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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

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