains matricellular proteins that have a therapeutic potential in the treatment of myocardial infarction. Additionally, being enriched in the biologically active fraction of the secretome, SPARC could be exploited as a molecular tag for therapeutic applications using the pericyte CM.

Material and Methods

Ethics

Studies complied with the principles stated in the “Declaration of Helsinki” and were covered by approval from the South West-Central Bristol Research Ethics Committee (REC reference 06/Q2001/197), the North Somerset and South Bristol Research Ethics Committee (REC 11/SW/0122) and the Independent Ethics Committee of the University Hospital of Udine. Adult patients and paediatric patients’ custodians gave written informed consent for inclusion in the study. Clinical characteristics of patients and cardiac samples’ donors are reported in **Supplemental Table III and IV**.

In addition, blood samples were obtained from the participants recruited for “Ischemic Heart Disease Study” (University of Otago Human Ethics Committee reference number H14/028). Clinical data are shown in **Supplementary Table V**. Inclusion criteria were subjects aged between 20 and 80 years old of both genders, with newly developed ischemic heart disease. Exclusion criteria were history of valvular heart disease, recent major surgery or diagnosed with cancer. Blood samples were also collected as a control from healthy participants of the “Dunedin microRNA study” (University of Otago Human Ethics Committee H13/015).

Derivation of primary human cells

APC primary lines were obtained from vein leftovers of coronary artery bypass graft surgery, expanded to passage 5 (P5) and characterized for purity using flow cytometry analysis of typical antigenic markers, as previously reported.(4,11) Cardiac pericytes were isolated and characterized for antigenic profile as previously described.(5) Atrial CSCs were expanded to P5 from leftovers of healthy hearts donated for transplantation, and their antigenic profile was assessed using flow cytometry as previously described.(3) HUVECs and human fibroblasts were purchased from Lonza (UK). Human coronary artery endothelial cells were purchased from Promocell (UK).

Production of pericyte-conditioned media

For collection of CM, confluent APCs and cardiac pericytes at P6/P7 were incubated in basal endothelial media with no growth factors or serum added (Lonza) for 48h. Cell supernatants were centrifuged at 1,000xg for 10 minutes at 4°C, aliquoted and stored at -80°C until use, avoiding freezing/thawing cycles.

Studies with iPS-derived human cardiac progenitor cells

iCell iPS-derived human CPCs were purchased from FUJIFILM Cellular Dynamics, Inc. (Madison, Wisconsin, USA) and cultured with William's E Medium (Gibco, Renfrewshire, UK) according to the manufacturer's instructions. For proliferation assays, cells were incubated with 5-Ethynyl-2'-deoxyuridine (EdU) (Click-iT® EdU Imaging Kit, Life Technologies, Paisley, UK) for 48 hours in the presence of the APC-CM or control. Revelation of EdU⁺ cells was performed according to the manufacturer's instructions. Immunostaining for Aurora B (Abcam – dilution 1:100) and β -tubulin (Cell Signalling Technology, London, UK – dilution 1:1000) was performed to identify the typical assembling of chromosomes and mitotic spindle during the four progressive stages characterising mitosis. The percentage of EdU and Aurora B positive CPCs were quantified and expressed as fold-change of UCM.

APC-CM, collected as specified above, was diluted 1:1 with CPC medium. For UCM, basal EBM2 (Lonza) was used. For all experiments, APC-CM (N=6 biological replicates) were assayed in triplicate. For UCM control, N=6 technical replicates were employed. Per each single replicate, at least 800 cells were analyzed. Cells were imaged with an Axio Observer Z1 microscope (Zeiss, Oberkochen, Germany).

Studies with induced pluripotent stem cells-derived human cardiomyocytes

iCell iPS-derived human cardiomyocytes were purchased from FUJIFILM Cellular Dynamics and cultured with “plating” followed by “maintenance” medium according to the manufacturer’s instructions. After one week, cells formed a syncytium monolayer with spontaneous synchronous beating, and were ready to enter the experimental procedures.

Proliferation assay

Cells were incubated with EdU for 48 hours in the presence of the APC-CM or control. Identification of EdU⁺ cells was performed according to the manufacturer’s instructions. Immunostaining for Aurora B and β -tubulin was performed to identify the typical assembling of chromosomes and mitotic spindle during the four progressive stages characterizing mitosis. An antibody anti- α sarcomeric actinin (Abcam - dilution 1:100) was used to identify cardiomyocytes sarcomeres. The percentage of EdU and Aurora B positive cardiomyocytes were quantified and expressed as fold-change UCM.

Apoptosis assay

An “ischemic assay medium” was employed to mimic the ischemic environment *in vitro* and prepared as follows: 93% DMEM (-) glucose and (-) glutamine (Life Technologies), creatine 5 mM, D-glucose 2.75 mM, glutamine 1X, HEPES 10 mM, L-carnitine 2 mM, non-essential amino acids 1X, sodium pyruvate 1X, taurine 5 mM, linoleic acid-BSA 1X (all from Sigma-Aldrich). Cells were incubated for 48 hours under either 20% or 1% oxygen in the presence of the APC-CM or UCM. A live cell staining for activated Caspase 3/7 (Cell Event Caspase 3/7 Green detection reagent, Invitrogen, Renfrewshire, UK) was performed at the end of the protocol according to manufacturer’s instructions. For experiments, APC-CM, collected as specified above, was diluted 1:1 with maintenance or ischemic assay medium. For UCM, basal EBM-2 (Lonza) was used. For all experiments, APC-CM (N=6 biological replicates) were assayed in triplicate. For UCM control, N=6 technical replicates were employed. Per each single replicate, at least 1,000 cells were analyzed. Cells were imaged using an Axio Observer Z1 microscope.

iCell antigenic characterization

iCell CPCs and cardiomyocytes antigenic profile was confirmed as recommended by the vendor. Briefly, cells were fixed with PFA 4% w/v, rinsed with PBS, permeabilised with Triton-X100 0.1% w/v for 10 min if required, and blocked with 5% v/v foetal bovine serum (FBS) for 30 min at 20-22 °C. All chemicals were purchased from Sigma Aldrich. Antibodies anti-cardiac troponin T, α sarcomeric actinin, GATA4, cKit, PDGFR- α , NKX2.5 (all diluted 1:100, from Abcam) were incubated for 16 hours at 4°C. Secondary antibodies conjugated with Alexa488 (Invitrogen) were incubated for 1 hour at 20-22 °C in the dark. Nuclei were counterstained with DAPI. Cells were analysed with an Axio Observer Z1 microscope.

Treatment of APC-CM for migration experiments

For selected experiments, the APC-CM was either pre-treated with proteinase K (100 μ g/ml for 1 hour) or RNase A (2, 20 or 200 Units/L for 1 hour), or denatured by boiling for 30 minutes at 120°C before use in the migration assays (all reagents from Sigma-Aldrich, Merck, Darmstadt, Germany). For determination of the molecular mass of the pro-migratory factor, APC-CM were fractioned according to molecular weight using Amicon® Ultra Centrifugal Filters units (cut-off 10 kDa and 30 kDa) (Sigma-Aldrich). Differential ultracentrifugation was employed to separate microvesicles and exosomes.

In selected experiments, APC-CM were supplemented with antibodies anti-human SDF1 or SCF, or with a cocktail of blocking antibodies anti-human HGF, FGF, VEGF, and Tie2 (all from Abcam, Cambridge, UK) for 6 hours prior use in migration assays.

Silencing of SPARC

Opti-MEM media (ThermoFisher Scientific, UK) and Lipofectamine RNAiMAX (Invitrogen, UK) were used to transfect APCs with On-target plus SPARC SMARTpool (a mixture of 4 siRNA in a single reagent, L-003710-00-0005, GE Healthcare, UK). Silencer® Select Negative Control No. 2 siRNA (ThermoFisher Scientific) was used as a control (final concentration 25nM for both). The transfection reagent was removed after 6 hours and replaced with fresh media overnight. Media was then change to EBM2 media and cells were incubated for a further 48 hours. RNA and conditioned media were then collected, or cells were re-seeded for caspase, calcein, and EdU protocols. Silencing was confirmed by ELISA on CM as described below.

Transwell Migration Assay

To test the capacity of the APC secretome to induce the migration of CSCs, HUVECs and lung fibroblasts (60,000 cells) were seeded in 24-well plates on a 6.5-mm Transwell with 8-µm pore polycarbonate membrane insert (Corning, UK) in serum-free EGM-2. In the bottom of the wells, 0.5 mL of APC-CM was added. Complete EGM-2 was used as reference control. Cells were incubated for 17 hours at 37°C. At the end of this period, the membrane inserts were washed with PBS, fixed with icecold methanol for 15 minutes at room temperature, stained with DAPI, and mounted on a slide. Membranes were analyzed with an epifluorescence microscope at x200 magnification. Migrated cells were expressed as a percentage. All experiments were performed in duplicate. For experiments with SPARC-silenced CM, we used the fluorometric CytoSelect 96-Well Cell Migration Assay (CBA-106, Cell Biolabs, UK), according to the manufacturer's instructions, with experiments being performed in triplicates. Recombinant SPARC (Peprotech, UK) was diluted in EBM2 to the indicated concentration.

Scratch (wound closure) migration assay

A scratch was produced in confluent CSCs in the centre of each well. Cells were washed with PBS to remove detached cells and incubated with the experimental medium. Cell proliferation was inhibited with mitomycin C or hydroxyurea (Sigma-Aldrich). The wound area was measured immediately after the creation of the scratch (D0) and after 8- and 16-hour incubation (D1). Percentage of wound closure was calculated as following: %wound closure = 100-(100*D1/D0). Experiments were performed in 48-well plates in triplicates. Recombinant SPARC (Peprotech, UK) was diluted in EBM2.

Cell proliferation and viability

Proliferation was measured using either a BrdU colorimetric (Roche, Switzerland) or an EdU fluorescent (ThermoFisher) kit. Apoptosis was assessed with a CaspaseGlo 3/7 (Promega, UK) assay. Cell viability/apoptosis was assessed using a Calcein-AM/EthDIII kit (Biotium, UK), all according to the manufacturer's instructions. Cells were exposed for 16-24 hours to the experimental conditions before the functional readouts. Experiments were performed in 96-well plates in triplicates. Recombinant SPARC (Peprotech, UK) was diluted in EBM2.

2D-Matrigel angiogenesis assay

Human coronary artery ECs (CAECs, Promocell) were seeded on the top of Matrigel (Corning® Matrigel® Growth Factor Reduced Basement Membrane Matrix) (4,000

cells/well) using Angiogenesis μ -Slides (IBIDI, UK). APC-CM was diluted 1:1 with fresh EBM2 medium. EBM2 was used as control. Recombinant SPARC (Peprotech, UK) was diluted in EBM2. Images were snapped after 5 hours, and the total tube length per imaging field was measured.

ELISA on APC or CP conditioned medium and peripheral blood

The APC or CP secretion of SPARC and TGFBI was confirmed by ELISA (both from R&D Systems). SPARC protein secretion was further assessed in cells collected following exposure to normoxia or hypoxia. In addition, immunoreactive SPARC levels in peripheral blood of patients with acute MI and healthy controls were measured using the same ELISA kit. APC production of Pro-Collagen 1 α 1 was measured by ELISA (R&D Systems), using APC-CM diluted 1:600 in reagent diluent.

Phospho-kinase array

For the study of intracellular signalling activated by APC-CM in CSCs, the latter were exposed to the APC-CM or UCM control for 1 hour. Cell lysates were then collected, and the assay performed according to the manufacturer's instructions (Human phospho-kinase array kit, R&D System). The same amounts of protein were loaded onto pre-made membranes.

Size exclusion liquid chromatography (SEC)

SEC was performed on an Agilent 1100/1200 HPLC (Agilent Technologies, Santa Clara, CA) detecting UV absorbance (280 nm). Multi-angle light scattering (MALS) measurements were made with a Wyatt DAWN EOS 18-angle MALS detector (Wyatt Technology Corp., Santa Barbara, CA) plumbed in series with the UV detector.

Three biological replicates of APC-CM and the control UCM were fractionated by SEC, all fractions corresponding to the active peaks were pooled and tested for pro-migratory action on CSCs. Analysis of the foetal calf serum (FCS) served to exclude all candidate factors that may derive from APC contamination with FCS present in the growth medium. The 2 peaks that exerted pro-migratory effects on CSCs underwent mass spectrometry analysis.

Liquid chromatography mass spectrometry (LCMS)

An aliquot from each size exclusion fraction pool was separated using SDS-PAGE until the dye front had migrated approximately 1cm into the separating gel. Each gel lane was then excised and subjected to in-gel tryptic digestion using a DigestPro automated digestion unit (Intavis Ltd.). The resulting peptides were fractionated using an Ultimate 3000 nano-LC system in line with an LTQ-Orbitrap Velos mass spectrometer (Thermo Scientific). In brief, peptides in 1% (vol/vol) formic acid were injected onto an Acclaim PepMap C18 nano-trap column (Thermo Scientific). After washing with 0.5% (vol/vol) acetonitrile 0.1% (vol/vol) formic acid peptides were resolved on a 250 mm $\times$ 75 μ m Acclaim PepMap C18 reverse phase analytical column (Thermo Scientific) over a 150 min organic gradient, using 7 gradient segments (1-6% solvent B over 1min., 6-15% B over 58min, 15-32%B over 58min, 32-40%B over 5min, 40-90%B over 1min, held at 90%B for 6min and then reduced to 1%B over 1min) with a flow rate of 300 nl min⁻¹. Solvent A was 0.1% formic acid and Solvent B was aqueous 80% acetonitrile in 0.1% formic acid. Peptides were ionized by nano-electrospray ionization at 2.1 kV using a stainless steel emitter with an internal diameter of 30 μ m (Thermo Scientific) and a capillary temperature of 250°C. Tandem mass spectra were acquired using an LTQ-Orbitrap Velos mass spectrometer controlled by Xcalibur 2.1 software (Thermo Scientific) and operated in data-dependent acquisition mode. The Orbitrap was set to analyze the survey scans at 60,000 resolution (at m/z 400) in the mass range m/z 300

to 2000 and the top twenty multiply charged ions in each duty cycle selected for MS/MS in the LTQ linear ion trap. Charge state filtering, where unassigned precursor ions were not selected for fragmentation, and dynamic exclusion (repeat count, 1; repeat duration, 30s; exclusion list size, 500) were used. Fragmentation conditions in the LTQ were as follows: normalized collision energy, 40%; activation q, 0.25; activation time 10ms; and minimum ion selection intensity, 500 counts.

The raw data files were processed using Proteome Discoverer software v1.4 (Thermo Scientific) and searched against the UniProt Human database (122604 sequences) or the uniprot Bos Taurus database (31717 sequences) using the SEQUEST algorithm. Peptide precursor mass tolerance was set at 10ppm, and MS/MS tolerance was set at 0.8Da. Search criteria included carbamidomethylation of cysteine (+57.0214) as a fixed modification and oxidation of methionine (+15.9949) as a variable modification. Searches were performed with full tryptic digestion and a maximum of 1 missed cleavage was allowed. The reverse database search option was enabled, and all peptide data was filtered to satisfy FDR of 1%.

SPARC expression by human cardiac pericytes

Cell preparation. Three human cardiac pericyte lines were cultured in complete EGM2 media (Promocell, UK) until confluent. At P5 they were incubated in basal EBM2 medium with no growth factors or serum added (Promocell, UK) for 48h under 21% O₂ (normoxia) and 2% O₂ (hypoxia). CAECs (Promocell, UK) were cultured as a known control source of the matricellular protein SPARC.

RNA Extraction and Quantitative Real-Time Analysis

Total RNA was extracted using miRNeasy kit (QIAGEN). RNA concentration and purity were assessed using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific). RNA was reverse transcribed using a High Capacity RNA-to-cDNA Kit (Applied Biosystems). cDNA amplification was performed on a QuantStudio 6 Flex (Thermo Fisher Scientific). Expression of *SPARC* was measured and normalized to *ACTIN*. mRNA expression level was determined using the $2^{-\Delta Ct}$ method. Each reaction was performed in triplicate.

Immunocytochemistry in cardiac pericytes and CAECs

SPARC was co-stained with antibodies against PDGFR α or PDGFR β to identify distinct populations of pericytes. Briefly, cells were fixed with PFA 4% w/v (Sigma-Aldrich, UK), washed with PBS (Life technologies, UK) and blocked with 5% v/v foetal bovine serum (FBS, Life technologies, UK) for 30 minutes at 20-22°C. Antibodies anti-SPARC (diluted 1:100, from Cell Signalling, UK), anti-PDGFR α (diluted 1:100, from Santa-Cruz, UK) and PDGFR β (diluted 1:50, from R&D Systems, UK) were incubated for 16 hours at 4°C. Secondary antibodies conjugated with donkey anti-rabbit Alexa 568, donkey anti-mouse Alexa 647 and donkey anti-goat 488 (diluted 1:200, from Invitrogen) were incubated for 1 hour at 20-22 °C in the dark. Nuclei were counterstained with DAPI (diluted 1:1000, Invitrogen, UK). For CAECs, anti-CD31 primary antibody was also used to confirm the endothelial nature of the commercial primary cells. Images were taken using 20x objectives of Zeiss Observer.Z1 microscope and Zen pro software.

Immunofluorescence of ischemic hearts

For the assessment of SPARC expression in different regions of human ischemic and donor hearts, 5- μ m-thick sections were cut and antigen retrieval was performed after deparaffinization using 0.02M Citrate buffer, PH=6 (sigma-Aldrich, UK), for 30 minutes at 98°C. To discriminate SPARC localization in the vessels, sections were incubated

with anti-SPARC primary antibody (diluted 1:100), and anti- α -Smooth Muscle Actin-Cy3 conjugated (α -SMA, 1:400, Sigma-Aldrich, UK) for 16 hours, at 4°C followed by a mouse monoclonal anti- α -sarcomeric actin primary antibody (Sigma-Aldrich, UK, 1:200), for 2 hours 37°C. Secondary antibodies conjugated with goat anti-rabbit Alexa 488 and goat anti-mouse IgM Alexa 647 were incubated for 1 hour at 20-22°C in the dark. Nuclei were labelled with DAPI and mounted with Fluoromount G (Thermo Fisher, UK). SPARC localization in the vessels and cardiomyocytes was analysed with 20x objectives of Zeiss Observer.Z1 microscope and Zen pro software.

Immunohistochemistry-DAB.

SPARC protein was assessed also in human and swine hearts using EnVision®+ Dual Link System-HRP (DAB+) kit (Dako, UK). The staining on paraffin-embedded sections of infarcted and donor controls was performed according to the manufacturer's procedures. DAB reagents without anti-SPARC primary antibody was used as negative control. Qualitative images were taken using 20x objectives of Zeiss Axio Observer.Z1 with Zen Blue software (Carl Zeiss Microscopy, LLC, US).

Acknowledgements

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List of abbreviations

APC	adventitial vein pericyte
BrdU	Bromodeoxyuridine / 5-bromo-2'-deoxyuridine
CHD	congenital heart disease
c-Kit	tyrosine-protein kinase KIT or CD117
CM	conditioned media
CP	cardiac pericyte
CPC	cardiac progenitor cell
CSC	cardiac stromal cell
DAPI	4',6-diamidino-2-phenylindole
EBM-2	endothelial basal medium
EdU	5-Ethynyl-2'-deoxyuridine
EGFR	epidermal growth factor receptor
EGM-2	endothelial growth medium
ELISA	enzyme-linked immunosorbent assay
FCS	foetal calf serum
FDR	false discovery rate
FGF	fibroblast growth factor
GATA4	GATA Binding Protein 4
GO	gene ontology
HF	heart failure
HGF	Hepatocyte growth factor
HUVECs	human umbilical vein endothelial cells
iPS	induced pluripotent stem cell
LC	liquid chromatography
LF	lung fibroblast
MI	myocardial ischemia
MS	mass spectrometry
NKX2.5	NK2 Homeobox 5
P	passage
PBS	Phosphate-buffered saline
PDGFR	platelet-derived growth factor receptor
PPI	protein-protein interaction
SCF	stem cell factor
SDF-1	stromal cell-derived factor 1
SDS/PAGE	Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis
SPARC	Secreted Protein Acidic and Cysteine Rich
STRING	Search Tool for the Retrieval of Interacting Genes/Proteins
TGFB1	Transforming Growth Factor Beta Induced
TGF- β	transforming growth factor beta
Tie-2	tyrosine kinase with immunoglobulin (Ig) and epidermal growth factor (EGF) homology domains 2
UCM	unconditioned media
VEGF-A	Vascular Endothelial Growth Factor A

Figure 1: The APC secretome promotes iCells cardiac progenitor cells (CPCs) proliferation. **A)** Experimental protocol: CPCs were cultured with unconditioned media (UCM) or APC-conditioned media (APC-CM) for 48 hours, in the presence of EdU. At the end of this time, proliferation analysis was performed. **B)** Fluorescence microscopy image (magnification: 200x) showing EdU⁺ CPCs in green fluorescence. Nuclei are identified by DAPI (blue). **C)** Bar graph shows quantitative data of EdU⁺ cells reported as fold-change of UCM. Data are mean ± SEM. **D)** Fluorescence microscopy images showing the typical Aurora B localisation (in green fluorescence) during the stages of mitosis. In magenta, β -Tubulin shows the arrangement of the mitotic spindle. Nuclei/chromatin are recognised by the blue fluorescence of DAPI. **E)** Bar graph reports quantitative data of Aurora B⁺ cells expressed as fold-change of UCM. Data are mean ± SEM. Comparison between the two groups was performed by unpaired Mann-Whitney test. The images are representative of the staining and were taken from the APC-CM group.

Figure 2: The APC secretome promotes iCells cardiomyocytes proliferation. **A)** Experimental protocol: Cardiomyocytes were cultured with UCM or APC-CM for 48 hours, in the presence of EdU. At the end of this time, proliferation analysis was performed. **B)** Fluorescence microscopy image (magnification: 200x) showing EdU⁺ cardiomyocytes in green fluorescence. Nuclei are identified by DAPI (blue). **C)** Bar graph shows quantitative data of EdU⁺ cells reported as fold-change of UCM. Data are mean $\pm$ SEM. **D)** Fluorescence microscopy images showing the typical Aurora B localisation (in green fluorescence) through progression of mitosis. In magenta (top panel), β -Tubulin shows the arrangement of the mitotic spindle. In red fluorescence (bottom panel), α -Sarcomeric Actinin identifies cell sarcomeres. Nuclei/chromatin are identified by DAPI (blue). **E-F)** Bar graphs report quantitative data of Aurora B⁺ cells expressed as fold-change of UCM. Data are mean $\pm$ SEM. The images are representative of the staining and were taken from the APC-CM group.

Figure 3: The APC secretome protects iCells cardiomyocytes from hypoxia-induced apoptosis. **A)** Experimental protocol. Apoptosis was induced in cardiomyocytes by culturing cells in hypoxia (1% oxygen). Control cells were cultured in normoxia (20% oxygen). Cells were cultured for 48 hours with the APC-CM or UCM as indicated. At the end of the protocol, an apoptosis assay was performed evaluating the expression of activated Caspase 3/7 in live cells. **B)** The fluorescence microscopy image shows activated Caspase 3/7-positive cells in red fluorescence. Nuclei are identified by the blue fluorescence of DAPI. Optical magnification: 200x. **C)** Bar graphs show quantitative data of cells expressing activated Caspase 3/7. Data are reported as fold-change of UCM under normoxia and presented as mean $\pm$ SEM. The image representative of the staining was taken from the UCM group.

Figure 4. APC-CM chemo-attractant properties. **A)** APC-induced cardiac stromal cell (CSC) migration assessed following addition of the conditioned medium (APC-CM) to both the upper and lower migration chambers (without gradient) or only to the lower chamber (with gradient). Bar graph with data presented as mean ± SEM. **P<0.01 and ***P<0.001 vs. unconditioned medium (UCM). **B)** APC-CM induced migration of HUVECs or CSCs. Data presented as mean ± SEM. **P<0.01 and ***P<0.001 vs. UCM. **C)** APC-CM induced migration of lung fibroblasts (LFs) or CSCs. Data presented as mean ± SEM. **P<0.01 and ***P<0.001 vs. UCM. **D&E)** APC-CM promotes CSC motility. CSCs grown to confluence were assayed in a scratch assay; Images were taken at 8 and 16h. Data presented as mean ± SEM. *P<0.05 vs. UCM. N = 6 biological replicates in all the assays.

Figure 5: Liquid chromatography analysis of the APC-CM secretome. Fractions from size exclusion chromatography of three APC lines (upper chromatograms) and unconditioned medium (UCM, lower chromatogram) were assessed in migration assays of human cardiac stromal cells (CSCs). Each fraction showing promigratory activity (A and B peaks) was subjected to liquid chromatography mass-spectrometry (LCMS) analysis to identify the proteins present. Human proteins common to two peaks with chemo-attractant property and absent from peaks lacking this property or from UCM were considered potential candidates. The bar graph shows the average values for each cell line on three CSC replicates. Data presented as mean $\pm$ SEM. **P<0.01 and ***P<0.001 vs. UCM.

Figure 6: Validation and network analysis of protein hits from LCMS-based discovery. **A)** Levels of SPARC (a protein very similar to B4DRV4) and TGFB1 in APC-CM as assessed by ELISA. Data presented as mean $\pm$ SEM. N=4 biological replicates. **B-D)** STRING analysis of protein networks associated with each or both proteins.

Action Types:

- activation
- inhibition
- binding
- catalysis
- phenotype
- posttranslational modification
- reaction
- transcriptional regulation

Gene Ontology Identifiers:

- GO:0043062 extracellular structure organization
- GO:0030198 extracellular matrix organization
- GO:0010595 positive regulation of endothelial cell migration
- GO:0005518 collagen binding

Figure 7. Expression of SPARC in human cardiac pericytes. **A)** Bar graph showing the levels of SPARC and TGFBI in conditioned media of cardiac pericytes (CPs). **B)** mRNA and protein expression of SPARC in human CPs under basal conditions and 48 hour exposure to 1% O₂ and growth factor deprivation (HYPO-GFD). Bar graph with results of qPCR. N=4 biological replicates. **C)** Representative immunofluorescence images of CPs expressing SPARC from an experiment on 3 cell lines. Bar = 100mm. Single staining shown in the inserts. **D-E)** Bar graph showing abundance of PDGFR α (**D**) and PDGFR β (**E**) CPs. **F)** Bar graph showing expression of SPARC in the two populations identified by the PDGFR subtypes. **G)** Representative immunofluorescence images of human coronary endothelial cells expressing SPARC, Bar = 100 μ m. Single staining shown in the inserts. Data presented as mean $\pm$ SEM.

Figure 8. Induction of SPARC by myocardial ischemia. A) Levels of SPARC in peripheral blood of patients with acute MI classified as small, medium, and large infarcts according to the levels of CKMB and compared with controls. PB was collected during the first 12 hours after acute STEMI. Analysis of covariance indicates SPARC is induced early after MI in patients with large infarct when considering age, sex, BMI, and comorbidities (diabetes and hypertension) as covariates. Interestingly, 5 patients in the small MI group and 4 in both the medium and large MI groups showed SPARC values within the range of controls (violet shadowed area of the columns), suggesting these subjects were unable to upregulate the protein after the ischemic event. **B)** Expression of SPARC in human heart. IF images showing SPARC expression (green) in the endothelium of vessels and cardiomyocytes of infarcted and remote areas compared to heart from healthy donors. α -SMA (red) used to discriminate SPARC localization in the vessels. **C).** DAB shows SPARC (brown precipitates) in vessels and infiltrating cells of the infarcted heart compared with healthy donors. Nuclei are stained with haematoxylin (violet). Inserts showing higher SPARC magnification. Scale bar: 60 μ m. DAB reagent without primary SPARC antibody is used as negative control. **D)** Expression of SPARC in swine hearts. DAB method showing SPARC localization in the vessels, infiltrating cells, and cardiomyocytes of swine infarcted heart. Healthy heart control shows weak brown precipitates in the endothelium and occasional precipitates in cardiomyocytes. Inserts showing higher magnification of SPARC. Scale bar: 60 μ m. Data presented as mean $\pm$ SEM.

A) Cardiomyocytes **B)****Cardiac Progenitor Cells**

Supplementary Figure I. Antigenic profile of iCells assessed by fluorescence microscopy. A) Cardiomyocytes are characterised by positivity to α -Sarcomeric Actinin and Cardiac Troponin T. **B)** Cardiac Progenitor cells are defined as positive for GATA-4, NKX 2.5 (both nuclear) and PDGFR- α (membrane localization), and negative for c-Kit, α -Sarcomeric Actinin and Cardiac Troponin T. All markers are identified by green fluorescence. DAPI identifies nuclei in blue. Images were taken at a x200 optical magnification.

Supplementary Figure II. Characterization of the chemical properties of the bioactive component of the APC-CM. **A)** APC-CM was treated with heat (120 °C), proteinase K, or both and then employed to induce migration of human cardiac stromal cells (CSCs) in a transwell-migration assay. **B)** In a similar experiment, RNase was employed to destroy RNAs. In (**A&B**) empty columns represent the migration using APC-CM and solid columns represent the migration using unconditioned medium. N=3 APC-CM, N=1 CSC. Comparison between groups was performed by unpaired Mann-Whitney test. **P<0.01. **C)** Migration was assessed using total CM or the CM fractioned according to molecular weight. Values are mean ± SEM. N=3 APC-CM, N=2 CSC. Comparison between groups was performed by unpaired Mann-Whitney test as indicated. ***P<0.001 vs. EGM-2, ###P<0.01 vs. the <10 KDa fraction, δδδ P<0.001 vs. 10 - 30 kDa group. **D)** A human phospho-kinase array showed that APC-CM activates Erk1/2 in CSCs. Each pair of spots (duplicate) represents a different kinase. Upper and lower left pair of spots on membrane A and upper right pair of spots on membrane B are reference spots.

Supplementary Figure III. Unbiased analysis of the APC-CM. **A)** Diagram recapitulating steps to obtain LCMS analysis results. **B)** APC-CM obtained from three different cell lines and unconditioned medium (UCM) were subjected to size exclusion liquid chromatography. **C)** Those fractions corresponding to a peak were pooled and assayed in a migration chamber. **D)** Each fraction showing promigratory activity was subjected to LCMS analysis to identify the proteins present. Human proteins common to two peaks with chemo-attractant property and absent from peaks lacking this property or from UCM were considered potential candidates.

Supplementary Figure IV. Annotated MS spectra and Ion Series information for proteins that were only identified by 1 or 2 peptides.

B2RAN2 Fraction A

B2RAN2 Fraction B

B4E1V0 Fraction A

B4E1V0 Fraction B

B4E1V0 Fraction B

Q494V2 Fraction A

Q494V2 Fraction B

P05543 Fraction A

P05543 Fraction A

P05543 Fraction B

F8VSC5 Fraction A

F8VSC5 Fraction B

Q8WUP2 Fraction A

Q8WUP2 Fraction B

Supplementary Figure V. Intrinsic effects of SPARC silencing on APCs. (A) Silencing of SPARC was confirmed using ELISA of APC-CM (n=3). Silenced and control APCs were screened for viability (B), apoptosis (C), and proliferation (D), all with N=3 biological replicates. Effects of SPARC silencing on collagen production was measured using ELISA (E). Data presented as mean $\pm$ SEM. **P<0.01.

Supplementary Figure VI. Effects of SPARC-silenced APC-CM on CSCs and CAECs. (A) Transwell migration, (B) scratch migration, (C) proliferation, and (D) apoptosis assays with CSCs showed no effect of the SPARC silencing on the functional readouts. Three biological replicates in all experiments. * $P < 0.05$, ** $P < 0.01$ as indicated. Likewise, (E) Proliferation, (F) apoptosis, and (G) angiogenesis assays with CAECs did not show any effect of the SPARC silencing. CAEC (one cell line used in technical triplicates) and APCs (three biological replicates). Data presented as mean $\pm$ SEM. * $P < 0.05$ as indicated.

	Accession	Description	Fraction	Score	Coverage	# Peptides	# AAs	MW [kDa]	calc. pI	#PSMs
1	P01871	Ig mu chain C region OS=Homo sapiens GN=IGHM PE=1 SV=3 - [IGHM_HUMAN]	A	21.55	2.65	1	452	49.3	6.77	8
			B	78.50	25.00	8	452	49.3	6.77	24
	Score average			50.02						
2	Q86TT1	Full-length cDNA clone CS0DD006YL02 of Neuroblastoma of Homo sapiens (human) OS=Homo sapiens PE=2 SV=1 - [Q86TT1_HUMAN]	A	7.49	4.27	1	375	41.2	6.79	2
			B	89.89	32.00	7	375	41.2	6.79	24
	Score average			48.69						
3	P09486 B4DRV4	SPARC cDNA FLJ55667, highly similar to Secreted protein acidic and rich in cysteine OS=Homo sapiens PE=2 SV=1 - [B4DRV4_HUMAN]	A	37.44	39.62	7	212	24.7	5.62	11
			B	37.58	44.81	8	212	24.7	5.62	11
	Score average			37.51						
4	G8JLA8	Transforming growth factor-beta-induced protein ig-h3 OS=Homo sapiens GN=TGFB1 PE=4 SV=1 - [G8JLA8_HUMAN]	A	44.25	17.13	8	683	74.6	7.25	13
			B	14.75	5.27	3	683	74.6	7.25	3
	Score average			29.50						
5	O95965	Integrin beta-like protein 1 OS=Homo sapiens GN=ITGBL1 PE=2 SV=1 - [ITGBL_HUMAN]	A	5.77	5.26	2	494	53.9	5.64	2
			B	30.62	17.41	6	494	53.9	5.64	9
	Score average			18.20						
6	P05121	Plasminogen activator inhibitor 1 OS=Homo sapiens GN=SERPINE1 PE=1 SV=1 - [PAI1_HUMAN]	A	18.95	8.21	2	402	45.0	7.20	5
			B	11.79	10.20	3	402	45.0	7.20	4
	Score average			15.37						
7	B2RAN2	cDNA, FLJ95014, highly similar to Homo sapiens vanin 1 (VNN1), mRNA OS=Homo sapiens PE=2 SV=1 - [B2RAN2_HUMAN]	A	20.65	3.70	1	513	57.0	5.57	6
			B	2.64	1.75	1	513	57.0	5.57	1
	Score average			11.65						

	Accession	Description	Fraction	Score	Coverage	# Peptides	# AAs	MW [kDa]	calc. pI	#PSMs
8	B4E1V0	cDNA FLJ54839, highly similar to Lactotransferrin (EC 3.4.21.-) OS=Homo sapiens PE=2 SV=1 - [B4E1V0_HUMAN]	A	14.98	4.00	1	300	33.0	7.64	3
			B	8.23	4.00	1	300	33.0	7.64	5
	Score average			11.61						
9	A0M8Q9	C1 segment protein (Fragment) OS=Homo sapiens GN=C1 segment PE=4 SV=1 - [A0M8Q9_HUMAN]	A	19.40	46.67	3	105	11.3	7.87	5
			B	3.27	14.29	1	105	11.3	7.87	1
	Score average			11.34						
10	Q494V2	Coiled-coil domain-containing protein 37 OS=Homo sapiens GN=CCDC37 PE=1 SV=1 - [CCD37_HUMAN]	A	2.49	1.15	1	611	71.1	7.11	7
			B	16.99	1.15	1	611	71.1	7.11	1
	Score average			9.74						
11	Q9HAT2	Sialate O-acetylsterase OS=Homo sapiens GN=SIAE PE=1 SV=1 - [SIAE_HUMAN]	A	2.48	2.29	1	523	58.3	7.33	1
			B	14.77	7.07	3	523	58.3	7.33	5
	Score average			8.62						
12	P05543	Thyroxine-binding globulin OS=Homo sapiens GN=SERPINA7 PE=1 SV=2 - [THBG_HUMAN]	A	7.79	5.06	2	415	46.3	6.30	3
			B	9.11	2.41	1	415	46.3	6.30	3
	Score average			8.45						
13	F8VSC5	SCY1-like protein 2 (Fragment) OS=Homo sapiens GN=SCYL2 PE=4 SV=1 - [F8VSC5_HUMAN]	A	7.79	2.64	1	681	77.0	7.05	3
			B	5.06	2.64	1	681	77.0	7.05	2
	Score average			6.43						
14	Q8WUP2	Filamin-binding LIM protein 1 OS=Homo sapiens GN=FBLIM1 PE=1 SV=2 - [FBLI1_HUMAN]	A	3.07	7.24	1	373	40.6	6.02	1
			B	3.81	7.24	1	373	40.6	6.02	1
	Score average			3.44						

Supplementary Table I. **Accession:** Displays by default the unique identifier assigned to the protein by the FASTA database used to generate the report. **Description:** Provides the name of the protein exclusive of the identifier that appears in the Accession column. **Fraction:** Identifier of the run fraction. **Score:** Displays the total score of the protein. This score is the sum of the scores of the individual peptides. **Coverage:** Displays by default the percentage of the protein sequence covered by identified peptides. **# Peptides:** Displays the number of unique peptide sequences. **MW [Da]:** Displays the calculated molecular weight of the protein. The Proteome Discoverer application calculates the molecular weight without considering post-translational modifications (PTMs). **calc. pl:** Displays the theoretically calculated isoelectric point, which is the pH at which a molecule carries no net electrical charge. **PSMs:** peptide spectral matches identified for each of those 14 proteins.

Supplementary Table II: Patients with STEMI classified according to infarct classes

Sample type	Sex (M:F)	Age (years)	Height (cm)	Weight (kg)	Diabetes (%)	Hypertension (%)	Systolic BP (mmHg)	Diastolic BP (mmHg)	Smoking (%)
Healthy control	8:2	69.6±7.3	169±7	84±11	30	70	131±15	75±9	60
Small Infarct	7:3	64.3±7.2	172±6	94±10	40	70	131±13	76±12	70
Medium Infarct	8:2	62.5±8.5	170±9	85±19	20	80	128±17	72±12	80
Large Infarct	10:0	63.4±8.2	173±7	88±21	30	70	126±21	76±10	70

Supplementary Table III: Clinical information of APC patients recruited to the study

Baseline clinical and demographic characteristics of the 16 donors	
Baseline characteristics	Screening (n = 16)
Male sex, n (%)	16 (100%)
Age, median [IQR] (n)	73 [56.75-76.5] (14)
Hypertension, n (%)	8 (73%)
Unknown	5
Diabetes mellitus, n (%)	1 (9%)
Unknown	4
Hyperlipidaemia, n (%)	7 (64%)
Unknown	5
Body mass index (kg/m ²), median [IQR] (n)	29 [27-33.5] (12)
Smoking habit, n (%)	
Non-smoker	4 (25%)
Previous	3 (19%)
Current	5 (31%)
Unknown	4 (25%)
Previous Myocardial Infarction	
Coronary Artery Disease, n (%)	
0 vessels affected	n/a
1 vessel affected	n/a
2 vessels affected	2 (12.5%)
3 vessels affected	10 (62%)
Unknown	4 (25%)
Angina classification (CSS score), n (%)	
Asymptomatic	2 (12.5%)
I	4 (25%)
II	2 (12.5%)
III	2 (12.5%)
IV	2 (12.5%)
Unknown	4 (25%)
NYHA classification, n (%)	
I	4 (25%)
II	5 (31%)
III	3 (19%)
Unknown	4 (25%)
Left ventricular function, n (%)	
Good (>50)	8 (50%)
Moderate (30-50)	2 (12.5%)
Low (<30)	n/a
Unknown	6 (37.5%)

Supplementary Table IV: Clinical information of CP and CSC donors recruited to the study

Cell type	Number of patients or donors	Gender (M/F)	Age (mean $\pm$ SD)	Pathology
CP	5	3/2	7 $\pm$ 5 months	Congenital heart disease
CSC	6	3/3	54 $\pm$ 9 years	Not applicable

Supplementary Table V: Patients with chronic ischaemic cardiomyopathy and control donors

	Controls (N=10)	ICM (N=40)
Age (years)	43 $\pm$ 13	61 $\pm$ 3
Sex (M/F)	4/6	37/3
Time from MI (months)	-	123 $\pm$ 82
NYHA class (%) III IV	- -	68 32
LVEF %	ND	23 $\pm$ 5
Smoke %	ND	51
Hypertension %	ND	43
Diabetes %	ND	40

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