Clinical Research

Serum response factor play a regulative role in the gene expression in heart failure

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Objective To investigate the relationship between transcription factor and the change of protein expression levels in heart failure. Methods Bioinformatic method was used to analyze the data of binding-sites on the 5′ flanking regions of four genes whose mRNA level changed in failing heart from three databases about nucleic acid-EMBL, transcriptional regulation factor-TRANSFAC and protein-SWISS PORT. The expression level of selected transcription factor was determined by immunohistochemical method. Results Nine transcription factors were inferred to influence the proteins' levels in occurrence and development of heart failure. Serum response factor (SRF) was selected from the nine factors and assayed. The results showed that there was a higher level of SRF in healthy group than in chronic heart failure (CHF), and the level was associated with the degree of CHF. It was also found that there was a relative higher level of SRF in the acute myocardial infarction (AMI) than that in CHF, but which was lower than the healthy. Conclusion It showed that SRF had a quantitative change in the development of heart failure, and suggested SRF might play an important regulative role in the gene regulatory network and protein-synthesis factors. Modulation of transcription of these genes and be responsible for the changes of mRNA level in CHF. But few transcription factors had been studied in CHF and the mechanism of their actions is unknown. This study used a bioinformatic method to analyze the 5′ flanking sequences to control the transcription of gene by binding to specific binding sites in the DNA regulatory regions (e.g. promoters, enhancers). Multiple transcription factors have been found to regulate the expression of cardiac specific genes7 or the cellular immediate-early genes (e.g. c-fos gene)18 in both physiological and pathological conditions via interactions with various cis-acting DNA elements. In rat and rabbit, molecular signals produced by pressure overload and β-adrenergic agonist stimulated the expression of β-MHC mRNA and other late response genes, such as ANP genes.19,20 Considerable evidence has accumulated to show that transcriptions of β-MHC, β-MHC and ALC-1 were developmentally regulated in tissue-specific and muscle-specific manners. Tissue-specific transcription factors modulate the transcription of genes through their interactions with cis-acting DNA elements which are frequently located upstream at the transcription initiation site.21,22 A number of positive or negative DNA regulatory sequences and their corresponding transcription factors had already been identified of the genes encoding α-actin, myosin light chains, cardiac troponin T, embryonic MHC, α-MHC and β-MHC.23 So tissue-specific transcription factors might be involved in the modulation of transcription of these genes and be responsible for the changes of mRNA level in CHF.

Key Words heart failure; gene expression; transcription factor; serum response factor; bioinformatics

Introduction

Congestive heart failure (CHF) is a multifactorial disease that may result from different initiating events. Multiple molecular pathways are believed responsible for transduction of the stimuli into gene expression during the process of heart failure. Changes of messenger RNA (mRNA) level of some myofibrillar proteins were related to changes in the protein content and/or myocardial function.1,11 Many studies have previously been made to identify genes differentially expressed in failing and nonfailing human hearts using oligonucleotide array or cDNA array4 and RT-PCR methods. Some proteins, whose mRNA levels were changed in the failing heart, are up-regulation of β isoform of myosin heavy chain (β-MHC),2 the essential myosin light chain (ALC-1),3 atrial natriuretic peptide (ANP),1 the Na+-Ca2+ exchanger,10 down-regulation of α isoform of myosin heavy chain (α-MHC),2,3 the sarcoplasmic reticulum Ca2+-ATPase (SERCA2a),11,12 phospholamban (regulatory protein of SERCA2a),13 the Ryanodine receptor,14 the β1 adrenergic receptor (β1-AR),15 and the angiotensin II AT-1 receptor subtype.16 Transcription factors provide their activation or repression to control the transcription of gene by binding to specific binding sites in the DNA regulatory regions (e.g. promoters, enhancers). Multiple transcription factors have been found to regulate the expression of cardiac specific genes7 or the cellular immediate-early genes (e.g. c-fos gene)18 in both physiological and pathological conditions via interactions with various cis-acting DNA elements. In rat and rabbit, molecular signals produced by pressure overload and β-adrenergic agonist stimulated the expression of β-MHC mRNA and other late response genes, such as ANP genes.19,20 Considerable evidence has accumulated to show that transcriptions of β-MHC, β-MHC and ALC-1 were developmentally regulated in tissue-specific and muscle-specific manners. Tissue-specific transcription factors modulate the transcription of genes through their interactions with cis-acting DNA elements which are frequently located upstream at the transcription initiation site.21,22 A number of positive or negative DNA regulatory sequences and their corresponding transcription factors had already been identified of the genes encoding α-actin, myosin light chains, cardiac troponin T, embryonic MHC, α-MHC and β-MHC.23 So tissue-specific transcription factors might be involved in the modulation of transcription of these genes and be responsible for the changes of mRNA level in CHF. But few transcription factors had been studied in CHF and the mechanism of their actions is unknown. This study used a bioinformatic method to analyze the 5′ flanking sequences to control the transcription of gene by binding to specific binding sites in the DNA regulatory regions (e.g. promoters, enhancers). Multiple transcription factors have been found to regulate the expression of cardiac specific genes7 or the cellular immediate-early genes (e.g. c-fos gene)18 in both physiological and pathological conditions via interactions with various cis-acting DNA elements. In rat and rabbit, molecular signals produced by pressure overload and β-adrenergic agonist stimulated the expression of β-MHC mRNA and other late response genes, such as ANP genes.19,20 Considerable evidence has accumulated to show that transcriptions of β-MHC, β-MHC and ALC-1 were developmentally regulated in tissue-specific and muscle-specific manners. 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of proteins whose mRNA level changed in the failing heart in order to find common transcription factors participating in the transcription regulation of these proteins. Nine transcription factors were identified to be involved in the regulation. A possible mechanism for their actions is that changes of the amount of transcription factor(s) might lead to changes of the transcription of the proteins related to myocardial function. A pilot study of serum response factor (SRF) was made by immunohistochemistry in myocardium specimens. The results show a decreasing expression of SRF in proportion to the worsening of the heart function, which might give a support for the possible mechanism above.

Materials and Methods

Sequence data collection

The receptor was not included in this study because of its complex function. The sequences of genes whose mRNA level changed during heart failure were searched in the EMBL Nucleotide Sequence Database (Database of European Molecular Biology Laboratory, Release 80, September 2004). The protein factors involved in the interaction with their DNA binding sites were chosen from the TRANSFAC Database (Release 4.0 public), on condition that their primary structure and function were accepted in the SWISSPROT Protein Sequence Database (Release 45.0 Oct 25th 2004).

Bioinformatical analysis

The GOLDKEY Software (a software for DNA and protein analysis, designed by JiaJin Wu) was used to analyze the sequence data. The DNA sequences were spliced to gain an entire 5' flanking sequence. The 5' regulation region was designed upstream at the initiation site of the mRNA encoding region. Some common binding sites on the 5' flanking sequences of the four genes were selected, on condition that both the complete bases and the core region have at least five continuous bases matched between the cis-acting element and 5' flanking sequences of genes. The corresponding transcription factors with exact descriptions in TRANSFAC and SWISSPROT databases were picked out.

Myocardium specimen preparation

The left ventricular myocardium tissues in the paraffin blocks were obtained from autopsies of 40 patients at the General Hospital of PLA from December 1997 to May 1999. All autopsies were preserved as quickly as possible in less than 24 hours except for five cases that were within 72 hours. All tissues were fixed in 10 percent formalin and embedded in paraffin wax. The CHF group included 15 men and 6 women (77.4±3.5 years). They had histories of ischemic cardiomyopathy (ICM), hypertension or chronic rheumatic heart disease. According to the New York Heart Association functional classification, thirteen patients were NYHA IV and eight patients were NYHA III. The acute myocardial infarction (AMI) group included 9 male patients who had ICM history more than 2 years before they suffered from AMI (83.4±2.0 years). The normal group consisted of 10 subjects (28.3±2.0 years). Six were donors (5 men and 1 woman) who were brain dead due to traumatic injury. The other four had no history of cardiac disorders.

Immunohistochemistry analysis

Immunohistochemical analysis was performed as previously described. The antibody directed against human SRF was purchased from Santa Cruz Biotechnology (Cat No.SC-335, Santa Cruz, CA). The SRF antibody concentration was 1.67 μg/ml.

Measurement of positive cells

At 1000× magnification, 500 cells were counted in 50 neighboring fields of vision (0.04 mm²/field) to calculate the percentage of positive cells. The positive cells displayed brown stain of the cytoplasm and nucleus.

Statistical analysis

Data were expressed as mean±SEM. And the mean values between different groups were compared. The statistical significance was assessed using the paired t-test. P<0.05 was considered as statistically significant. Spearman’s rank correlation coefficient was calculated by STATA software (a statistical software written by Computer Resource Center of the USA, release 4.0).

Results

The whole sequences of four genes were found by sequence comparison and splicing using Goldkey software (Table 1). One hundred and six common binding sites were located on the 5' flanking region of the four genes by the bioinformatical analysis. Seventy-six binding sites which have their corresponding factors in TRANSFAC database were selected from the 106 sites, and 38 factors were further reserved because their primary sequence or information exist in SWISSPROT database.

Because irrelevant factors may be chosen by chance by base matching, twenty-nine factors were rejected for the following reasons: non-mammal proteins; the same protein in different species; the same protein with different names; and transcription initiators (e.g. TFIIA). Finally, 9 factors were retained in this study, including SRF, SP1, NF-Y, C/EBP, PEA3, CTCF, NF-1, CDP and CTF. Additional information concerning these factors were used to identify their putative binding sites in the sketch of β-MHC gene and α-MHC gene (Fig. 1).

SRF was picked out since it had been of great interest in previous studies. Immunohistochemistry method was used to display the anti-SRF positive cells in the human myocardium. A brown precipitate appeared in both the cytoplasm and the nucleus of positive cells stained by 3,3'-diaminobenzidine tetrahydrochloride (DAB). The percentage of positive cells was
Table 1 The data of four genes in EMBL

<table>
<thead>
<tr>
<th>Gene</th>
<th>Species</th>
<th>Full length (bp)</th>
<th>CDS</th>
<th>Selected length (bp)</th>
<th>Exon I</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-MHC</td>
<td>HSBMYHC</td>
<td>25000</td>
<td>4394</td>
<td>4394</td>
<td>&lt;4394</td>
</tr>
<tr>
<td>ALC-1</td>
<td>HSMC1G1</td>
<td>2572</td>
<td>2099</td>
<td>2099</td>
<td>2024-2233</td>
</tr>
<tr>
<td>ANP</td>
<td>S77079</td>
<td>2610</td>
<td>/</td>
<td>1-2608</td>
<td>&lt;2695</td>
</tr>
<tr>
<td>α-MHC</td>
<td>HSCAMHCA</td>
<td>31462</td>
<td>5535</td>
<td>5535</td>
<td>4485-4506</td>
</tr>
</tbody>
</table>

* human atrial natriuretic factor gene (complete CDS )

![Diagram of β-MHC and α-MHC genes]

Fig 1. The binding-sites of nine transcription factors on β-MHC gene and α-MHC gene

calculated to quantify the SRF level. It was shown that there was extremely higher level of SRF in normal hearts than in CHF. Anti-SRF positive cardiocytes appeared in the normal hearts nearly 90% and strong stain could be clearly seen in positive cells, especially in the donors' hearts. The SRF level was markedly lower in CHF than in the normal hearts, and the difference was significant (Table 2). There was a negative correlation between the amount of SRF and the NYHA functional class, which was calculated by the STATA software with the Spearman’s rank (correlation coefficient: -0.7963) (P<0.01). The SRF level of the AMI group was in the middle of the normal and the CHF. The value of the AMI was statistically different from both that of the normal and that of the CHF (P <0.05).

Discussion

Nine common transcription factors were found that might infer tissue-specific or developmental patterns of gene expression during the development of heart failure. In the sketch of the binding sites of the nine transcription factors on the β-MHC gene and α-MHC gene (Fig.1), most of the sites were located upstream near the TATA box (about 1000 nt). The upstream region near the TATA box is believed to be rich in transcriptional regulation sequences, including the upstream promoter elements, such as the CAAT box and the GC box. So these factors might have an influence on the expression of gene relative to CHF. The layout in fig.1 suggests that one transcription factor might have multiple sites and multiple factors might bind to the same one.

Table 2 The percentage of anti-SRF positive Cardiocytes in each group

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of cases</th>
<th>Mean (%) ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>The normal</td>
<td>10</td>
<td>91.6±10.05</td>
</tr>
<tr>
<td>NYHA II-IV</td>
<td>21</td>
<td>28.73±12.79</td>
</tr>
<tr>
<td>The AMI</td>
<td>9</td>
<td>45.3±13.3</td>
</tr>
</tbody>
</table>
Many documents had been studied to identify the effects of the 9 factors on gene expression in CHF. SRF, SP-1, NF-Y and C/EBP are related to heart failure, in which SRF was most notable. In murine, the SRF mRNA had the highest level in adult skeletal and cardiac muscles, but it was barely detected in liver, lung and spleen tissues. As the 5' regulatory boundary, the 310bp in SRF promoter region, located upstream at the cap site, was required for muscle-restricted expression.  

SRF had been observed to modulate expression of human cardiac-specific genes, such as the α-cardiac actin gene.  

Hemodynamic stress and α1 adrenergic agonists, both of which are known to produce cardiac hypertrophy, rapidly provoked c-fos gene expression through protein kinase C (PKC) activation in ventricular myocardium. In addition, the serum response element (SRE) of c-fos was responsible for the expression of the PKC-induced c-fos gene. The p38 mitogen-activated protein kinase (p38 MAPK) was found to be a key regulator of cell growth and the cardiac-specific gene induction that occurred in response to potentially stressful stimuli in cardiac myocytes. A promoter-proximal SRE which bonds SRF, was shown to be critical for ANP induction in primary cardiac myocytes transfected with the selective p38/MAPK activator.  

Electrical stimulation of contractions (pacing) of primary neonatal rat ventricular myocytes activated a hypertrophic growth program that included the expression of the cardiac-specific gene ANP. Pacing stimulated ANP-promoter activity approximately 10-fold and proximal SRF and SP1 binding sites were required for the effects of pacing on the ANP promoter. In addition, SP1 was involved in the transcriptional regulatory mechanism during the course of pressure overload-induced cardiac hypertrophy. SP1 also played an important role in the transcriptional regulation of the cardiac sarcoplasmic reticulum Ca2+-ATPase gene.  

Nuclear protein NF-Y shared in the transcriptional regulation of the phospholamban gene, whose mRNA decreased in human failing myocardium. The region from -96bp to -78bp of the phospholamban gene played a critical role in its expression, and was regulated by binding of NF-Y. Nitrogen oxide (NO) was found to be an endothelium-derived vasorelaxing factor which directly affected myocardial function. C/EBP affected NO synthase induction via its interaction with the CAAT box.  

PEA3 participated in regulating myogenins in adult muscle. CTF and NF-1 usually bond to the CAAT box in eucaryotic cells of different tissues. CTCF and CDP were found to have a wide regulatory effect on eucaryotic cells as a transcription repressor, not just in the heart.  

SRF was picked out for further study because there are so many reports about it. SRF has shown in both the cytoplasm and the nucleus by Gauthier-Rouvire staining. The same distribution was also observed in this study. SRF has been identified to interact with SRE, which acted as a positive regulatory element located from -344bp to -156bp in rat α-MHC gene. A comparison of rat and human α-MHC promoters showed that the sequence from -340bp and +20bp was highly conserved. In this study, the putative binding site of SRF was located at both the 5' flanking region and intron 2 of human α-MHC gene. The site in 5' flanking region was at the 220 bp upstream of the TATA box, which was almost the same as that in the rat α-MHC gene. The results suggest that SRF might have a similar effect on human α-MHC gene as on rat α-MHC gene. Because of the down-regulation of α-MHC mRNA in CHF and the positive regulation of SRE to α-MHC, the level of SRF was supposed to decrease in myocardium during CHF. The result of immunohistochemical analysis indicated a higher level of SRF-positive cells in the normal group than that in the CHF group, which supported that the SRF level would surely change markedly with the development of CHF. The decrease of SRF might lead to the decline of α-MHC gene expression in CHF. The negative correlation between the level of SRF and heart function suggested SRF have a tendency to decrease during the transition from normal cardiac function to heart failure. If the supposition is significant, the level of SRF protein might be a sensitive marker to identify potential CHF. In this study, the age distribution of normal group was significantly younger than that of the CHF and AMI groups, it is necessary to identify whether SRF level might be altered with age. Interestingly, the basal expression of SRF protein was increased in the hearts of old rats compared with young adult animals.  

But the SRF level of the normal is higher than that of the other groups, suggesting that it is not the age but heart function might contribute to the observed decrease of SRF expression. The percentage of SRF-positive cells in the AMI group was statistically lower than that in the normal, but statistically higher than that of the CHF group (P<0.05). In a recent study, acute ischemia was demonstrated to induce the expression of immediate early-acting genes in the heart, such as the c-fos gene. SRE is located at 320 bp upstream of the initiation site of the c-fos gene and plays a major role in the regulation of c-fos transcription. Lu observed that the SRF level had changed in response to acute cardiac ischemic stress in a study of c-fos gene in rat heart. As a transcription factor binding to SRE, SRF was expressed in response to acute ischemia. which could explain the higher level of SRF in the AMI group than that in the CHF group.  

In summary, 9 transcription factors, which may modulate the transcription of CHF-related genes, were identified by bioinformatics analysis. Their transcriptional regulation might depend on quantitative change in their levels. Although immunohistochemistry counting is not an accurate quantifiable method, it showed a statistical difference of SRF level between the CHF heart and the normal heart, which gave a support to our supposition about the importance of the transcription factor. Transcriptional regulation of a gene is not only the effect of one transcription factor with its binding element, but depends on the coactions of multiple factors. Therefore, it could not definitely explain the regulatory mechanism of a transcription factor in CHF in this study. Further study should be needed to identify whether SRF directly regulates the expression of proteins associated with CHF or whether it cooperates with other factors.
References


