Cluster Analysis of Transcription Factor Binding Sites in the Promoter Regions of Cardiac Genes in Failing Hearts

Masaharu Nakayama\(^1\),\(^2\)  
nakayama@cardio.med.tohoku.ac.jp  
Hiroko Tada\(^1\)  
htada@cardio.med.tohoku.ac.jp  
Yasuhide Asaumi\(^1\)  
asaumi@cardio.med.tohoku.ac.jp  
Hiroaki Shimokawa\(^1\)  
shimo@cardio.med.tohoku.ac.jp

\(^1\) Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine  
\(^2\) Department of Medical Informatics, Tohoku University Graduate School of Medicine  
1-1 Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan

Keywords: transcriptome, heart failure

1 Introduction
Heart failure is a disorder in which the heart is unable to pump blood effectively to the body. Extensive studies have revealed that cardiac gene expression is altered in the progression of heart failure and the pattern of gene expression varies, depending on the cause of heart failure, such as myocardial infarction, pressure overload, and neurohormonal activation. Microarray technique has recently been used for large-scale genomic approach to describe the molecular mechanisms involved in the processes in heart failure [1,2]. However, those studies showed only lists of genes altered in heart failure but did not entirely clarify the upstream of the alteration in gene expression.

The interaction between transcription factors and their binding sites in the promoter region of genes plays a key role for gene regulation. Thus, this study was designed to identify critical transcription factors that alter gene expression in heart failure by clustering of transcription binding sites in the upstream sequences of co-regulated genes in the different models of heart failure.

2 Method and Results
Cardiac gene expression was examined in three different heart failure models in mice, including myocardial infarction (MI) with a permanent coronary artery ligation, pressure overload by aortic banding (B), and neurohormonal activation by angiotensin II infusion. Six-weeks-old male mice were anesthetized with an intraperitoneal injection of pentobarbital sodium (0.04 mg/g body weight) and were ventilated using an endotracheal tube and a ventilator. Each mouse underwent one of the following procedures; left coronary artery ligation (MI), banding of the transverse aorta (B), angiotensin II infusion (2.0 mg/kg/day) with an implanted osmotic mini-pump (AT), or sham operation. One week after the operation, total RNA was isolated from the heart using the Trizol reagent. Double-stranded cDNA was synthesized from 5 μg RNA. Each double-stranded cDNA was subsequently used as a template to make biotin-labeled cRNA and 15 μg of fragmented, biotin-labeled cRNA from each sample was hybridized to an Affymetrix mouse genome 430A 2.0 array. Among genes labeled as “significant” analyzed using Affymetrix criteria, we extracted those genes that displayed 2-fold increase in the heart failure groups compared to the sham-operated group. The number of the genes extracted was 1,288 (MI), 511 (B), and 13 (AT). We collected the sequences of 1500bp upstream of the translation start sites of 18,218 genes on mice from DBSTT [3]. We also collected the motifs of transcription factor binding site retrieved from the TRANSFAC database [4]. We then calculated binomial probability and found significantly frequent transcription factor binding sites in the promoter regions of the genes induced in the heart failure among the 18,218 genes (significant p-value is 0.05).
Table 1 shows the significantly frequent transcriptional binding sites in the promoter region of genes with altered expression in the 3 heart failure models, including myocardial infarction (MI), aortic banding (B), and infusion of angiotensin II (AT). GATA binding sites were significantly noted in the upstream sequence of the genes whose expressions were augmented in all the heart failure models. Eight sites were related with both MI and Banding model. Each model had specific transcriptional binding sites (5 sites in MI, 5 sites in B, and 2 sites in AT).

3. Discussion
In this study, we assumed that the altered cardiac gene expression is directly or indirectly co-regulated by a limited number of transcriptional factors associated with the different cause of heart failure. To describe the critical transcription factors in heart failure, we compared the frequency of transcriptional binding sites in the promoter region of the genes whose expression was increased in the different types of heart failure with that of the genes whose expression were unaltered. Twenty-one transcription factor binding sites were significantly frequent in the heart failure models. GATA binding sites were related with all the heart failure modes. This is feasible because GATA binding sites existed in the promoter region of the cardiac genes commonly related to heart failure, such as atrial natriuretic factor, B-type natriuretic peptides, and alpha and beta myosin heavy chain. Each model of heart failure had specific transcriptional factor binding sites. NF-κB is known to mediate oxidative stress that may play an important role in the progression of heart failure [5]. AP2 triggers myocardial apoptosis [6]. Neither Myb nor HNF4 has been reported in the heart failure. Further study is required to validate the molecular and pathological function of the transcription factors that we identified in this study on heart failure. In conclusions, specific transcriptional factor binding sites are found in the promoter region of the genes associated with the different types of heart failure. This approach may be useful to identify therapeutic targets of heart failure.

References