Transcriptional profiling of left ventricle and peripheral blood mononuclear cells in a rat model of postinfarction heart failure

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Abstract

Background: Myocardial infarction (MI) often results in left ventricular (LV) remodeling followed by heart failure (HF). It is of great clinical importance to understand the molecular mechanisms that trigger transition from compensated LV injury to HF and to identify relevant diagnostic biomarkers. The aim of this study was to investigate gene expression in the LV and to evaluate their reflection in peripheral blood mononuclear cells (PBMCs).

Methods: MI was induced in rats by ligation of the proximal left coronary artery. Rats with small, moderate, and large MI size were included into the experiment two months after the operation. The development of heart failure was estimated by echocardiography and catheterization. Microarrays were used to compare the LV and PBMCs transcriptomes of control and experimental animals.

Results: Only rats with a large MI developed extensive LV remodeling and heart failure. 840 transcripts were altered in LV of failing hearts, and especially numerous were those associated with the extracellular matrix. In contrast, no significant gene expression changes were seen in LVs of rats with moderate or small MI that had compensated LV injury. We showed that ceruloplasmin was similarly overexpressed in the heart and blood in response to HF, whereas downregulation of tetraspanin 12 was significant only in the PBMCs.

Conclusion: A large size of infarcted area is critical for progression of LV remodeling and HF development, associated with altered gene expression in the heart. Ceruloplasmin and tetraspanin 12 are potential convenient markers in readily obtainable PBMCs.

Keywords: Left ventricular remodelling, Heart failure, Myocardial infarction, Extracellular matrix, Gene expression profiling, Microarrays, Ceruloplasmin, Tetraspanin 12

Background

Left ventricular (LV) remodelling is crucial in the development of heart failure (HF) and is associated with increased mortality after myocardial infarction (MI). It is characterized by complex structural alterations connected with infarct expansion, scar formation, wall thinning, progressive chamber dilation and hypertrophy [1]. Initially all these changes help maintain normal cardiac function but in time they become maladaptive and can be responsible for systolic dysfunction and progressive decompensation of LV [2]. LV remodelling involves numerous cellular changes, but is essentially connected with dynamic alterations in the extracellular matrix (ECM) [3]. Proper organization and composition of the ECM is necessary for structural (size, shape) and mechanical (strength, contractility) support, and also for communication between different cardiac cells [4]. Numerous transcriptional studies have been performed to investigate alterations in cardiac gene expression related to the progression of heart failure [5-7]. To investigate the mechanisms of heart failure animal models are mostly used (rodents [8-10], dogs [11], swine [12]) as well as human heart tissues [13,14] from patients with severe failing hearts classified to transplantation. Studies on human HF usually concentrate on the end-stage of heart failure and drug-treated myocardium. Therefore, they miss factors that uniquely mark the transition of LV hypertrophy to heart failure, making animal heart failure models irreplaceable.

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Peripheral blood mononuclear cells (PBMCs), being easily obtainable, could be used as a proxy to study changes associated with heart diseases. Blood permeates the heart with infiltration of leukocytes into the muscle tissue under the certain conditions. Thus, PBMCs contribute to the inflammatory response in the infarcted myocardium and are also considered a source of factors crucial in matrix remodelling, such as matrix metalloproteinase 9 [15].

The aim of the present study was to investigate gene expression in the left ventricle in a well validated model of post-infarcted HF and to evaluate their reflection in PBMCs. We decided to use the rat model since it is the most commonly used animal model of myocardial infarction and heart failure and had convincingly been shown to mimick the mechanisms of human HF.

Methods
Rat myocardial infarction model and samples processing
The study conformed to the Guide for the Care and Use of Laboratory Animals by the US National Institutes of Health (NIH publication No. 85–23, revised 1996) and was approved by the local Ethics Committee.

Myocardial infarction (MI) was induced in 40 male Wistar rats (275–300 g) by ligation of the proximal left coronary artery, as described previously [16]. In brief, rats were anaesthetised with ketamine HCl and xylazine (100 mg/5 mg/kg body weight), left thoracotomy was performed and a silk suture was tightly tied around the proximal left coronary artery, approximately 2 mm from its origin. The sham-operated group (control group, n = 6) was subjected to the same protocol, except that the suture was not tied.

Two months after the operation the rats were anaesthetised with ketamine HCl and xylazine (75 mg/3.5 mg/kg body weight) and echocardiography was performed to evaluate the LV function and dimensions and to estimate the infarct size. Later a micromanometer-tipped catheter (Millar Instruments) was advanced through the right carotid artery into the LV for recording of LV pressures. Finally, the heart was dissected and blood samples were obtained. Whole blood was collected via cardiac puncture under anaesthesia and transferred to EDTA-K2 collection tubes (Medlab Products, Warsaw, Poland). PBMCs (peripheral blood mononuclear cells) were isolated by centrifugation through HISTOPAQUE® 1083 (Sigma-Aldrich, St Louis, MO, USA) according to the manufacturer’s instructions and resuspended in RNALater® Solution (Ambion Inc., Austin, TX, USA). LV from rats with induced MI was divided into a well visible fibrotic scar tissue and apparently healthy myocardial tissue. For further examinations, whole LV from sham-operated rats and the morphologically unaffected part of LV from MI rats were placed in RNALater® Solution and stored at −80°C until RNA extraction.

For determination of infarct size regional LV wall motion abnormalities were quantitated as described previously [16]. The contractility of twelve wall segments visualized in the midpapillary short-axis view and eleven segments visualized in the long-axis view was graded as 1 (normal) or 0 (abnormal) and the total wall motion index (WMI) was calculated. Normal hearts had WMI = 23. Our previous results [16] revealed that WMI closely correlated with infarct size, thus post-MI rats were divided into three groups: large MI (L-MI, ≥ 40% of LV area, n = 5), moderate MI (M-MI, 20–39% of LV area, n = 6) and small MI (S-MI, <20% of LV area, n = 6).

All results are given as means ± SEM. The statistical significance was determined by one-way ANOVA followed by a Dunnett test in case of significance, with the Statistica software package (version 6.0, StatSoft, Poland). The difference was considered significant at P < 0.05.

RNA isolation
Total RNA was isolated from PBMCs and heart samples using MagNA Pure Compact System (Roche Diagnostics GmbH, Germany) according to the manufacturer’s recommendations. Heart tissues were homogenized using MagNA Lyser Instrument (Roche Diagnostics GmbH, Germany). The amount of isolated total RNA was quantified by UV absorption (Nanodrop, LabTech International, UK). The quality of these samples was determined using an Agilent 2100 Bioanalyzer© and RNA 6000 Nano Kit (Agilent, Santa Clara, CA, USA). For all RNA samples the RIN (RNA integrity number) value was above eight.

cDNA microarrays
GeneChip® Rat Gene 1.0 ST Arrays (Affymetrix, Santa Clara, CA, USA) were used. RNA from LV and PBMCs samples obtained from L-MI and sham-operated rats were processed according to standard Affymetrix manufacturer’s protocols. First, the samples were reverse transcribed and amplified, then fragmented and biotinylated. Hybridization to the GeneChips® was conducted for 16 h at 45°C. After hybridization the microarrays were washed and stained on a fluidics station and scanned in an Affymetrix GCS 3000 GeneArray Scanner.

Data analysis of microarrays
After a robust multiarray average (RMA) normalization of microarray data, differences in gene expression levels were calculated by comparing the group with myocardial infarction with the sham-operated group. Lists of differentially expressed genes, principal component analysis (PCA) and hierarchical clustering were generated using the Partek Genomics Suite software (Partek Inc, St. Louis, MO, USA). The fold change (FC) of gene expression ratios ≥1.3 and P ≤ 0.05 corrected by false discovery rate (FDR) were set as significant criteria for heart tissue. For PBMCs the
Quantitative real-time RT-qPCR
The real-time reverse transcription-polymerase chain reaction (RT-qPCR) was used to validate the microarray data. Each total RNA sample (400 ng) was reverse transcribed using Quantitect Reverse Transcription (Qiagen, Germany) according to the manufacturer’s recommendations. Primer sets for selected genes were designed using Clone Manager Suite software; their sequences and reaction conditions are available in additional files (Additional file 1). Specificity of the amplified product was demonstrated by melting curve analysis and agarose gel electrophoresis (data not shown). Each sample was run in triplicate in 96-well plates using LightCycler®480 and LightCycler®480 FastStart SYBR Green I Master (Roche Diagnostics GmbH, Germany). Quantification cycles (Cq) were calculated using the fit point method (LightCycler®480 Software, Version 1.5 provided by Roche). The fold change in gene expression levels, corrected by efficiency, was analyzed using REST-MCS©-version 2 software [18]. The expression data were normalized to the reference glyceraldehyde-3-phosphate dehydrogenase (Gapdh) and hypoxanthine-guanine phosphoribosyltransferase (Hprt1) genes, which in a GeNorm algorithm-based selection [19] were the most stable among the four that we tested (M-value; the highest M-value means the least stable gene). Therefore, the expression data were normalized to the reference glyceraldehyde-3-phosphate dehydrogenase (Gapdh) and hypoxanthine-guanine phosphoribosyltransferase (Hprt1) genes, which in a GeNorm algorithm-based selection [19] were the most stable among the four that we tested (M-value; the highest M-value means the least stable gene).

Results
Physiological characteristic of rats
Two months after MI induction rats with large MI (L-MI, mean infarct size 49.8%) had extensive LV dilation, ejection fraction (EF) reduction and a highly elevated left ventricular end-diastolic pressure (LVEDP), a recognized marker of heart failure in the rat MI model (Table 1). On the other hand the rats with small (S-MI) or moderate myocardial infarction (M-MI) (mean infarct size 10.5% and 25.8%, respectively) had only mild LV dilation and EF reduction, but normal LVEDP.

This indicates that while the rats with large MI developed extensive LV remodelling and heart failure, the rats with small or moderate infarction developed only mild LV remodelling and a condition that could be called compensated LV injury.

Microarrays results from heart tissues
The microarray data after normalization were subjected to PCA analysis to visualize gene expression differences between control and treated animals. The PCA plot (Figure 1) demonstrates a distinct separation between sham-operated rats and rats with L-MI in contrast to animals with S-MI and M-MI, which clustered together with the sham group.

Genes expressed differentially between groups were determined using the cutoff criteria described in Materials and Methods. The expression of 840 transcripts was altered in L-MI rats 2 months after the operation. Consistent with the results of the PCA analysis, no gene expression differences were identified between rats with S-MI or M-MI and the control group. From the total 840 transcripts altered in the L-MI group, 814 (553 upregulated, 261 downregulated) were supported with gene annotations provided by RefSeq, GeneBank (http://www.ncbi.nlm.nih.gov) or Ensembl (http://www.ensembl.org). Their full list is available in additional files (Additional file 2).

The genes identified as significantly altered in the L-MI group were further subjected to a gene ontology analysis (AmiGO) to find significant shared Gene Ontology terms. Figure 2 presents only the GO terms with the most significant p-values, whereas in additional files we present complete results (Additional file 3). The analysis revealed that the molecular function of these genes is essentially connected to GO:0005488 binding and particularly to GO:007367 carbohydrate derivative binding, GO:0005539 glycosaminoglycan binding, GO:1901681 sulfur compound binding, GO:0008201 heparin binding, GO:0005102 receptor binding and GO:0005515 protein binding. The biological processes are mainly associated with response to stimuli: GO:0010033 response to organic substance, GO:0009611 response to wounding and GO:0070887 cellular response to chemical stimulus. Cell adhesion GO:0007155 and small molecule metabolic process GO:0044281 were also at the top of significantly altered biological processes. Cellular component classification showed that most of the differently expressed genes were involved in GO:0005576 extracellular region (GO:0044421 extracellular region part, GO:0005615 extracellular space).
and GO:0031012 extracellular matrix (GO:0005578 proteinaceous extracellular matrix, GO:0044420 extracellular matrix part).

Extracellular matrix remodelling is the critical event in pathology of HF. For that reason, a focused hierarchical clustering analysis was done for transcripts annotated as GO:0031012 extracellular matrix (Additional file 4) to show their expression profiles in LV samples from individual rats (Figure 3). This analysis revealed a clearly distinct gene expression profile of the selected genes in rats with L-MI that clustered separately from the other groups. Interestingly, all these genes were upregulated in the L-MI group, with one exception - vitronectin (Vnt), although they are involved in both ECM deposition and degradation.

Microarray results from PBMCs
PBMCs are considered to be an ideal surrogate tissue to reflect changes occurring in major human organs [14]. Therefore, a further microarray analysis was performed to evaluate if and how the progressive heart failure in rats with L-MI affected the gene expression patterns in PBMCs. Global transcriptomic profiles analysed by PCA demonstrated a separation between the sham-operated rats and rats with L-MI that was less clear-cut than that found for the heart tissue (Figure 4). A list of differentially expressed genes was determined with the cutoff criteria described in Materials and Methods. The expression of 72 (38 - upregulated, 34 - downregulated) well-annotated transcripts was altered in PBMCs in the L-MI rats 2 months after the operation in comparison with sham-operated rats (Additional file 5). The AmiGO analysis showed no

| Table 1 Body weight and echocardiographic parameters in post-myocardial infarction and sham-operated rats 2 months after surgery |
|---------------------------------------------------------------|
| **Sham, n = 6** | **Infarct** |
| **Small, n=6** | **Moderate, n=6** | **Large, n=5** |
| BW [g] | 419 ± 14 | 411 ± 8 | 424 ± 22 | 377 ± 4 |
| Infarct size [%] | 0 | 10.5 ± 1.6*** | 25.8 ± 1.6*** | 49.8 ± 1.5*** |
| LVEDD [mm²] | 58.3 ± 1.0 | 63.8 ± 2.1 | 67.5 ± 1.1*** | 99 ± 6.3*** |
| LVEDD [mm²] | 26.5 ± 0.7 | 35 ± 1.5** | 39.5 ± 0.8*** | 76 ± 6.0*** |
| EF [%] | 54.5 ± 0.9 | 45.2 ± 0.7*** | 41.5 ± 0.5*** | 23.2 ± 2.8*** |
| LVEDP [mm Hg] | 6.0 ± 0.3 | 5.8 ± 0.3 | 6.8 ± 0.3 | 21.1 ± 0.5*** |

BW - body weight, LVEDD - left ventricular end-diastolic diameter, LVEDP - left ventricular end-diastolic pressure.

Data are presented as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001.
Figure 2 (See legend on next page.)
significantly enriched GO terms, while the DAVID Functional Annotation Tool identified several categories. The molecular function of those genes is mainly connected to GO:0005488 binding, especially GO:0005529 sugar and GO:0005537 mannose binding. Biological processes are associated with response to stimuli: GO:0014070 response to organic cyclic substance and GO:0009607 response to biotic stimulus (GO:0009617 response to bacterium stimuli, GO:0051007 response to other organism). Cellular component classification showed that most of those genes were connected with GO:0016020 membrane. Complete results are shown in Additional file 6.

A comparison of the list of differentially expressed genes in LV tissue and PBMCs from rats with L-MI revealed five transcripts (Klra7 - killer cell lectin-like receptor, subfamily A, member 7; Clr7 - C-type lectin-related protein 7; Cp - ceruloplasmin; Ptgs2 - prostaglandin-endoperoxide synthase 2 and Tspan12 - tetraspanin 12) similarly altered in both tissues (Additional file 7).

Validation of microarrays with RT-qPCR

Reverse transcription-qPCR was used to validate the microarray data. Several genes important for heart functioning were assayed and the results are demonstrated in Tables 2 and 3.

First, to verify the main conclusion drawn from the microarray results for LV samples, the expression levels of genes coding for cardiac neurohormones (Nppa - natriuretic peptide A precursor, Nppb - natriuretic peptide B precursor, Edn1 - endothelin 1) and the enzyme responsible for converting pro-ANP and pro-BNP to mature ANP and BNP (Corin - corin, a serine peptidase) were determined. We confirmed that those genes were upregulated in the L-MI group in contrast to the S-MI and M-MI groups,

Figure 2 Enriched GO categories among differentially expressed genes in large-size infarction compared to sham-operated rats.

Expression level were determined in left ventricle. Graphs show top results for the biological process (A), molecular function (B) and cellular component (C). In round brackets the p-value obtained from the AmiGO is shown, in square brackets the number of differentially expressed genes related to a particular GO term.

Figure 3 Heat map representing hierarchical clustering of 73 differentially expressed genes related to extracellular matrix (GO:0031012). Each column corresponds to a single microarray whereas each row represents expression profile of a single gene. Red stand for the positive values in the gene expression and green for the negative ones. Sham - sham-operated; S-MI - small-size infarction; M-MI - moderate-size infarction; L-MI - large-size infarction.
which indicated that only in the L-MI group the myocardial infarction progressed to heart failure.

We also tested transcript levels of other genes known to be associated with cardiac hypertrophy and left ventricular remodelling. Expression of these transcripts was altered significantly in our model only in rats with a large MI.

Further, changes in the expression level of genes connected with cardiac extracellular matrix were confirmed. In addition to the genes whose upregulation was indicated by microarray data, additional matrix metalloproteinase and tissue inhibitor of metalloproteinase genes (Mmp9, Mmp13, Mmp16, Mmp23, Timp3, Timp4) were checked. The levels of the Mmp9 and Mmp13 transcripts were close to the detection limit of the platform (Cq values around cycle 35) in sham-operated, S-MI, and M-MI rats, but were substantially higher (cycle 32 and 29 respectively) in the L-MI group, indicating their up-regulation after large infarction.

Overall, the RT-qPCR results were qualitatively consistent with the results of the microarray analysis. However, the RT-qPCR analysis tended to give higher up-regulation levels than those calculated from the microarray data. The highest change was found for peristin (Postn), osteopontin (Spp1) and thrombospondin 4 (Thbs4) (20.8, 52.3, and 19.4 -fold, respectively).

In addition, RT-qPCR was used to quantitate the five transcripts found earlier to be differentially expressed following HF in both heart and blood. The results are shown in Table 3 for L-MI and in Additional file 8 for all examined MI groups. Overexpression of ceruloplasmin (Cp) was significant ($P<0.05$) in both tissues, whereas significant downregulation of tetraspanin 12 (Tspan12) was confirmed in PBMCs but not in LV.

**Discussion**

The transition from LV injury to heart failure is poorly understood. Only a small percentage of patients develop heart failure immediately after MI, but with time many more eventually progress to heart failure. What triggers this process remains a matter of speculation. Therefore it could be of great clinical relevance to understand the differences between stable compensated LV injury and heart failure at the gene expression level and to identify potential triggers of the decompensation of compensated LV injury.

The rat MI model has been used extensively to study the pathophysiology of cardiovascular disease owing to its good reproducibility and similarity to the course of the human disease [21]. It has previously been shown in this model that large MI results in progressive LV remodelling...
Table 2 Comparison of results from microarrays and RT-qPCR

| Gene symbol | Large-MI | | Moderate-MI | | | Small-MI | |
|-------------|---------|---|---------|---|---|---------|---|
| | Results from RT-qPCR | Results from microarrays | | Results from RT-qPCR | Results from microarrays | | Results from RT-qPCR | |
| | Fold change | P-value | Significance | Fold change | P-value | Significance | Fold change | P-value | Significance | Fold change | P-value | Significance |
| End1 | 1.9 | 0.043 | * | 1.3 | 0.005 | *** | −1.1 | 0.412 | ns | −1.3 | 0.147 | ns |
| Nppa | 19.2 | 0.001 | *** | 3.7 | 1.3e-5 | *** | 1.1 | 0.884 | ns | 1.2 | 0.694 | ns |
| Nppb | 2.2 | 0.001 | *** | 1.4 | 0.14 | ns | −1.1 | 0.849 | ns | −1.5 | 0.496 | ns |
| Others related to cardiac hypertrophy and left ventricular remodelling | | | | | | | | | | | | |
| Corin | 2.6 | 0.020 | * | 1.4 | 3e-5 | *** | 1 | 0.788 | ns | 1 | 0.934 | ns |
| Hcn4 | 3.6 | 0.013 | * | 2.1 | 0.001 | *** | 1 | 0.951 | ns | −1.4 | 0.343 | ns |
| Pparg | −1.7 | 0.017 | * | −1.4 | 1.4e-4 | *** | 1.1 | 0.643 | ns | 1 | 0.865 | ns |
| TgfB1 | 1.4 | 0.071 | ns | 1.3 | 1.6e-5 | *** | 1.1 | 0.613 | ns | 1.1 | 0.712 | ns |
| TgfB2 | 3.1 | 0.010 | ** | 2 | 7.8e-5 | *** | −1.1 | 0.762 | ns | 1 | 0.874 | ns |
| ECM – Extracellular matrix components | | | | | | | | | | | | |
| Col1a1 | 3.9 | 0.003 | ** | 2.3 | 6.1e-6 | *** | 1 | 0.767 | ns | 1 | 0.852 | ns |
| Col1a2 | 2.3 | 0.011 | ** | 1.6 | 5.1e-5 | *** | −1.1 | 0.685 | ns | 1 | 0.852 | ns |
| Col3a1 | 5.1 | 0.001 | *** | 2.1 | 3.1e-8 | *** | −1.1 | 0.579 | ns | 1.1 | 0.619 | ns |
| Col8a1 | 2.4 | 0.001 | *** | 2 | 1.0e-7 | *** | 1.1 | 0.628 | ns | 1.1 | 0.891 | ns |
| Col18a1 | 2.2 | 0.001 | *** | 1.3 | 7.6e-5 | *** | −1.1 | 0.668 | ns | −1.1 | 0.310 | ns |
| Eln | 2.2 | 0.001 | *** | 1.4 | 1.8e-5 | *** | 1.2 | 0.304 | ns | 1.1 | 0.689 | ns |
| Fn1 | 3.8 | 0.005 | ** | 2.9 | 6.2e-8 | *** | 1.2 | 0.384 | ns | 1.1 | 0.522 | ns |
| Lamc1 | 1.3 | 0.014 | ** | 1.3 | 5.1e-7 | *** | 1.1 | 0.451 | ns | 1 | 0.773 | ns |
| Prg4 | 2.1 | 0.004 | ** | 1.7 | 2.4e-5 | *** | 1 | 0.907 | ns | −1.1 | 0.430 | ns |
| Vtn | −1.4 | 0.015 | * | −1.4 | 2.3e-6 | *** | 1 | 0.874 | ns | −1.1 | 0.167 | ns |
| Matricellular proteins | | | | | | | | | | | | |
| Mmp2 | 1.6 | 0.001 | *** | 1.4 | 7.5e-7 | *** | 1 | 0.855 | ns | 1.1 | 0.343 | ns |
| Mmp9 | 2.7 | 0.351 | ns † | 1.73 | 0.037 | * | 1.2 | 0.660 | ns | 1 | 0.871 | ns |
| Mmp13 | 62.2 | 0.001 | *** † | 2 | 0.021 | * | −1.2 | 0.358 | ns | 1 | 0.862 | ns |
| Mmp14 | 2.3 | 0.019 | ** | 1.3 | 0.002 | ** | 1.1 | 0.529 | ns | −1.1 | 0.465 | ns |
| Mmp16 | 2.9 | 0.020 | ** | 1.3 | 0.006 | *** | −1.4 | 0.204 | ns | −1.3 | 0.334 | ns |
| Mmp23 | 1.8 | 0.027 | ** | 1.3 | 1.0e-4 | *** | −1.1 | 0.572 | ns | −1.2 | 0.438 | ns |
Table 2 Comparison of results from microarrays and RT-qPCR (Continued)

| Gene  | Ratio | P-value | Log2 Fold Change | P-value | NORM | Ratio | P-value |
|-------|-------|---------|------------------|---------|------|-------|---------|
| Postn | 20.8  | 0.001 ** | 8  | 1.2e-9 ** | 1 | 0.988 ns  | 1.1 | 0.738 ns |
| Spp1  | 52.3  | 0.003 ** | 4.1 | 2.3e-5 ** | 2.7 | 0.157 ns  | 2.3 | 0.212 ns |
| Thbs4 | 19.4  | 0.003 ** | 6.1 | 8e-9 ** | −1.7 | 0.272 ns  | −1.3 | 0.348 ns |
| Timp1 | 3     | 0.008 ** | 2.4 | 2.8e-7 ** | 1.2 | 0.287 ns  | 1.4 | 0.041 * |
| Timp2 | 1.7   | 0.037 ** | 1.5 | 4.6e-7 ** | −1.1 | 0.666 ns  | 1.0 | 0.936 ns |
| Timp3 | 1.9   | 0.043 *  | 1.2 | 7.8e-5 ** | −1.4 | 0.288 ns  | −1.3 | 0.321 ns |
| Timp4 | −1.5  | 0.151 ns | −1.4 | 0.006 ** | −1.3 | 0.307 ns  | −1.1 | 0.716 ns |

Expression level of selected genes was measured 2 months after operation in left ventricle tissues of all infarcted rats and compared to sham-operated group (statistical significance: *P < 0.05, **P < 0.01, ***P < 0.001, ns = non significant, † expression level in sham-operated rats close to the limit of detection). Results were normalized to Gapdh and Hprt.
and periostin. We confirmed a significant up-regulation of Thbs4, Edn1, and Klrk1 in the L-MI group, while moderate or small MI resulted in compensated LV injury. The transcripts altered in the L-MI group were related to diverse GO categories, of which the most striking was upregulation of extracellular matrix genes. It is well established that an imbalance between ECM components and disturbances in collagen metabolism are crucial in the progression of heart failure [30,31]. Therefore, detailed understanding of the alterations of key matrix proteins is essential in deciphering the mechanisms of LV remodelling after MI.

Matrix metalloproteinases (MMPs), also known as matrixins, are proteinases crucial in both normal tissue remodelling (development, wound healing) and in pathological conditions (arthritis, scar formation after MI, LV remodelling). There is ample evidence that MMPs modulate myocardial remodelling underlying heart failure progression [32,33]. From our results, Mnbp2, Mnp14, Mmp16 and Mmp23 appeared to be constitutively expressed in the heart and overexpressed in volume overload conditions, whereas Mnbp9 and Mnp13 transcripts were abundant only in the failing heart. To the best of our knowledge, this is the first report of an elevated expression of Mmp16 ("membrane type", synonym MT3-MMP) connected with heart failure. The interstitial tissue homeostasis is determined by an interplay between MMPs and their tissue post-translational inhibitors (TIMP). TIMPs not only bind the active MMP domain, but can also interact with the pro-MMP domain which can facilitate MMP activity [33]. MMP/TIMP profiles can be informative in the context of HF. Our study showed significant overexpression of Timp1, -2 and -3, similar to the end-stage human heart failure [31]. An upregulation of Timp1 was also found in studies on the transition from hypertrophy to HF in rats [34], and an increased serum level of TIMP1 protein was related with HF progression [35] and mortality risk [36].

Peripheral blood mononuclear cells (PBMCs) are more easily accessible than a tissue biopsy. Numerous studies have shown that circulating blood cells are involved in the pathogenesis of many diseases and may serve as biomarkers of pathological changes occurring in other tissues [17,37]. PBMCs also contribute to LV remodelling by expressing key proteins, such as matrix metalloproteinase

| Gene symbol | GenBank ID | Results from RT-qPCR | Results from microarrays | Results from RT-qPCR | Results from microarrays |
|-------------|------------|----------------------|-------------------------|----------------------|-------------------------|
| Chr7        | EU128749.1 | 1.4                  | 0.129**                 | 1.6                  | 0.014*                  |
| Cp          | NM_012532  | 2.3                  | 0.039*                  | 1.2                  | 0.019*                  |
| Klrk1       | XM_578407.4| 1.6                  | 0.112**                 | 1.4                  | 0.048*                  |
| Ptgs2       | NM_017232  | 2.4                  | 0.073**                 | 1.3                  | 0.037*                  |
| Tspan12     | NM_001015026| -3.22                | 0.019*                  | -3.0                 | 0.011*                  |

Statistical significance: *P < 0.05, **P < 0.01, ***P < 0.001, ns = non significant, near = near significance. Results were normalized to Gapdh and Hprt.
In the present study we did not observe significant changes in the expression of MMPs or TIMPs in PBMCs (data not shown), but found other gene alterations of interest.

Our analysis indicated five HF-affected genes in the both examined tissues, but only one - ceruloplasmin (Cp) - showed statistically significant changes in expression (Table 3). Ceruloplasmin is a plasma metalloprotein synthesized in hepatocytes and in activated monocytes/macrophages and secreted into the serum, with a copper ion incorporated during biosynthesis. It acts as an antioxidant through its ferroxidase activity and also regulates nitric oxide homeostasis in the blood plasma by converting NO to NO$_2$, thus an elevated level of CP may lead to decreased NO bioavailability and endovascular dysfunction [38]. Ceruloplasmin level increases in various acute inflammatory conditions, including acute graft-versus-host-disease (aGvHD) after allogeneic hematopoietic stem cell transplantation [39], cancer [40], and cardiovascular disease [41]. Recent studies indicate serum CP as an independent cardiac risk factor [42] and show its association with heart failure [43,44]. Our results strongly support those findings, since large infarction in rats raised the level of Cp mRNA in the heart and PBMCs. We showed that both PBMCs and failing myocardium could be a major source of ceruloplasmin that is subsequently found in the serum of patients with heart failure. Accordingly, we propose that CP is important in the progression of heart failure due to its property of an acute phase protein, antioxidant function and regulation of nitric oxide homeostasis.

Another interesting gene responding to HF was tetraspanin 12 (Tspan12). In contrast to Cp, it was downregulated substantially in the PBMCs but showed no response in the heart tissue. Members of the tetraspanin superfamily regulate key cellular processes such as adhesion, migration, and fusion. Tetraspanins play particularly important roles in cancer, by modulating tumor cell growth. Tetraspanins...
are expressed in vascular and haematopoietic cells and are involved in both physiological and pathological processes related to angiogenesis, vascular injury, thrombosis, and haemostasis. Previous studies suggest that tetraspansins directly regulate the development and functioning of the vascular system and the pathogenesis of vascular diseases [45]. In addition, mutations in the Tspan12 gene are associated with familial exudative vitreoretinopathy, a human disease caused by failure of peripheral retinal vascularization. Our results for the first time linked directly Tspan12 downregulation with heart failure development. To better understand the biological relevance of this finding we performed an Ingenuity Pathway Analysis of the molecular interaction networks for the genes differentially expressed in PBMCs. The major functional network with the highest score (IPA score = 47) and with 20 up- or downregulated genes, including Tspan 12, was associated with Cardiovascular System Development and Function, Cellular Function and Maintenance, Cellular Growth and Proliferation (Figure 5). This network analysis indicates that altered expression of tetraspanin 12 and other identified molecules may result in a dysfunction of the cardiovascular system by disturbance of their direct and indirect functional interactions. Moreover, tetraspanin 12 was found recently to regulate the activity of membrane metalloproteinases, MT1-MMP/MMP14 [46] and ADAM10 (disintegrin and metalloproteinase domain-containing protein 10) [47]. Affected functions of MT1-MMP/MMP14 influence fibronectin proteolysis and extracellular matrix degradation. ADAM10 is responsible for the proteolytic release of the ectodomains of numerous protein substrates, including specific ligands of EGFR (epidermal growth factor receptor) regulating the subsequent EGFR-dependent signal transduction pathways involved in cardiac hypertrophy [48]. Interestingly, ADAM10 mediates also cleavage of corin that may be important in regulating corin activity and hence ANP processing [49].

On the other hand, it is well known that heart remodeling after myocardial infarction, especially after a large one, is accompanied by extensive neurohumoral activation. An increased plasma norepinephrine concentration due to a marked increase in the sympathetic nerve activity is a common finding in experimental heart failure models as well as in humans. Moreover, the increased wall stress after myocardial infarction and increased plasma norepinephrine level result in the activation of the renin-angiotensin-aldosterone system (RAAS) followed by an increase of the renin and angiotensin II plasma levels. Additionally, increased levels of vasopressin and endothelin have been found in heart failure. [50]. It is possible that these neurohormones circulating in the plasma could influence the expression of diverse genes in PMBCs through activation of the receptors present on the surface of these cells. Besides, PMBCs are mononuclear cells recruited into the infarcted myocardium [15] and the invading monocytes differentiate into macrophages also during tissue repair after myocardial infarction [51]. We therefore hypothesize that the altered expression of Tspan12 in PBMCs may influence the pathological processes occurring in the failing heart, especially that tetraspansins are present not only on the plasma membrane but also in exosomes, which are often released by cells as messenger vehicles for intercellular communication [52].

Conclusions

We showed that a large size of infarcted area is critical for progression of LV remodelling and HF development, associated with altered gene expression in the heart. In contrast, compensated LV injury does not lead to significant changes of the transcriptome as evaluated two months after the injury. Transcriptional profiling of failing myocardium and peripheral blood mononuclear cells simultaneously is a useful approach for robust markers of heart failure. We identified genes (Cp, Tspan12) whose expression is altered in the readily obtainable blood cells in response to progressive heart failure. To our knowledge, this is the first report on a possible association of Tspan12 expression with HF development.

Additional files

- Additional file 1: Primer sequences and parameters used to validate the microarray analysis by RT-qPCR.
- Additional file 2: Transcripts differentially expressed in LVs between rats with large-sized infarction and sham-operated ones.
- Additional file 3: AmiGO Term Enrichment analysis of differently expressed transcripts in LVs between rats with large-size of infarction and sham-operated ones.
- Additional file 4: Differentially expressed genes related to GO:0031012: extracellular matrix.
- Additional file 5: Transcripts differentially expressed in PBMCs between rats with large-size of infarction and sham-operated ones.
- Additional file 6: Chart report from DAVID analysis to determine GO term enrichment of BP (biological process), MF (molecular function) and CC (cellular component) and their associated genes differentially expressed in PBMCs between L-MI and sham operated group.
- Additional file 7: Genes that were altered similarly in LVs and PBMCs - microarray results.
- Additional file 8: Comparison of results from RT-qPCR of genes that were altered similarly in LVs and PBMCs.

Competing interests

The authors declare that they have no competing interests.

Author contributions

MG, BB, UM and MM conceived and designed the experiments. UM, MM carried out the animal work and co-wrote the paper. MG and BB performed the microarray experiments. DT and AM performed the RT-qPCR experiments. DT analyzed the data and wrote the paper. MG, BB critically reviewed the manuscript. All authors read and approved the final manuscript.
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