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Original article

Functional genomic fabrics are remodeled in a mouse model of Chagasic cardiomyopathy and restored following cell therapy

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Abstract

We previously found that, in a mouse model of Chagas cardiomyopathy, 18% of the 9390 quantified unigenes were significantly regulated by *Trypanosoma cruzi* infection. However, treatment with bone marrow-derived mononuclear cells (MNCs) resulted in 84% transcriptomic recovery. We have applied new algorithms to reanalyze these datasets with respect to specific pathways [Chagas disease (CHAGAS), cardiac muscle contraction (CMC) and chemokine signaling (CCS)]. In addition to the levels of expression of individual genes we also calculated gene expression variability and coordination of expression of each gene with all others. These additional measures revealed changes in the control of transcript abundances and gene networking in CHAGAS and restoration following MNC treatment, not accessible using the conventional approach limited to the average expression levels. Moreover, our weighted pathway regulation analysis incorporated the contributions of all affected genes, eliminating the arbitrary cut-off criteria of fold-change and/or p-value for significantly regulated genes. The new analyses revealed that *T. cruzi* infection had large transcriptomic consequences for the CMC pathway and triggered a huge cytokine signaling. Remarkably, MNC therapy not only restored normal expression levels of numerous genes, but it also recovered most of the CHAGAS, CMC and CCS fabrics that were altered by the infection.

Keywords: American trypanosomiasis; Chagas disease; Gene expression; Gene networks; Pathway restoration

1. Introduction

Life-long persistent infection with *Trypanosoma cruzi* results in clinically significant chronic Chagas cardiomyopathy (CCC) in about 30% of infected individuals, manifested by congestive heart failure (CHF), arrhythmias, thrombo-embolic events and apical ventricular aneurysm [1]. While endemic in several areas of Latin America, CCC has now gone global due to migration of infected individuals [2]. Myonecrosis, myocytolysis, inflammation and extensive interstitial fibrosis are observed [3−7]. Replacement of myocytes and/or vascular cells by fibrotic tissue [8,9] results in myocardial thinning and hypertrophy of the surviving cardiac myocytes. In many aspects, CCC is not different from other dilated cardiomyopathies. Therefore, optimizing therapy based on stem cell transplant and administration of novel anti-inflammatory agents in CCC is significant not only for this specific disease

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occurring mainly in Latin America, but to a whole range of dilated cardiomyopathies and congestive heart failures affecting almost 5 million people in the US.

Current therapeutic strategy for CCC is inadequate, using drugs and devices to modulate symptoms of CHF rather than targeting the underlying etiology. For many patients, heart transplantation is the only available cure, despite the problems associated with donor shortage, immune rejection and exacerbation of the infection. Alternative therapies are therefore needed, and use of cell therapy has emerged as an exciting novel strategy [10–13].

Studies on animal models of cardiac diseases have provided striking evidence that cell therapy improves cardiac function and survival through repair and regeneration of damaged myocardium [14]. Clinical studies suggest that transplantation of mesenchymal stem cells from various sources leads to cardiac rehabilitation, improved left ventricular function, reverse remodeling, and decreased scar size in myocardial infarction [15,16].

Our studies of bone marrow-derived mononuclear [12,13,17,18] and mesenchymal cell therapy [19] in experimental murine Chagas disease [20,21] indicated substantial functional and structural improvement of the heart. Moreover, the use of expression microarrays revealed that the cell therapy was remarkably successful in restoring pre-infection expression levels of individual genes [18]. Initial inspection of the datasets indicated that certain pathways were particularly vulnerable to infection and raised the possibility that focused evaluation of changes in relationships among pathway components might promote mechanistic understanding of underlying changes. We have reanalyzed the original datasets using new algorithms applied to components of functional pathways defined by the Kyoto Encyclopedia of Genes and Genomes (http://www.kegg.jp). This analysis reveals unexpected linkages among expression of genes within pathways, suggesting that manipulated changes in expression of key genes might effectively bypass pathway blockage in pathological conditions.

2. Materials and methods

2.1. Mice

The original dataset was obtained from male 4 week old C57Bl/6 mice that were infected or not with trypomastigotes of the Colombian strain of *T. cruzi* [22] obtained from culture supernatants of infected LCC-MK2 cells as previously described [21] to obtain control (CTR group) and infected (INF) animals. Six months after infection, mice were injected intravenously with either bone marrow mononuclear cells (MNC) obtained from femurs and tibiae of C57BL/6 mice (INF + TRE group) or saline alone (INF + SAL group) [18]. Four not infected mice were also subjected to MNC treatment (CTR + TRE group). Mice of CTR, INF + SAL, CTR + TRE and INF + TRE groups were sacrificed 8 months after infection and hearts removed and quickly frozen and stored in liquid nitrogen until RNA extraction using the method as described in Ref. [23].

2.2. Microarray data

Microarray data obtained from 4 hearts of each group, deposited in https://www.ncbi.nlm.nih.gov/geo as GSE17363 (included in Ref. [21]) and as GSE24088 (included in Ref. [18]) were used in this re-analysis.

2.3. Data analyses

2.3.1. Weighted Pathway Regulation (WPR)

We here introduce a novel metric to assess whether a particular pathway (denoted by Π) was significantly regulated in hearts of mice of condition α (= INF + SAL, INF + TRE) with respect to that of their control (CTR) counterparts. WPR assigns to each gene a SPECIFIC WEIGHT proportional to its normal (control) average expression, net fold change and statistical relevance of its regulation.

$$\mathrm{WPR}_{\Pi}^{(\alpha)} = \langle \mu_i^{(\mathrm{CTR})} \left(\left| x_i^{(\alpha)} \right| - 1 \right) \left(1 - p_i^{(\alpha)} \right) \rangle_{i \in \Pi}, \quad (1)$$

where:

$\langle\rangle$ = median of the computed values for individual gene values within the pathway
$\mu_i^{(\mathrm{CTR})}$ = average expression level in control samples
$x_i^{(\alpha)}$ = fold-change (negative for down-regulation) of gene i in condition α (= INF + SAL, INF + TRE)
p = Bonferroni-type corrected p-value of the heteroscedastic t-test

As previously described [24], the p-values were computed with a Bonferroni-type correction for each set of spots probing redundantly the expression of the same gene. This approach is a reasonable statistical compromise between the less conservative Benjamini–Hochberg FDR and the too stringent Bonferroni correction of the entire data set. Note that the pathway-specific gene ensemble is analyzed together and that the arbitrary cut-offs of fold-change (typically 1.5×) and/or p-value (typically 0.05) are no longer applied. Thus, WPR not only takes into account ALL pathway genes are but each gene has a specific weighted contribution. This is an important departure from the common approach of determining the percentage of significantly regulated genes without discriminating their levels of regulation.

2.3.2. Pathway Restoration Efficiency (PRE)

We previously introduced the parameter TRE (transcriptomic recovery efficacy) to evaluate the extent to which a treatment restores normal gene expression levels after a perturbing event such as Chagasic cardiomyopathy [18] or myocardial infarction [25]. TRE considered both the percentage of regulated genes in infected mice for which expression level was restored to normal levels following the treatment α and the side effects (i.e. genes that were unregulated in infected but regulated in treated mice). We now introduce a novel ensemble measure, PRE (pathway

restoration efficiency) that is not restricted to the significantly regulated genes but incorporates all expression alterations by the treatment. PRE can be computed for a particular pathway Π as well as for the entire transcriptome:

$$PRE_{\Pi}^{(\Theta)} = \left(1 - \frac{WPR_{\Pi}^{(INF+TRE)}}{WPR_{\Pi}^{(INF+SAL)}}\right) \times 100\% \quad (2)$$

2.3.3. Expression Coordination Analysis

The transcriptomic networks in each of the three conditions (CTR, INF + SAL, INF + TRE) were established by computing pair-wise Pearson correlation coefficients of the (log_2) expression levels of each pair of pathway genes in the biological replicas. As in previous papers (e.g.: [26,27]), we considered only the (p-value < 0.05) significant synergistic, antagonistic and independent expressions.

2.3.4. Pathway Network Alteration

We here define the Pathway ("Π") Network Alteration $PNA_{\Pi}^{(\alpha)}$ in condition α (SAL or TRE) samples with respect to their control counterparts as

$$PNA_{\Pi}^{(\alpha)} = \left| \frac{\sqrt{\sum_{i,j\in\Pi} \left(\rho_{i,j}^{(\alpha)} - \rho_{i,j}^{(CTR)}\right)^2}}{\sqrt{\sum_{i,j\in\Pi} \left(\rho_{i,j}^{(CTR)}\right)^2}} - 1 \right| \times 100\%, \quad (3)$$

α = INF + SAL, INF + TRE

$\rho_{i,j}$ = Pearson correlation coefficient between expressions of genes *i* and *j* in condition α

2.3.5. Transcriptomic landscape

We used our Pair Wise Relevance analysis [28–31] to determine the topology of the "transcriptomic landscapes" of the genomic fabrics associated to the three KEGG pathways (cardiac muscle contraction, Chagas disease and chemokine signaling) in each condition β (= CTR, SAL, TRE). The analysis reveals the contributions of all gene pairs that can be formed to the expression level, control and coordination of the fabric. In such a "landscape" each gene pair (*i,j*) is represented by a peak whose height (PWR) is proportional to their absolute expression coordination $\left|\rho_{i,j}^{(\beta)}\right|$ and relative (to the pathway median) expression levels (μ_i) and controls (constant/CV_i).

$$PWR_{i,j}^{(\beta)} = \frac{\langle (CV)_{\Pi}^{(\beta)} \rangle}{\langle \mu_{\Pi}^{(\beta)} \rangle} \sqrt{\frac{\mu_i^{(\beta)} \mu_j^{(\beta)}}{(CV)_i^{(\beta)} (CV)_j^{(\beta)}}} \left|\rho_{i,j}^{(\beta)}\right|, \quad (4)$$

μ = average expression level in condition β

CV = coefficient of variation in condition β

⟨⟩ = median of individual gene values within the pathway

3. Results

In order to visualize the impact of each condition at the functional level, we mapped the differences in gene expression between groups onto the KEGG-determined pathways of cardiac muscle contraction (CMC, http://www.genome.jp/kegg-bin/show_pathway?org_name=mmu&mapno=04260&mapscale=&show_description=hide), Chagas disease (CHAGAS, http://www.genome.jp/kegg-bin/show_pathway?org_name=mmu&mapno=05142&mapscale=&show_description=hide) and chemokine signaling (CCS, http://www.genome.jp/kegg-bin/show_pathway?org_name=mmu&mapno=04062&mapscale=&show_description=hide).

Fig. 1 presents how Chagas disease KEGG-selected genes were regulated in hearts of infected mice with and without cell therapy with respect to the uninfected controls. When infected hearts were compared to controls, of the 103 genes in the Chagas disease pathway, 25 genes were not captured on the array, 8 (*Ccl3, Fasl, Gna15, Il1b, Il6, Pik3cd, Pik3cg, Tlr9*) were turned on (i.e. not detected in any of the controls but quantifiable in all infected hearts) and one (*Cflar*) was turned off, 31 were upregulated, two (*Akt2, Mapk10*) were down-regulated and 35 were not affected. When hearts of treated mice were compared to those of infected controls, all but one gene (*Ccl5*) turned on by infection was turned back off; it is especially notable that all 31 genes upregulated in infection were restored to normal levels in the treated group. Comparing treated not infected and control mice revealed that less than 1% of genes were affected by the treatment alone. Among others, *Fos* (FBJ osteosarcoma oncogene) was up-regulated and mitogen activated protein kinase kinase 4 (*Map2k4*) and myeloid differentiation primary response gene 88 (*Myd88*) were down-regulated.

MAPPFinder standardized difference scores between the observed and expected numbers of significantly regulated genes in the selected pathways under hypergeometric distributions were: $z_{CMC}^{(INF+SAL)} = 0.048$, $z_{CHAGAS}^{(INF+SAL)} = 0.377$, $z_{CCS}^{(INF+SAL)} = 1.647$ before treatment and $z_{CMC}^{(INF+TRE)} = -1.270$, $z_{CHAGAS}^{(INF+TRE)} = -1.054$, $z_{CCS}^{(INF+TRE)} = -0.962$ after treatment. The positive values in INF + SAL indicate that the analyzed pathways experienced larger than average regulation at the level of the entire transcriptome, while the negative values in INF + TRE indicate the substantial recovery of these pathways after the treatment.

We compared the alterations of Chagas disease, CMC and CCS pathways in INF + SAL mice and INF + TRE mice using both percentage of regulated genes (Fig. 2A) and WPR (Fig. 2B) with respect to CTR mice. Both measures indicated that the overall alteration of the analyzed pathways was much lower in hearts of the infected mice treated with stem cells than in the INF + SAL. In spite of the largest percentage of regulated genes found for CCS pathway, WPR revealed that actually the highest degree of dysregulation occurred in the CMC pathway, consistent with the cardiomyopathic nature of Chagas disease.

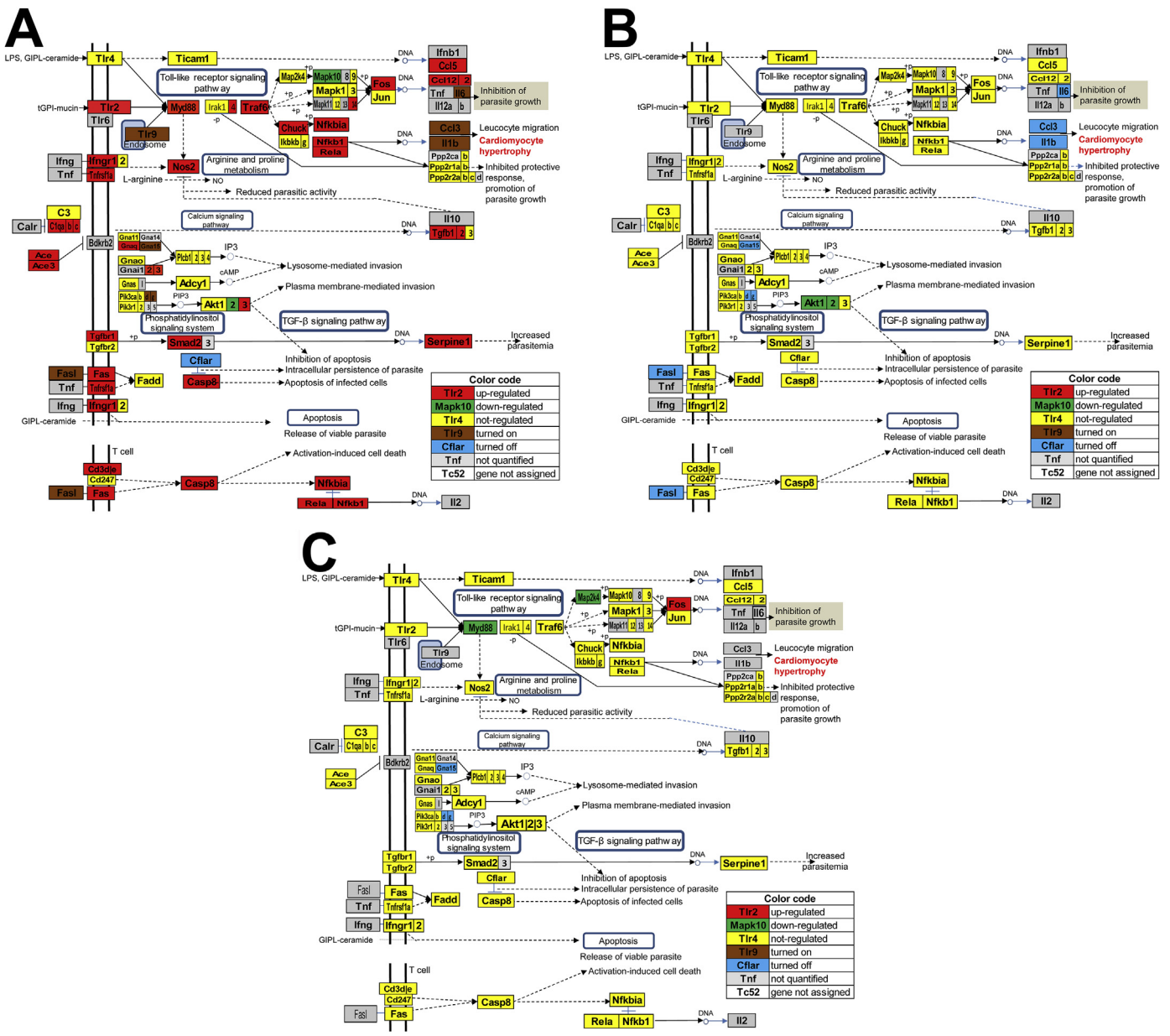


Fig. 1. KEGG-determined mouse Chagas disease pathway (CHAGAS) indicating the genes regulated by *T. cruzi* infection and recovered by bone marrow-derived mononuclear cell treatment (data from Refs 50 and 51). With respect to control animals, gene symbol background color indicates whether that gene was up- (red), down-(green) or not (yellow) regulated, while dark orange that it was turned on by infection and light blue turned back off by cell therapy. A. Gene expression consequences of *T cruzi* infection (comparison of heart samples from INF + SAL and CTR mice). Note the high numbers of regulated genes (red: up; green: down) B. Gene expression recovery after cell therapy (comparison of heart samples from INF + TRE group and CTR group). Here, turned on/off indication indicates how the treatment modified the status of that gene in infected mice. Thus, *Ccl3* turned on by the infection is turned off back by the treatment. C. Gene expression alteration in not infected mice subjected to the cell therapy (CTR + TRE) with respect to CTR mice.

We then determined the transcriptomic recovery following the treatment and found that almost 90% of genes (Fig. 2C) restored their normal expression levels (100% for CMC genes). This very optimistic finding was somehow tempered by computing the (more realistic in our opinion) Pathway Restoration Efficiency (PRE, Fig. 2D). For each pathway, the degree of restoration toward control values was between 60 and 72%, with the Chagas pathway being more restored than the others. Overall restoration of the entire transcriptome was about 50% efficient.

In order to evaluate the advantages of WPR over the standard percentage of regulated genes, we analyzed their dependence on the arbitrarily introduced threshold to consider a gene as significantly regulated and the impact of the regulation magnitude and significance. Fig. 2E and F shows that percentage of regulated genes is affected while WPR stays the same when the fold-change cut off is switched from 1.5× to 2.0× or the p-value cut-off is changed from 0.05 to 0.01. In contrast, Fig. 2G and H shows that in the standard measure each regulated gene has the same

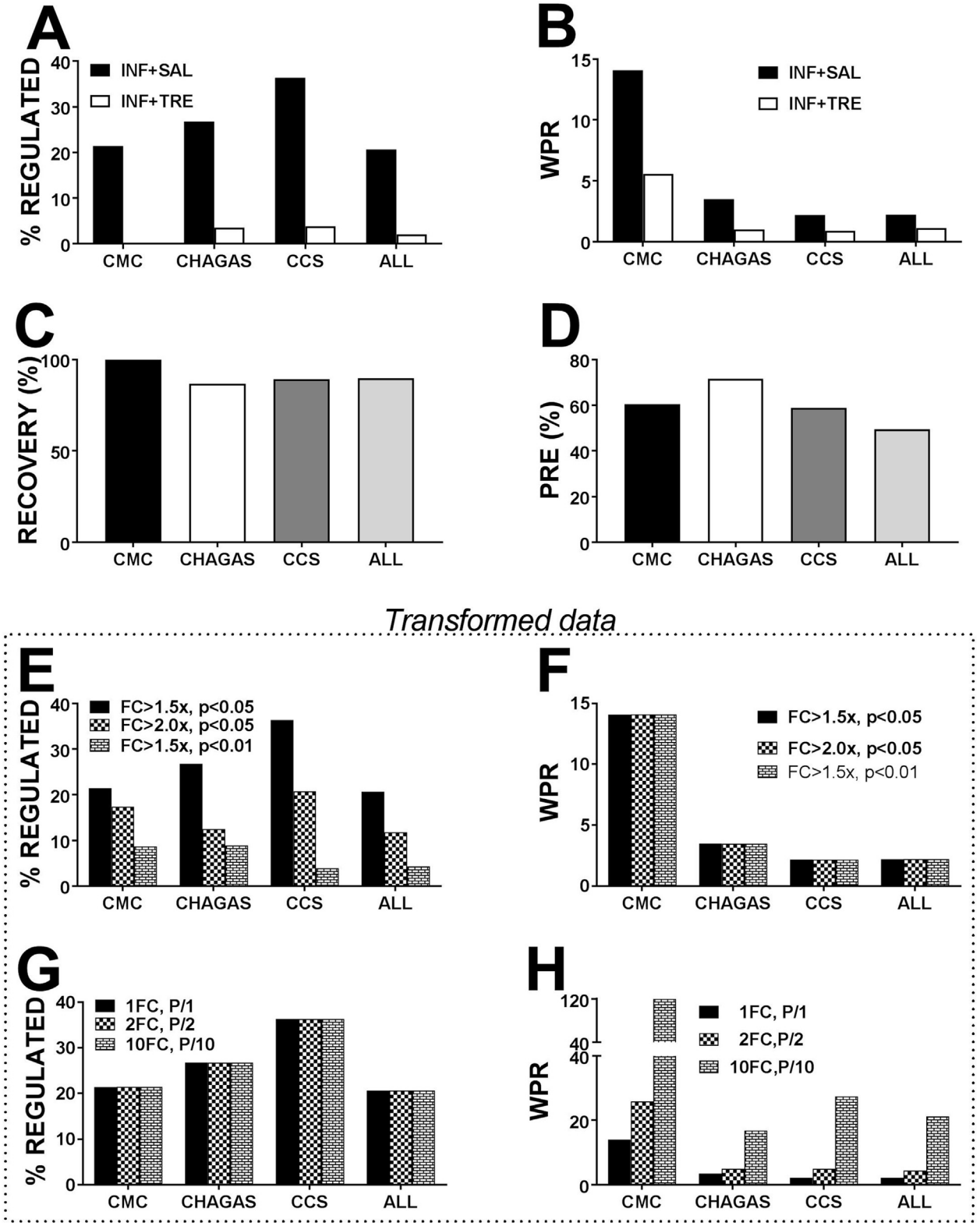


Fig. 2. Quantification of parameters assessing impact of *T cruzi* infection and cell therapy on selected KEGG pathways (cardiac muscle contraction, CMC; Chagas disease, CHAGAS; and chemokine signaling, CCS) and on the overall transcriptome. A. Percentage of significantly regulated genes in CMC, CHAGAS and CCS pathways and in the entire transcriptome (ALL) with and without cell treatment. Note the substantial reduction (by 100% for CMC) of the percentage of regulated genes. B. Weighted pathway regulation (WPR), a measure of how extensively the pathway genes were altered by each treatment compared to uninfected controls. Note that for each pathway WPR is substantially lower in the cell therapy group than in infected counterparts. C. Percentage of significantly regulated genes in INF + SAL condition that are no longer considered as significantly regulated in INF + TRE condition. Note the 100% recovery of CMC genes. D. Pathway restoration efficiency (PRE) of the analyzed pathways. Selected pathways recovered between 60 and 72% of their normal transcriptome. E–F. Simulated data showing that change of the (arbitrarily introduced) thresholds affects the percentage of regulated genes but not WPR. FC = absolute fold-change. Percentage of

contribution, while WPR incorporates the magnitude and significance of their regulation.

A prominent feature of gene networks is that expression of individual genes within the pathway is coordinated with that of other genes and these networks can be extensively remodeled under disease and treatment conditions. For example, in the Chagas disease pathway under control conditions the gene *Ace* (Angiotensin I converting enzyme (peptidyl-dipeptidase A) 1) is synergistically expressed with 10 other genes and antagonistically or independently expressed with no gene (Fig. 3). Following *T. cruzi* infection, the network is profoundly remodeled, such that gene *Ace* is synergistically expressed with 14 genes, antagonistically expressed with 3 genes (*Gna11, Gnao1, Gnas*) and independently expressed with 3 other genes (*Il6, Map2k4, Pik3r2*). In response to cell therapy, the network organization is partially restored as number of coordinated genes so that *Ace* is synergistically expressed with 11 genes without any antagonistically or independently expressed partner. Some of the normal expression coordinations (such are those with *Ifngr1, Pik3r2, Rela, Tnfrsf1a*) that disappeared in infection were restored after the treatment. Thus, the treatment of infected mice resulted into a (slightly) different but functional configuration of the transcriptome in which the overall coordination degree is similar to that of CTR although the coupled genes are not exactly the same.

As emphasized in previous papers [26,32,33], the coordination analysis in one condition predicts how the genes may be regulated under the next one. Thus, *Ace* and 9 out of its 10 synergistically expressed partners in CTR (*C3, Casp8, Gnai2, Ifngr1, Pik3r2, Rela, Tgfb2, Tgfbr2, Tnfrsf1a*) were similarly regulated (up) in INF + SAL. This is 90% accurate prediction. For the transition from INF + SAL to INF + TRE, the prediction was 100% accurate since the expression of *Ace* and of all its coordinately expressed partners were restored back to normal (although the independently expressed *Il6* was turned off).

Our finding that coordinated expression of genes within pathways is fundamentally altered in disease and following cell therapy raised the question of whether entire gene networks are coordinately expressed. To evaluate this issue, we created maps in which genes composing two pathways were compared, with common genes positioned as hubs between the networks. Fig. 4 presents the coordination of A) cardiac muscle contraction (CMC) genes with Chagas disease pathway genes and B) Chagas disease pathway with cytokine signaling (CCS) genes, under each experimental condition. In each case, networks were determined by identifying the genes that are synergistically or antagonistically co-expressed. Genes shared by distinct pathways may serve as operators by which one fabric modulates the other. For example, in INF + SAL samples the Chagas disease gene *Serpine1* that increases blood parasitemia [34] is synergistically expressed through the common node *Ppp2r2d* (that inhibits the immune response via T-cell proliferation with the sodium/hydrogen exchanger *Slc9a1,* essential for cardiac muscle contraction). Of note is the profound rearrangement of the gene networks in infected mice and the substantial recovery in response to the transplantation of bone marrow mononuclear cells. For instance, *Gnai3* (Guanine nucleotide binding protein, alpha inhibiting 3), common node Chagas disease and chemokine signaling pathways is synergistically expressed with *Gna11* (Guanine nucleotide binding protein, alpha 11) in control, antagonistically in INF + SAL and again synergistically in INF + TRE.

Fig. 5 presents the percentage of synergistically and antagonistically expressed gene pairs from Fig. 4 maps and the Pathway Network Alteration (PNA) for the analyzed pathways as well as for the entire transcriptome (ALL). Note in Fig. 5A–D that the treatment restored most of the initial percentage expression coordination. A common observation is that the infection antagonized the expression of numerous gene pairs (note the substantially larger percentage of antagonistically expressed pairs in INF + SAL compared the other two conditions) while the treatment restored their normal synergistic expression. Although the PNA induced by infection was significantly reduced by the treatment (e.g. from 37.5% to 18.5 for CMC), the gene networks after the treatment are still different than the control ones. Since the treated mice exhibited a significant (although not complete) recovery from the Chagas cardiomyopathy, these differences indicate the versatility of the cardiac gene networks in adapting the new conditions through multiple pathway remodeling. Thus, INF + TRE could be considered as a functional alternative to (CTR) physiological state.

The maps of intra- and inter-pathways transcriptomic networks revealed that coordination among gene expression remodeled with disease and partially recovered following cell therapy. A more comprehensive way to visualize the transcriptomic changes is to construct the Pair-Wise Relevant landscapes of the genomic fabrics. We show these landscapes for each of the pathways associated to cardiac muscle contraction, Chagas disease and chemokine signaling in the hearts of control, infected and treated mice (Fig. 6).

As expected, the fabrics of Chagas disease and chemokine signaling exhibited higher peaks in infected than in control mice, whereas PWR of the cardiac muscle contraction fabric in infected mice was almost flat. The flatness of CMC landscape indicates low expression coordination among muscle contraction genes in *T. cruzi* infection, consistent with Chagas disease being a severe heart condition. The treatment with bone marrow-derived MNCs had a remarkable restoring effect on each of the fabric landscapes. All these fabric topologies indicate that Chagas disease has massively pleiotropic effects

regulated genes changes when the threshold (FC > 1.5×, $p < 0.05$) is switched to (FC > 2.0×, $p < 0.05$) or to (FC > 1.5×, $p < 0.01$). G–H. Simulated data showing that WPR but not percentage of regulated genes is sensitive to changes of magnitude and statistical significance of expression regulation beyond the cut-offs. 1FC, P/1 = initial fold-changes and p-values, 2FC, P/2 = the fold-changes were doubled and the p-values divided by 2 for all regulated genes (and only for them), 10FC, P/10 = the fold-changes were increased 10 times and the p-values were divided by 10 for all regulated genes.

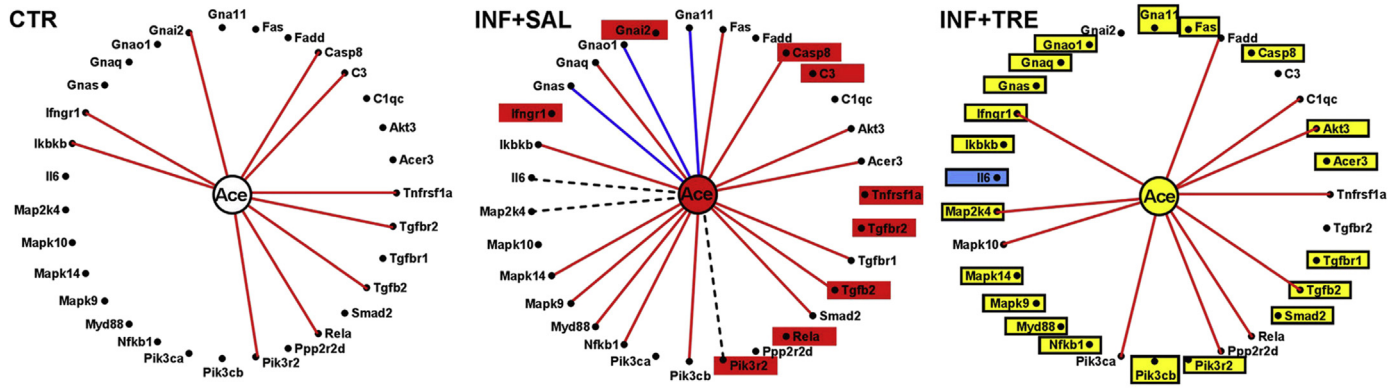


Fig. 3. Expression correlation of the Chagas pathway gene *Ace* (Angiotensin I converting enzyme (peptidyl-dipeptidase A) 1) with other genes of the pathway in the hearts of CTR, INF + SAL and INF + TRE mice. A continuous red/blue line indicates that the connected genes are synergistically/antagonistically expressed, while a dashed black line indicates that the two genes are independently expressed. Missing lines mean that for those gene pairs we have not enough evidence to asses neither (synergistic/antagonistic) coordination nor independence. Red/yellow background of a gene symbol indicates up-/no-regulation with respect to the control group of the genes coordinately expressed with *ACE* in the previous (CTR or INF + SAL) condition. Blue background indicates that expression of *Il6* was turned off. Note the profound change of synergistically and antagonistically expressed partners in INF + SAL and INF + TRE conditions.

and substantially increases the cytokine expression levels and coordination but causes a major decrease in cardiac function.

4. Discussion

4.1. Prior studies

Our studies of bone marrow-derived mononuclear [12,13,17,21] and mesenchymal cell therapy [19] in experimental Chagas disease [22,35,36] indicated substantial functional and structural improvement of the heart. Moreover, the use of expression microarrays as an unbiased measure of recovery revealed that the cell therapy was remarkably successful in restoring pre-infection levels of gene expression [18]. Despite this success in restoring function after treating mice with mononuclear cells, a double-blind multi-center randomized trial in patients with end-stage heart disease indicated that autologous mononuclear cell therapy provided no benefit beyond the positive effect of placebo [37]. The unsuccessful outcome of the clinical trial could have been due to a number of factors including clinical status of patients (severe end-stage disease), stem cell type used (mononuclear) and number transplanted (proportionally lower in patients than in mouse studies), route (intracoronary vessel) and timing of administration (not controlled with respect to disease onset in human patients).

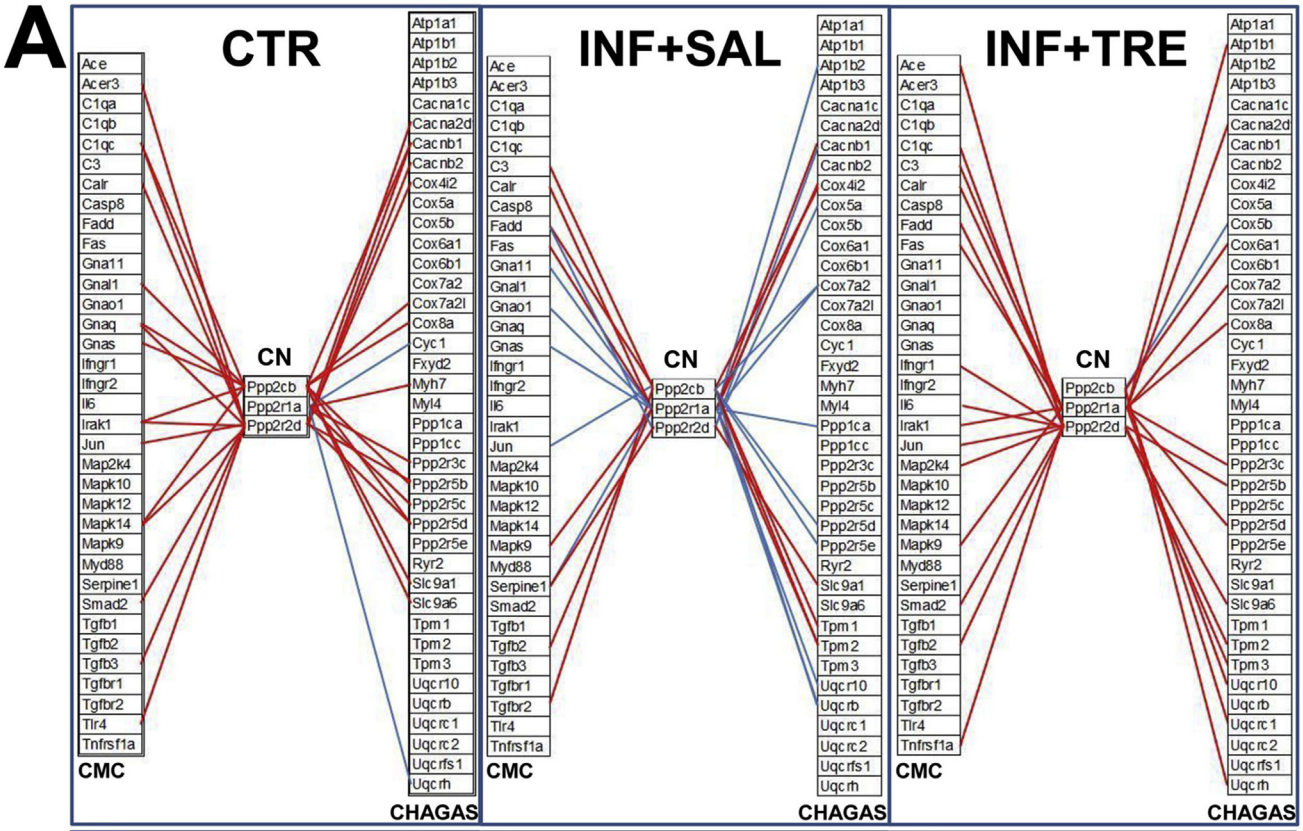
We believe that the fundamental issue, which is certain to impact therapy, is understanding the mechanisms by which cell therapy was so successful in our studies in the mouse model. One such mechanism likely involves reduction of inflammation/fibrosis, a hallmark feature of CCC. Our gene expression studies on cardiac tissues and cells from murine models of CCC [18,20–22,35,36,38] revealed extensive up-regulation of pro-inflammatory and fibrosis-related genes. Mononuclear cell therapy achieved a remarkable 84% restoration of normal gene expression patterns (including almost all pro-inflammatory and fibrosis genes, [12]). Notably, among the transcriptomic "side effects" (i.e. genes exhibiting expression alteration in treated but not in untreated CCC mice) was up-regulation of anti-inflammatory and other cytokine signaling suppressor genes such as *Socs2* [37].

Recent studies of cell therapy in heart disease have emphasized the role of cytokines in rescue and repair of damaged tissue through anti-inflammatory/anti-fibrotic pathways [38–45]. Such mechanisms include secretion of chemokines [46] and other paracrine factors that mobilize endogenous stem cell populations and ameliorate deleterious remodeling processes while enhancing beneficial mechanisms.

4.2. Extracting new insights from old datasets

In this report, we have re-analyzed previously published gene expression data [18,21] on a murine model of Chagas Cardiomyopathy (CCC) treated with bone marrow-derived mononuclear cells from the perspective of the genomic fabric paradigm (GFP; [47]). In previous papers [48–50], we have defined the genomic fabric as the transcriptome associated to the most interconnected and stably expressed gene network responsible for a particular functional pathway. GFP provides the most comprehensive approach of the transcriptome for it considers all three independent measures that can be obtained for each gene in a 4+ biological replicas experiment: average expression level, expression stability and expression coordination with each other gene. Thus, GFP makes visible aspects for which the conventional approach is blind. We interpret the gene networks constructed from the pairwise correlations to reflect "transcriptomic stoichiometry" [27] by which levels of proteins encoded by these genes are optimally maintained. The analysis from the GFP perspective revealed the massive alteration of the topology of major cardiac functional pathway caused by the *T. cruzi* infection and their substantial restoration in response to the cell treatment.

We grouped the genes in several KEGG-determined pathways of interest: cardiac muscle contraction, Chagas disease


A
CTR
INF+SAL
INF+TRE
CN
CMC
CHAGAS
Ace
Acer3
C1qa
C1qb
C1qc
C3
Calr
Casp8
Fadd
Fas
Gna11
Gnal1
Gnao1
Gnaq
Gnas
Ifngr1
Ifngr2
Il6
Irak1
Jun
Map2k4
Mapk10
Mapk12
Mapk14
Mapk9
Myd88
Serpine1
Smad2
Tgfb1
Tgfb2
Tgfb3
Tgfbr1
Tgfbr2
Tlr4
Tnfrsf1a
Ppp2cb
Ppp2r1a
Ppp2r2d
Atp1a1
Atp1b1
Atp1b2
Atp1b3
Cacna1c
Cacna2d
Cacnb1
Cacnb2
Cox4i2
Cox5a
Cox5b
Cox6a1
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Cox7a2
Cox7a2l
Cox8a
Cyc1
Fxyd2
Myh7
Myl4
Ppp1ca
Ppp1cc
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Ppp2r5b
Ppp2r5c
Ppp2r5d
Ppp2r5e
Ryr2
Slc9a1
Slc9a6
Tpm1
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Adrbk1
Adrbk2
Bcar1
Ccl11
Ccl6
Ccl8
Ccl9
Ccr5
Cdc42
Crk
Cxcl12
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Nfkbib
Nras
Pard3
Pf4
Prkaca
Prkacb
Prkcd
Ptk2
Pxn
RAC1
Rac2
Raf1
Rap1a
Rhoa
Rock1
Rock2
Shc1
Sos2
Stat1
Stat2
Stat3
Stat5b
Wasl
Akt1
Akt2
Akt3
Chuk
Gnai2
Gnai3
Ikbkb
Mapk1
Mapk3
Nfkb1
Nfkbia
Pik3ca
Pik3cb
Pik3r1
Pik3r2
Plcb3
Plcb4
Rela

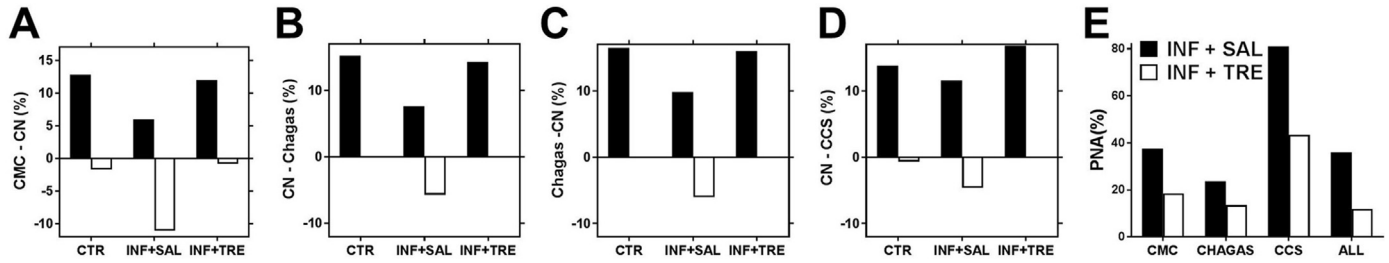

Fig. 5. Quantification of the remodeling and recovery of gene networks. (A–D) Percent of synergistically (positive black columns) and antagonistically (negative hallow columns) expressed gene pairs from Fig. 4 maps. Note that *T. cruzi* infection greatly affected the expression coordination of the analyzed subsets of genes and how similar the coordination percentages were between control and treated samples. E. Pathway Network Alteration (PNA) for the analyzed pathways as well as for the entire transcriptome (ALL). Note the substantial reduction of PNA in treated mice, albeit the differences with respect to controls are still large.

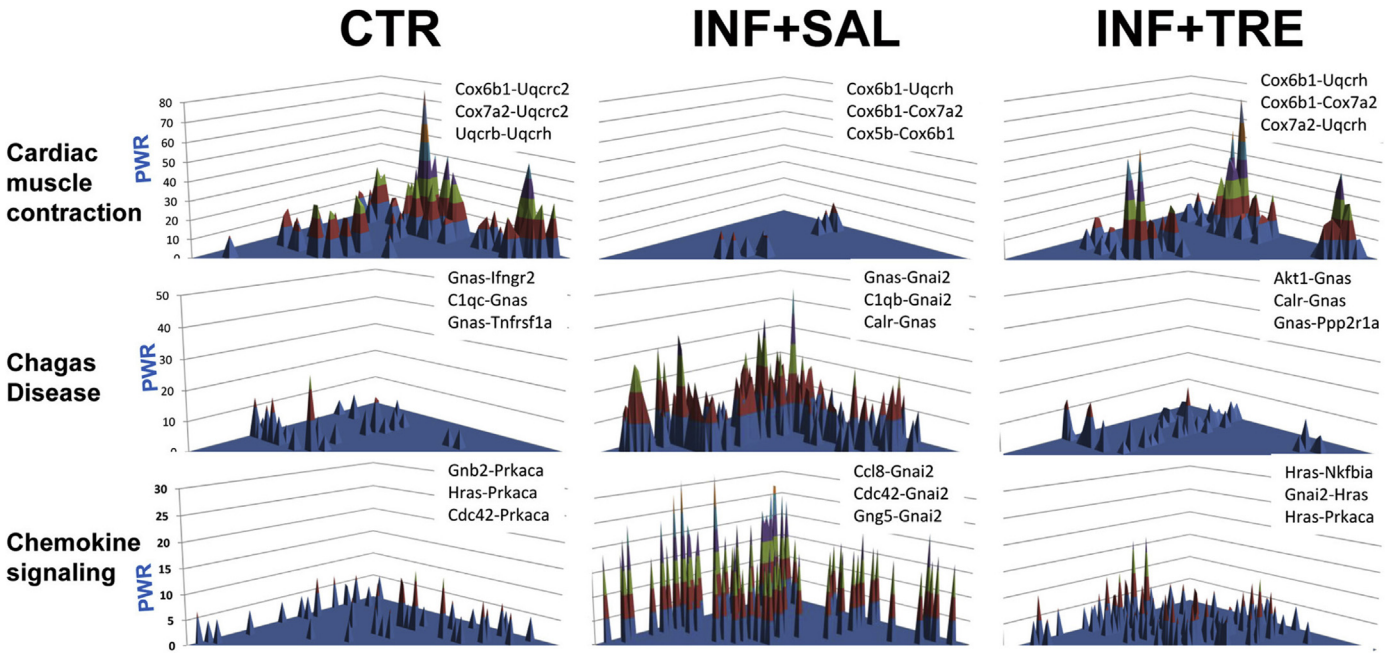

Fig. 6. Pair-Wise Relevance landscapes of the KEGG-determined functional pathways for Cardiac Muscle Contraction, Chagas Disease and Chemokine Signaling in hearts of CTR, INF + SAL and INF + TRE mice. Note that *T. cruzi* infection profoundly altered the landscapes of the three pathways and that the cellular treatment restored most of the original gene pair-wise relevance to control levels. PWRs in each condition were normalized to the maximum fabric value (for instance CMC scores were normalized to the PWR of *Cox6b1-Uqcrh* from treated samples). The most relevant three pairs were listed together with their percent PWR for each fabric in each condition.

and chemokine signaling. Alteration of these pathways in infected treated and untreated mice with respect to healthy counterparts and recovery following the treatment were quantified using two novel measures: the Weighted Pathway Regulation (WPR) and the Pathway Restoration Efficiency (PRE).

WPR goes beyond the metrics limited to the significantly regulated genes that treat all regulated genes as equals, regardless of their normal expression level and fold-change and statistical significance of their alteration. By choosing the median of the individual genes' alterations we were able to evaluate and compare the regulations of most pathway genes avoiding the bias introduced by the outliers.

Expression Coordination Analysis was completed with the Pathway Network Alteration (PNA) in infected/treated samples with respect to their control counterparts. This measure goes beyond counting the number of statistically significant synergistic and antagonistic expression coordination by quantifying the absolute coordination change for each gene pair. We found that, although the treatment mostly restored the

Fig. 4. *T. cruzi* infection altered the gene networks and the bone marrow-derived mononuclear cell treatment partially restored them. CN = connecting nodes. A. Expression coordination between KEGG-determined pathways of cardiac muscle contraction and Chagas disease through common gene nodes. B. Expression coordination between KEGG-determined pathways of Chagas disease and chemokine signaling thorough their common gene nodes. A red/blue line indicates ($p < 0.05$) significant synergistic/antagonistic expression coordination of the linked genes. Missing lines means that corresponding genes were not demonstrated to be coordinately expressed with p-value < 0.05.

number of correlations, the gene networks were substantially remodeled with genes changing the synergistically and antagonistically coordinately expressed partners.

Finally, our Pair-Wise Relevance Analysis determines the contribution of each gene pair to the genomic fabric expression level, stability and inter-coordination, explaining the recovery effect of the cell therapy for Chagasic mice.

Conflicts of interests

The authors have no conflict of interests with the present work.

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