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Identification and functionality of proteomes secreted by rat cardiac stem cells and neonatal cardiomyocytes

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Abstract

In the heart, the proteomes secreted by both cardiac stem cells (CSCs) and cardiac myocytes could act synergistically, but the identification and functionality of the proteins comprising the individual secretomes have not yet been described. In this study, we have identified proteins present in the media obtained from cultured rat CSCs and from cultured neonatal rat ventricular myocytes (NRVMs) and compared them to proteins identified in the media alone. 83 unique proteins were identified after analysis by reversed phase liquid chromatography and mass spectrometry (MS). 49 % and 23 % were NRVM-specific or CSC-specific proteins, respectively, and 63 % of total 83 proteins were integral plasma membrane and/or known secreted proteins. 15 proteins met our criteria for paracrine/autocrine factors: i) robust protein identification, ii) cell specific and iii) known to be secreted. Most of these proteins have not been previously linked to stem cells. NRVM-specific proteins atrial natriuretic factor (ANP) and connective tissue growth factor (CTGF), and CSC-specific protein interleukin-1 receptor-like 1 (ST2), were found to affect rat CSC proliferation. These findings suggest that relative concentration of each protein may be crucial for cellular intertalk and for the final outcome of cardiac cell therapy.

Keywords

paracrine secreted proteins; cardiac stem cells; neonatal cardiomyocytes; proteomics; secretomes; atrial natriuretic factor; interleukin-1 receptor-like 1; connective tissue growth factor

1. Introduction

As the heart has a limited capability for self-renewal and regeneration, stem cell-based therapy appears to be an efficient approach for myocardial disease treatment. Thus, embryonic stem cells [1–2] and adult stem cells from various tissues have been tested and

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used as candidate sources for myocardial repair, assessing their differentiation into cardiac cells and engraftment [3–7]. The discovery of cardiac stem/progenitor cells (CSCs), a small fraction of the total cells residing in the adult heart, opened the possibility for autologous heart repair. The first reports about CSCs appeared in 2003 [8–10] and from that time, many protocols have been developed to find the ideal procedure for their isolation and expansion into numbers suitable for therapeutic purposes. CSCs have now been shown to differentiate into all cell types of cardiac tissue and they are self-renewing, clonogenic and multipotent [9,11]. They have been identified in mouse/rat [9,11], dog [12], pig [13] and human [8,11,13–14] hearts. Recently, successful methods for CSC isolation were reported [9–11,13–14]. In the report of first successful isolation of adult CSCs from human cardiac biopsies [11], undifferentiated cells, growing either from explants of postnatal atrial/ventricular human biopsy specimens or from murine heart tissues, form multicellular clusters (cardiospheres, CSps) in semi-suspension culture. They express stem cell and endothelial progenitor cell antigens and markers. The original culture method [11] was further modified to obtain clinically suitable numbers of cells from the small specimen (biopsy) in a timely manner [13]. It is now possible to routinely replat CSps to yield a monolayer of cardiosphere-derived cells (CDCs). CSps and CDCs express antigenic characteristics of stem cells at each stage of processing, as well as proteins vital for cardiac contractile and electrical function [13]. Importantly, human CDCs acutely injected into the border zones of myocardial infarcts promoted cardiac regeneration and improved LV function in a mouse infarct model [11,13].

It has been proposed, that the clinical benefit of stem/progenitor cells may also arise from proteins secreted into the vicinity of the injury that can modulate the infarct microenvironment and play important roles in functional improvement. This was supported by the observation, that the correlation between the number of new cells forming at the site of injury and the degree of observed functional improvement are often hardly proportional [13,15]. Thus, an alternative hypothesis was created in which paracrine/autocrine mechanisms, mediated by factors secreted by either endogenous or transplanted exogenous stem cells, are meant to contribute to regeneration and repair processes [15–16]. Soluble proteins selectively secreted (secretomes) from stem cells can serve as interactive signals to the local environment, influencing survival, differentiation and engraftment. They regulate a multitude of physiological processes that make them potential therapeutic targets and biomarkers. Several groups have investigated paracrine/autocrine factors secreted by adult stem cells, e.g. by mesenchymal stem cells (MSCs), and their effects on cardiac functional improvement [15,17–18]. Studies were also published [19–21] in which a proteomic approach was used for identification of stem cell secreted proteins. However, very little is known about autocrine or paracrine factors that are secreted by CSCs.

In the present study, we investigate and compare proteins secreted into conditioned media by cultured CSCs (CSps and CDCs; isolated from adult rat hearts by method described in Ref. [13]) to proteins secreted by neonatal rat ventricular myocytes (NRVMs) after analysis by reversed phase liquid chromatography (RPLC) and identification by mass spectrometry (MS), in order to compare the specificity and functionality of both secretomes. Since proteins secreted from either or both CSCs and NRVMs *in vivo* can play a role after heart injury, we aimed to test and compare both types of cells – unique aspect of this study. Results obtained could provide valuable insight into secretome regulation and into novel paracrine/autocrine factors that protect, and regenerate the myocardium.

2. Materials and methods

2.1 Sample processing

Cell culture—Specimen preparation, CSps, CDCs and NRVMs culturing and harvesting were described previously in details [13]. Briefly, cardiac tissue specimens from septum or left ventricle of Wistar Kyoto rats, 12 to 16 weeks old, were cut into small pieces, washed by PBS, partially enzymatically digested and grown in primary cultures as explants on fibronectin (from human plasma, BD Biosciences) coated Petri dishes in complete explant medium (CEM), consisting of IMDM medium (Iscove's Modified Dulbecco's Medium, Invitrogen) supplemented with 20% fetal bovine serum (FBS), 1% penicillin-streptomycin, 1% L-glutamine and 0.1 mmol/L 2-mercaptoethanol at 37 °C and 5% CO₂. After 1 to 2 weeks, round phase-bright cells, migrating over a layer of fibroblast-like cells arisen from explants, were harvested by mild trypsinization and seeded at 2×10^4 cells/cm² on poly-D-lysine (BD Biosciences) coated wells in CSp-growing medium (CGM), composed of 35% IMDM/65% DMEM-Ham F-12 Mix (Invitrogen) and supplemented with 3.5 % FBS, 1% penicillin-streptomycin, 1% L-glutamine, 0.1 mmol/L 2-mercaptoethanol, thrombin (1 unit/mL, Sigma), 2% B-27 (Invitrogen), basic fibroblast growth factor (bFGF, 80 ng/mL, PeproTech), epidermal growth factor (EGF, 25 ng/mL, PeproTech) and cardiotrophin-1 (4 ng/mL, PeproTech). Five to six days after plating, CSps were collected, replated on fibronectin coated flasks and grown as monolayers, obtaining CDCs. Neonatal rat ventricular myocytes (NRVMs) were isolated by routine methods with overnight trypsin digestion from 2 days old Sprague-Dawley rats and plated confluent on fibronectin-coated multi-wells in Medium 199, 10% for 48 hours, then 2% FBS, 10mmol/L HEPES, 0.1 mmol/L MEM non-essential aminoacids, 19.4 mmol/L glucose, 2 mmol/L L-glutamine, 0.8 µg/mL vitamin B12 and 2 unit/mL penicillin.

Conditioned media collection—After 3 PBS washes, media in CSp, CDC and NRVM cultures were replaced with CEM (1% or 2% FBS) and conditioned for 48 hours or 10 days. Media were then collected and concentrated in SpeedVac concentrator, approximately 8 to 10 fold. Basal un-conditioned CEM (1% or 2% FBS) was processed the same way as the control. Samples were stored at -80 °C before further analysis. During optimization experiments, each biological experiment has been done as a single analysis, but once protocol has been optimized, three independent biological replicates have been carried out.

Viability assessment by 7AAD/AnnexinV labelling—Cells were collected by trypsinization, incubated for 15 minutes at room temperature with Annexin V-FITC and 7AAD (BD Biosciences), and analyzed with a FACScan flow cytometer and CellQuest software (BD Biosciences). Unlabelled cells were used as a control. The percentages of cells that were necrotic or in the early stages of apoptosis were determined in triplicate for each biological sample.

2.2 Reversed phase liquid chromatography (RPLC)

After collection and concentration, the media were mixed with solvent to a final concentration of 20% (v/v) acetonitrile (ACN), 1% (v/v) trifluoroacetic acid (TFA), pH 2.3 and centrifuged at 18×1000 g for 30 min at 4 °C. 200 or 800 µg (total protein) were injected into reversed phase C18 column (50 mm, nanoporous particles, HPRP modul of ProteomeLab PF 2D, Beckman Coulter, CA, USA) in consecutive runs of 100 µg/run, in order to avoid the clogging of the column due to high amount of albumin. The proteins were separated using a linear AB gradient from 0 to 100% B over 35 min, where solvent A was aqueous 0.1% TFA (v/v) and solvent B was ACN/0.08% TFA (v/v). The elution chromatogram was monitored at 214 nm. 68 fractions were collected per run from 7 to 24 min (0.25 min/fraction) and combined into 9 pooled fractions. Note that fractions containing

major amounts of albumin (between 16.4 min and 17.4 min of elution time, 50–53% acetonitrile) were excluded. Pooled fractions were dried in SpeedVac concentrator, neutralized by ammonium bicarbonate, trypsin digested (250 ng/fraction, Promega, WI, USA) at 37 °C overnight and stored at –80 °C.

2.3 Mass spectrometry and protein identification

Tryptic peptides were dissolved in 8 µl of 0.1% TFA (v/v) and analyzed by ThermoFinnigan LTQ ion trap or LTQ Orbitrap mass spectrometer (both instruments by Thermo Electron Corporation, MA, USA). The C18 column (120 mm, 75 µm id, YMC ODS-AQ 5 µm particles with 120 Å pore size) was used in gradient mode (5–60% of 0.1% formic acid/90% acetonitrile) over 30 or 60 minutes with a flow rate of 300 nl/min and a data-dependent MS/MS mode was used. Data obtained from MS spectra were submitted to NCBI nr_20060615 database search by using MASCOT search engine (Matrix Science Mascot Daemon, V2.1.3 -max. missed cleavages 2, peptide tolerance ± 1.5 Da and MS/MS tol. ± 0.8 Da, $p < 0.05$, all species). After Mascot Daemon search, the files were transferred to Scaffold software (Version Scaffold-01_06_06, 2006 Proteome Software Inc., OR, USA) for Mascot result validation, visualization and comparison of protein identifications between individual samples. All identified proteins were further examined for peptide and protein redundancy. The protein amino-acid sequence was blasted against UniProt Knowledgebase (Swiss-Prot + TrEMBL) by using SIB BLAST network service (ExPASy). In case of protein multiple names or homology, only one protein name was used after the original peptide sequences obtained from our MS results were checked back for matching that protein by using multiple sequence alignment program ClustalW (EMBL-EBI). Also, the confirmation of a protein isoform was done based on matching a tryptic peptide fragment to a unique amino-acid sequence of each intact isoform. In case the same protein name was identified for different species, the peptide sequences were checked and multiple protein names were included only when peptide sequence(s) was unique to species.

An Expanded Materials and Methods section is available in the Online Supporting Information in which the methods of Western blotting and proliferation assay are described in details.

3 Results

The experimental design of protein analysis in the conditioned media alone or that obtained from the cultured CSCs and NRVMs is shown schematically in Figure 1. This was achieved through initial optimization of both sample preparation and technology used. To optimize and establish the most effective procedure and methods leading to maximum secretome coverage, preliminary experiments were carried out in which several conditions were tested and evaluated: i) concentration of FBS in conditioned media and conditioning time, ii) type of heart tissue used as CSC source (septum or left ventricle), iii) concentration of conditioned media prior to analysis by RPLC and amount of protein injected, and iv) optimal solvent for sample mixing and for RPLC gradient run.

Protocol optimization

The list of proteins identified during preliminary optimization experiments, with the corresponding number of peptides, is shown in Online Supporting Information (Supplemental Table S1). Different concentrations of FBS in conditioned media (1 % or 2 % FBS; data not shown) were tested in order to minimize serum addition without increasing cell death rates: Supplemental Figure S1 shows that cells cultured in 1% FBS had similar or lower amount of necrosis, compared to conditions of 2% FBS. The effect of beating (48 hours of culture after isolation) vs non-beating (10 days of culture) NRVMs on the

secretome was analyzed to determine the best time to harvest NRVMs and whether changes were induced with time of culture or conditions of myocytes. Although the number of peptides detected in beating and non-beating NRVM secretomes (not found in control media; Supplemental Table S1) was similar for the same individual proteins (except for 2 collagen isoforms), more proteins were detected in the beating NRVM secretome (30 proteins vs 23 proteins; 14 new proteins found in the beating NRVM compared to non-beating NRVM secretome). Optimal results (i.e. maximum protein coverage and minimal cell death) were obtained after 48 hour of conditioning with 1% FBS. We compared results obtained from analyses of conditioned media of CDCs and CSps from heart left ventricle (LV) or septum, but since the results were not straightforward, in terms of number and types of proteins and number of peptides identified (see Supplemental Table S1), we opted to use specimens from heart left ventricle as a source of CSCs for further experiments. In preliminary experiments, we separated 200 µg of protein of conditioned media only from CDCs by RPLC, but to be able to identify more proteins and to obtain higher peptide coverage for individual proteins, protein amount was increased to injection of 800 µg and in addition, conditioned media from both CDCs and CSps were analyzed in following experiments, which resulted in additional 19 proteins (63%) identified in CSps from all 30 proteins found in CSCs during optimization experiments.

Proteomic optimization

RPLC was effective for desalting, concentrating the media and separating the proteins in a single step. RPLC was used to separate albumin from the other proteins (elution time from 16.4 to 17.4 minutes), allowing enhanced detection of low-abundance proteins. Typical chromatograms obtained by RPLC with 9 final fractions used for MS analysis are presented for conditioned media from CDCs in Figure 2A and for control media, conditioned media from NRVMs, CDCs and CSps in Figure 2B. Fraction containing albumin was excluded from downstream analysis. 800 µg of protein were injected into chromatographic column in consecutive runs of 100 µg/run in order to avoid the clogging of the column due to the high amount of albumin. The excellent reproducibility in the elution profile of 8 independent RPLC runs for separation of the same sample: media obtained from CDCs, is shown in Online Supporting Information (Supplemental Figure S2).

Secretome analysis

Conditioned media from CDCs, CSps and NRVMs were analyzed in triplicate, together with control media in duplicate, by MS by using the same aforementioned optimized experimental conditions. The complete list of 137 proteins identified is provided in Online Supporting Information (Supplemental Table S2). In order to distinguish proteins secreted into conditioned media by cells from proteins present in control media alone (1% FBS), only proteins found in media of CSCs and NRVMs and not identified in control media were further considered. The resulting 83 non-redundant proteins secreted by CSCs and NRVMs into conditioned media are listed in Online Supporting Information (Supplemental Table S3). The peptides identifying these proteins, their sequences, charges and calculated masses, are shown in Online Supporting Information (Supplemental Table S4). Two proteins found in control media but greatly increased in quantity in CSC/NRVM conditioned media (more than 5 fold, based on the number of peptides identified) were included as well (actin and collagen). Figure 3A illustrates the secreted protein distributions among media from CSCs (CSps and CDCs) and NRVMs. 41 (49.4%) and 19 unique proteins (22.9%) were secreted into media by NRVMs and CSCs, respectively, with 23 proteins (27.7%) overlapped between both of them. Among the total 42 proteins secreted by CSCs, 22 and 9 proteins were found in media from CSps or CDCs, respectively, with 11 proteins common to both of them. Protein databases (NCBI, UniProt, Human Protein Reference Database HPRD) and literature search revealed that 63% of the 83 proteins identified are known to be secreted (33

proteins) and/or integral plasma membrane proteins (20 proteins) and 37% are known to be intracellular proteins (Figure 3B).

15 proteins complied with our selection criteria for candidate paracrine/autocrine factors: i) robust protein identification (protein was identified by MS based on 2 or more peptides of unique amino-acid sequences, i.e. non-redundant); ii) cell specificity (i.e. proteins observed only in NRVM or CSC conditioned media and not in control media), and iii) protein is known to be secreted (Table 1). Many of these proteins have ascribed functions in stem cell differentiation or cardiovascular disease. In addition, 2 proteins (adrenomedullin and CTGF) have been suggested previously as potential stem cell paracrine factors.

Effects on CDC proliferation of 3 selected proteins

Positive/negative effects related to 3 proteins, CTGF, ANP and ST2, on rat CDC proliferation are shown in Figure 4, together with the proteomic data. For each protein in Figure 4, accession number, length of protein sequence, molecular weight, number and type of unique peptides, as obtained from MS analysis, are included. Cell specificity of individual proteins as detected in media of CSps, CDCs or NRVMs is shown in accompanied small table (Figure 4). Although ST2 (detected in CDC conditioned media) inhibited rat CDC proliferation down to about 40% (depending on protein concentration) compared to untreated controls, its ligand, interleukin 33 (IL-33), had no effect on rat CDC proliferation (data not shown). Of note, we did not observe IL-33 in any media, indicating that it is either not secreted or secreted at very low concentrations below our detectability. CTGF (exclusively secreted by NRVMs in our study) increased proliferation of rat CDCs up to 100% compared to control, and ANP (secreted by NRVMs as well) inhibited rat CDC proliferation down to 60–70%. ANP is synthesized as a pre-prohormone (152 aa; 16556 Da) and within the endoplasmic reticulum the signal peptide is cleaved to produce the prohormone (128 aa) with ANP hormone consisting of 28 aa (from C terminus) with molecular weight of 3063 Da. Western blots of ANP carried out on media or lysate of NRVMs, CDCs and CSps, with recombinant protein as control (200 and 500 ng loaded; rat ANP, 1–28 aa from C-terminus, 3063 Da; A4152-05, US Biological, MA, USA) are shown in panel A and B of Figure 4. Three bands corresponding to pre-proANP, proANP and ANP peptide were detected in media of NRVMs, but none of ANP bands were found in conditioned media from CSps and CDCs. These findings confirmed our MS results in which 3 peptides were exclusively identified only in media of NRVMs: 2 peptides covering a part of ANP prohormone sequence and 1 peptide matching the partial sequence of ANP hormone.

4 Discussion

The hypothesis that endogenous or transplanted exogenous stem cell can secrete proteins into the vicinity of injury, which can modulate cardiac regeneration, has been supported by increasing numbers of recent studies [22–29]. In this work, we show that: i) rat CSCs secrete proteins, ii) rat cardiomyocytes (in our case neonatal myocytes) secrete proteins, iii) most of these secreted proteins are cell specific, and iv) at least some of these proteins (even proteins unique to cardiomyocytes) alter rat CSC proliferation rate. This supports the concept that these proteins could act as autocrine or paracrine factors *in vivo* and that there is a potential for a cross talk between cardiomyocytes and CSCs.

The autocrine/paracrine hypothesis is supported by the fact, that mesenchymal stem cells (MSCs) secrete proteins (e.g. growth factors and cytokines) that can influence cardiac improvement even in the absence of cell transplantation [22]. Also, endothelial progenitor cells (EPCs) secrete soluble factors (e.g. VEGF, SDF-1 and IGF-1) into the cell culture supernatant and EPC conditioned media significantly stimulates the migration of cardiac

resident progenitor cells *in vitro* [23]. Furthermore, stem cells genetically modified release therapeutic paracrine factors and show better reparative potential in the heart compared to unmodified stem cells, such as MSCs with regulated heme oxygenase-1 vector [24], Bcl-2 engineered MSCs [25] or MSCs with overexpressed Akt [26]. Studies have shown that secreted proteins appear to modulate stem cell differentiation into cardiac cell lineages [27–28], but although the trigger and cellular mechanisms of stem cell response is not known, changes in the temporal and spatial concentrations of paracrine/autocrine proteins in the environment are most likely involved [29]. Importantly, a cross talk between different cell types might occur, as the secretome of one cell type alters the function or activates another cell type [22,30–31]. Despite all this, actual protein candidates have been rarely suggested.

In this study, we have identified 83 proteins secreted by CSCs (CSps and CDCs) and NRVMs, and many of them are known secreted and/or integral plasma membrane proteins that can be considered as potential paracrine/autocrine factors or targets. 41 proteins were exclusively detected in the NRVM secretome, 19 proteins in CSC secretome and 23 proteins were secreted by both NRVMs and CSCs. 15 proteins that we selected, based on our previously mentioned criteria (Table 1), including exclusivity to CSC or NRVM secretome, were further searched in protein databases and literature in order to find their possible correlations with stem cells, or their function as paracrine/autocrine factors in the heart. Commercially available proteins were subjected to functional assay to test their effect on proliferation of rat CDCs. Interestingly many proteins in Table 1 were found to have functions that suggest a potential role in stem cell differentiation or in cardiovascular disease.

Whereas ANP is well known for its blood pressure lowering, natriuretic, diuretic, and/or kaliuretic (i.e. potassium excreting) properties, its function has not been linked previously to either stem cells nor to a potential paracrine factors. As shown in Figure 4, NRVM-specific secretome protein ANP decreases CDC proliferation. This suggests that under physiological conditions, when an increase in ANP level occurs, ANP would suppress endogenous CSC proliferation. This anti-proliferation/growth property of ANP has been previously observed in fibroblasts, vascular endothelial and smooth muscle cells [32–34]. In recent publication, ANP was shown to increase endothelial tube formation abilities *in vitro*, which the authors speculate could be related to enhanced angiogenesis *in vivo*, a very desirable property for a paracrine factor involved in therapy [35]. Thus, in pathological conditions where ANP may be elevated, it could be linked to poor outcome due to poor proliferation of endogenous stem cells. Interestingly, the majority of ANP in conditioned media from NRVMs occurs in the form of pre-prohormone (Figure 4).

CTGF has previously been suggested to be a paracrine factor as it is able to induce osteoblast and fibroblastic differentiation of MSCs [15,36]. CTGF is upregulated by Wnt3A and bone morphogenetic protein BMP-9 at the early stage of osteogenic differentiation and downregulated as the differentiation progresses [36], suggesting that tight regulation of CTGF expression may be essential for osteoblast differentiation of MSCs. Our data showed that NRVM-specific protein CTGF also alters rat CDC proliferation rates in a paracrine manner, i.e. it affects the proliferation of CSCs and, interestingly, its effect is opposite compared to ANP (ANP inhibited proliferation rates, while CTGF increased them). These findings suggest that the relative concentration of each protein will drive the final outcome.

Another candidate protein in our study is ST2 which was identified in the conditioned media from CSCs. ST2 belongs to the interleukin-1 receptor family and it is expressed in 2 isoforms: membrane-bound isoform A (Fit-1M) and soluble isoform B (Fit-1S; secreted). MS results showed that both ST2 isoforms might be present in our conditioned media samples, since 2 identified unique peptides matched the amino-acid sequences of either

isoform. Increased quantities of ST2 is known to occur with neurohormonal and biomechanical stress activation, and increased serum levels of ST2 predict mortality and clinical outcome in patients with MI [37–39]. Recently, IL-33 was identified as a specific ligand for ST2 [40] and IL-33/ST2 signaling was shown to be a mechanically-activated cardioprotective fibroblast-cardiomyocyte paracrine system [41]. Whereas IL-33 exhibited no effect on rat CDCs proliferation in our experiments (results not shown), ST2 acted as an autocrine factor since it was secreted into media by CDCs and had an inhibitory effect on rat CDCs proliferation (Figure 4).

The functions, potential roles in stem cell differentiation and/or in cardiovascular diseases, as found in literature, for additional proteins included in Table 1, are described in Online Supporting Information (Supporting information to Table 1) in more details. It is noteworthy, that the majority of the proteins secreted by either NRVMs or CSCs are involved in the regulation of extracellular matrix. However, the proteins are unique to each cell type which may reflect the difference in the role of the extracellular matrix to these cell types. For example, NRVMs secrete specifically CTGF, periostin, extracellular matrix protein 1 and two specific isoforms of collagen which would provide structural support that is unique characteristic of the muscle.

In conclusion, we identified by proteomic approach 15 proteins exclusively secreted into conditioned media by CSCs (6 proteins) and NRVMs (9 proteins). From wide database and literature search, these proteins were found to have various known functions: they positively/negatively affect the cardiac system (adrenomedullin, ANP, extracellular matrix protein 1, periostin, fibrinogen, ST2, osteopontin, (pro)collagen, SPARC and tropoelastin), they are related to stem cells and their functions (CTGF, periostin, osteopontin and SPARC) and they can act as stem cell paracrine/autocrine factors (adrenomedullin and CTGF). In the present study, three proteins identified in secretomes were shown to affect rat CDCs proliferation: CTGF increased proliferation up to 100% (in a concentration-dependent manner), whereas ANP and ST2 inhibited CDC proliferation down to 70% and 40%, respectively. In addition, nine proteins identified are known to be involved with extracellular matrix structure and function. They are not only important in providing structural support: in fact extracellular matrix proteins are also part of signaling pathways and they can actively influence behaviors of cells and their responses to various stimuli. The biology of cardiac extracellular matrix and its protein composition can help understanding the pathologic alterations of heart function. Although several proteins have already been identified in secretomes of stem cells, e.g. of human ESCs (various collagen types, Pcolce, SPARC and periostin) [42], only adrenomedullin and CTGF (2 proteins identified in our study as well) had been previously suggested as stem cell paracrine/autocrine factors [15]. To our knowledge, this is a first report displaying the direct comparison of rat CSC- and NRVM-specific secretomes with their unique functionality profiles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Strategy of protein analysis in conditioned media of CSCs and NRVMs.

Figure 2.

Representative chromatograms obtained by RPLC analysis.

A - Chromatogram of CDC conditioned media analyzed in 8 subsequent runs (100 μ g/run) with 9 final fractions used for MS protein identification after trypsin digestion. Eight chromatograms are overlayed to demonstrate the reproducibility. Fraction containing albumin was excluded. B - Comparison of chromatograms obtained during analysis of control media and conditioned media of NRVMs and CSCs (CDCs and CSps), respectively, in time window from 14 to 22 minutes.

Figure 3.
A - Distribution of 83 unique proteins present in the conditioned media from CSCs and/or NRVMs. B - Cellular locations of 83 unique proteins as found in protein databases.

Table 1

Proteins secreted by either CSCs or NRVMs that complied with our selection criteria for candidate paracrine/autocrine factors.

Protein name	Secretome	Effect on CSC proliferation	Function ^a	Refs.
Adrenomedullin	NRVMs	ND	Cardiovascular disease Paracrine/autocrine factor	[2–3] online
Atrial natriuretic factor	NRVMs	Down	Cardiovascular disease	[32–35]
Connective tissue growth factor	NRVMs	Up	Stem cells Paracrine/autocrine factor	[15,36]
Extracellular matrix protein 1	NRVMs	ND	Cardiovascular disease	[23] online
Extracellular matrix protein periostin	NRVMs	ND	Cardiovascular disease Stem cells	[5,7–10] online
Fibrinogen beta chain	CSCs	ND	Cardiovascular disease	[17–18] online
Hemopexin	CSCs	ND		
Histidine-rich glycoprotein	CSCs	ND		
Interleukin-1 receptor-like 1	CSCs	Down	Cardiovascular disease	[37–41]
Osteopontin	CSCs	ND	Cardiovascular disease	[13–16] online
Pcolce protein	NRVMs	ND	Cardiovascular disease	[22] online
Collagen alpha-1(VI)	NRVMs	ND	Cardiovascular disease	[20–21] online
Procollagen alpha-2(VI)	NRVMs	ND	Cardiovascular disease	[20–21] online
SPARC	CSCs	ND	Cardiovascular disease Stem cells	[24–28] online
Tropoelastin	NRVMs	ND	Cardiovascular disease	[29–30] online

ND: not determined

^a as found in protein database and/or literature (related to stem cells, paracrine/autocrine function and/or function in cardiovascular disease)

Refs. online: corresponding references can be found in Online Supporting Information (Supporting information to Table 1).