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RAPID COMMUNICATION

Integration of ATAC-Seq and RNA-Seq identifies the key genes in myocardial ischemia

Myocardial ischemia (MI) is a common disease with high mortality and morbidity worldwide. Since the pathological process of MI is very complicated, a comprehensive understanding of its pathogenesis is the key to the treatment of MI. As chromatin plays a crucial role in regulating gene expression, and gene regulation is a fundamental process in developing and disease progression, combined analysis of the chromatin and gene can further reveal the pathological mechanism of MI. In this study, Assay for Transposase-Accessible Chromatin with high throughput sequencing (ATAC-seq) was used to identified open chromatin, RNA-seq was applied to detected differential expression profiling. The combined analysis of ATAC-seq and RNA-seq showed that inflammatory response was critical in MI injury. Analysis of transcription factors (TFs) found a key TF, STAT2 (signal transducers and activators of transcription 2). The annotation analysis of target genes predicted by *Stat2* found most of these genes were involved in inflammation, which indicated *Stat2* was a key regulator of inflammatory response after MI. This study reveals a deeper regulatory mechanism of inflammation after MI from the levels of chromatin, TFs, and genes; *Stat2* may be the core of this regulator network. These results provide a more specific target for early intervention of inflammation after MI.

ATAC-seq was developed in 2013 and has been widely used to detect the open chromatin regions. This technology uses the Tn5 transposase to detect accessible open chromatin and cut the exposed DNA regions for sequencing.[1] Potential TFs and target genes will be obtained by analyzing the motifs of open chromatin regions. RNA-seq is a necessary method for understanding the gene expression profile. Through the integration analysis of ATAC-seq and RNA-seq, the effect of chromatin accessibility obtained by ATAC-seq on gene expression can be verified, and the molecular mechanism of gene expression regulated by TFs can be researched. To further understand MI's pathological processes and target genes, we combined ATAC-seq and RNA-seq to research the regulator mechanism of MI from the open chromatin and gene transcription level.

This study performed MI models by ligating the left anterior descending, a classic mouse MI model.[2] We evaluated MI injury using ECG, TTC staining, echocardiography, and myocardial enzyme levels and found that the cardiac function on day 5 after MI was significantly defective. TUNEL, Masson and HE staining found that cell apoptosis, myocardial fibrosis, and inflammatory response were obvious after MI. Considering the complex pathological process of MI, we collected the heart tissues of mice in Control and MI groups for ATAC-seq and RNA-seq detection, respectively.

Firstly, we found that the chromatin accessibility and gene expression profiles were significantly changed after MI. By analyzing differentially accessible regions (DARs) in the control and MI group, we found 22,635 increased DARs and 1,935 increased DARs in MI conditions. RNA-seq results showed that 5,083 genes were differently expressed in the MI group compared to the control group; of these, 2,774 genes were up-regulated, and 2,309 were down-regulated (Fig. 1B). Then we integrated the ATAC-seq data with RNA-seq data to determine whether the changes in DARs regulated the gene expression. The result showed that most of the up-regulated genes in the MI group were also involved by increased peaks. The GO analysis found these genes were enriched in the biological process, such as inflammatory response, angiogenesis, and response to hypoxia, which have been proved to play a role in MI injury (Fig. 1C). KEGG analysis found these genes involved in the chemokine signaling pathway, phagosome, and regulation of actin cytoskeleton (Fig. 1D). All these analyses revealed that inflammatory response might be the critical pathological process on day 5 after MI.

ATAC-seq can predict the binding of transcription factor (TF), while RNA-seq can reveal the transcription of TF and predict the possible target genes. Therefore, the combined analysis of ATAC-seq and RNA-seq can determine which differentially expressed TFs are binding on which

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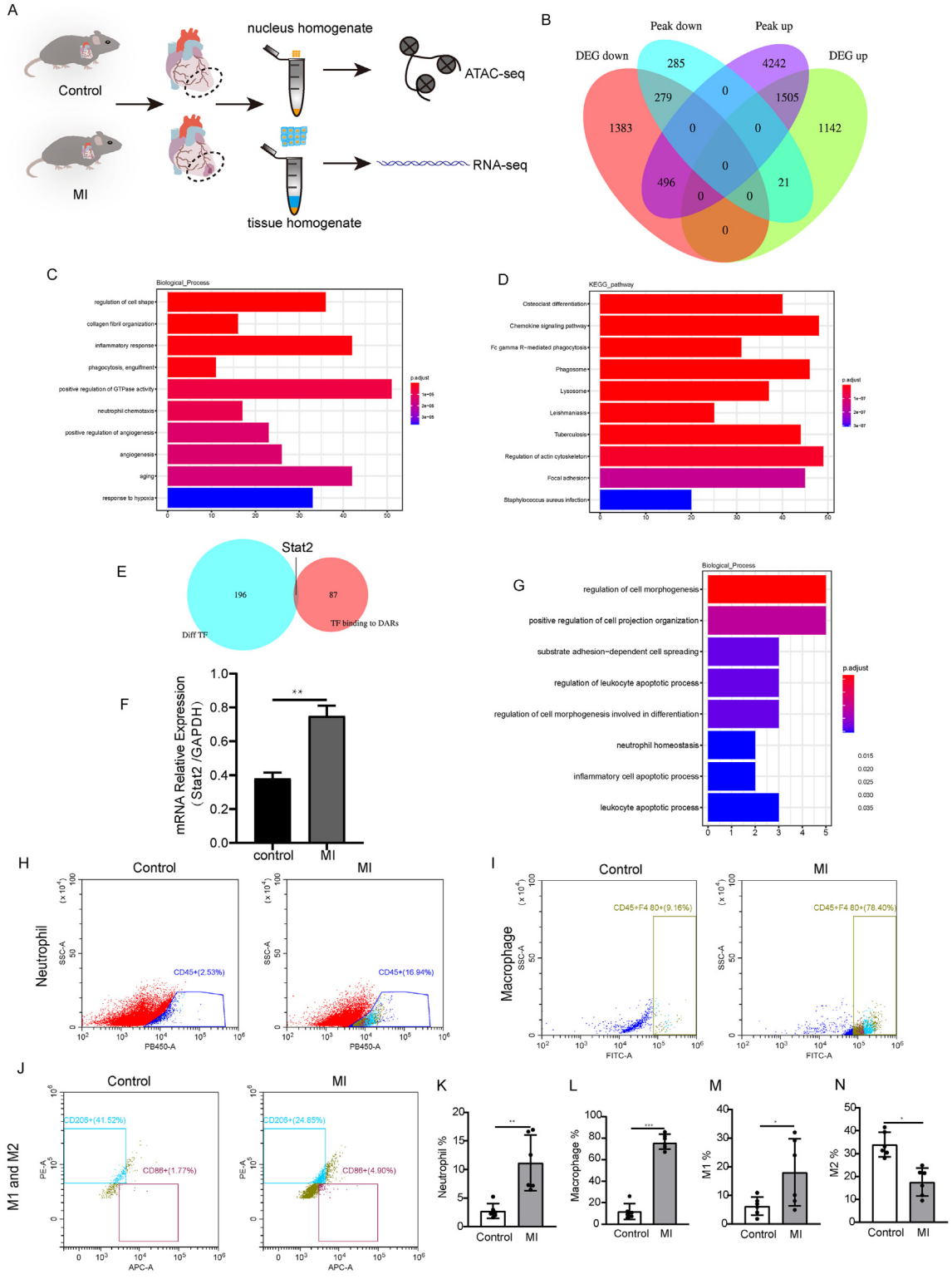

Figure 1 Integration of ATAC-Seq and RNA-Seq identified *Stat2* as a possible regulator of inflammatory response in MI. **(A)** Experimental workflow. Heart tissues from Control and MI groups were collected, then nuclei homogenate and tissue homogenate were extracted and processed for ATAC-seq and RNA-seq, respectively. ($n = 3$) **(B)** Venn Diagram of DEG down, DEG up, Peak down, and Peak up. **(C)** GO terms (biological process, BP) of DEGs which involved increased peaks. **(D)** KEGG pathway analysis of DEGs involved with increased peaks. **(E)** Venn Diagram of TFs in DEGs and TFs annotated by DARs. **(F)** The mRNA expression of *Stat2* in control and MI groups. ($n = 3$). **(G)** GO analysis (BP) of target genes predicted by *Stat2*. **(H–J)** Flow cytometric analysis of neutrophils, macrophage, M1 macrophage and M2 macrophage. **(K–N)** The ratio of neutrophils, macrophage, M1 macrophage and M2 macrophage. ($n = 6$).

differentially peak regions. These TFs may play a more critical role at both the accessibility and transcription levels. Through the combined analysis of ATAC-seq and RNA-seq, a differentially expressed TF binding on differentially peak regions was found, STAT2 (Fig. 1E). Although the results of RNA-seq in this study were shown that the expression of other members of the STAT family, including *Stat1* and *Stat5*, differentially in the MI group (Table S1), *Stat2* was the only differentially expressed gene binding on differentially peak regions. We also detected the expression of *Stat2*, and found that the expression of *Stat2* was increased in the MI group compared to the control group, consistent with the result of RNA-seq (Fig. 1F). As an essential member of the STAT family, *Stat2* has been shown to regulate the inflammatory response in many diseases[3,4]; however, unlike other member, such as *Stat3* and *Stat5*, that have been well studied in MI, the regulating effect of *Stat2* in MI has not been fully understood. In this study, we focused on the analysis of genes binding to STAT2. The result of GO analysis found that these genes enriched in some biological processes involved in inflammation, including the leukocyte apoptotic process, neutrophil homeostasis, inflammatory cell apoptotic process, and leukocyte apoptotic process (Fig. 1G). This result proved the regulating effect of *Stat2* on the inflammatory response in MI.

Integrating ATAC-seq and RNA-seq data, we found that inflammatory response was an essential pathological change on day 5 after MI. We further observed the inflammatory cells in this phase. As the neutrophil infiltration and macrophage polarization were major inflammatory response changes after MI and *Stat2* was necessary for pro-inflammatory differentiation of macrophages,[5] we examined neutrophil recruitment and M1/M2 macrophage polarization by flow cytometry. We found that the infiltration of neutrophils (Fig. 1H, K) and macrophages (Fig. 1I, L) increased on day 5 after MI. Further analysis of macrophages revealed that MI injury could induce the polarization towards M1 macrophages (Fig. 1J, M, N). It suggested that recruitment of neutrophils and macrophages to polarize M1 macrophages was the major pathological process on day 5 after MI injury. And *Stat2* may play a vital role in the inflammation after MI by regulating the macrophage's polarization.

This study provided a more comprehensive profile of open chromatin and transcriptome under MI conditions. The findings of this study suggest that the pathological processes of MI were very complicated; in all of these pathological changes, the inflammatory response may be the most critical process on day 5 after MI. And the TF, STAT2, maybe a crucial regulator of inflammation. These findings provided a more specific target for the treatment of myocardial ischemia. Further studies should focus on the effect of *Stat2* on the regulation of inflammation and the possible downstream target genes of *Stat2*.

Conflict of interests

Authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2022.05.013.

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Jing Yuan [1], Jun-Meng Wang [1], Zhi-Wei Li, Cheng-Shun Zhang, Bin Cheng, Su-Hao Yang, Ding-Jun Cai *, Shu-Guang Yu **

Acupuncture and Tuina School/Third Teaching Hospital, Chengdu University of Traditional Chinese Medicine, Chengdu, Sichuan 610075, China

*Corresponding author. Acupuncture and Tuina School/Third Teaching Hospital, Chengdu University of Traditional Chinese Medicine, Chengdu, Sichuan 610075, China.

**Corresponding author. Acupuncture and Tuina School/Third Teaching Hospital, Chengdu University of Traditional Chinese Medicine, Chengdu, Sichuan 610075, China.
E-mail addresses: djcai@cdutcm.edu.cn (D.-J. Cai), ysg@cdutcm.edu.cn (S.-G. Yu)

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[1] These authors contributed equally to this work.