Integrated Analysis of Immunocyte Infiltration and Differential Gene Expression in Hypertrophic Obstructive Cardiomyopathy

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Research

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Abstract

Objective: Hypertrophic obstructive cardiomyopathy (HOCM) is one of the main reasons for sudden cardiac death (SCD) of young people. Researches have revealed that immune-related genes are closely relevant with HOCM. Therefore, it is important to explore the key immune regulatory mechanisms and biomarkers of HOCM.

Methods: We used many bioinformatics methods, including linear models for microarray analysis (LIMMA), protein-protein interaction (PPI) network, Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes pathway (KEGG), and CIBERSORT to assess the key pathway and hub genes involved in HOCM. Furthermore, expression levels of hub genes were validated in human tissue.

Results: Our results showed that the degree of infiltration of five immune cells were linked to HOCM, including monocytes, macrophages M2, NK cell resting, B cells native, and T cells regulatory (Tregs). A total of 7 hub genes (CCL2, CXCL8, FOS, MAP2K1, NFKBIA, STAT3, and TNFRSF1A) were identified and validated by qt-PCR. The core genes including CCL2, MAP2K1, NFKBIA, STAT3, and TNFRSF1A are closely related to monocytes infiltration in HOCM.

Conclusion: Taken together, our research will provide useful information to explore the immune mechanisms underlying HOCM and the potential targets for therapy. The candidate genes CCL2, MAP2K1, NFKBIA, STAT3, and TNFRSF1A were involved in the regulation of monocytes tissue infiltration, which is closely related to the HOCM.

1. Introduction

Hypertrophic cardiomyopathy (HCM) has been one of the main reasons for sudden cardiac death (SCD) of young people [1, 2]. The primary cause of SCD in HCM is the left ventricular outflow tract obstruction (LVOTO) [3, 4]. About 25–70% of HCM patients demonstrate LVOTO, which is called hypertrophic obstructive cardiomyopathy (HOCM) [5–7]. In HCM, most patients remain asymptomatic or mildly symptomatic in the whole life, whereas patients have apparent symptoms in HOCM, consisted of dyspnea, exercise intolerance, chest pain, palpitations, and syncope. Moreover, SCD might be the initial symptom of unfortunate young individuals with HOCM. Therefore, it is essential to study the mechanism of HOCM.

In the heart, there are many cell types, composed by cardiomyocytes and non-cardiomyocytes including immune cells, endothelial cells and fibroblasts [8]. Multiple reports suggest that rolling and infiltration of circulating and resident immune cells, particular of monocytes, macrophages, and natural killer T lymphocytes, is a critical initiating event in the pathogenesis of HOCM [9, 10]. Understanding the specific functional phenotype of immune cells is as necessary as the recognition of specific immune cell populations. Resident and circulating immune cells can produce numerous cytokines associated cardiac hypertrophy and heart failure [10]. HOCM is a complex process involving a variety of cell types that interact with each other in the heart and circulation. It is very important to fully understand the recognition and the role of immune-related cell types in pathophysiological response.
In this study, we used many bioinformatics methods to assess the immune-related pathways, and immune cell subtypes in HOCM (Figure 1). Furthermore, we used human samples to validate our results. We explored the mechanisms of the infiltration of immune cells and provided useful information to explore the potential targets for therapy in HOCM.

2. Materials And Methods

2.1 Materials

The GSE36961 dataset contributed by Hebl VB et al. were downloaded from the Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo/) database for subsequent analysis. The dataset contained a total of 145 tissue samples, including 39 normal interventricular septum (IVS) samples and 106 HOCM IVS samples. The HOCM samples were obtained during surgical septal myectomy because of LVOTO, and the normal IVS samples were donor cardiac tissues. Meanwhile, 10 patients diagnosed with HOCM according to the American College of Cardiology Foundation and American Heart Association (ACCF/AHA) guidelines were enrolled in Guangdong Provincial People’s Hospital (Guangzhou, China) [11]. All samples were taken from IVS during surgical septal myectomy because of LVOTO, and the 5 control samples were donor cardiac tissues. Written informed consent was granted by patients. The research was approved by the Research Ethics Committee of Guangdong Provincial People’s Hospital, Guangdong Academy of Medical Sciences. Statistical analysis were performed with the R (version 3.6.0).

2.2 Identification of differentially expressed genes

Raw data was processed using “affay” package of R language. Next, the Benjamini-Hochberg method was used to adjust p-values and thus the false discovery rate (FDR) and fold-change (FC) were calculated [12, 13]. Finally, genes expression values of the $|\log_2 FC| > 1.5$ and adjusted $p < 0.05$ for filtering differential expressed genes (DEGs) were set.

2.3 Gene Ontology (GO) and pathway analysis

GO enrichment of DEGs was performed using the clusterProfiler algorithm. The results of GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment for DEGs were obtained from Metascape (http://metascape.org/gp/index.html) database. GO includes biological process (BP), cellular component (CC) and molecular function (MF). GO terms for which $p < 0.05$ were considered to be significant. And, the filtered the genes enriched in KEGG pathway associated with immunity and inflammation were subsequently used to construct the protein-protein interaction (PPI) biological networks based on the STRING online database (V 11.0) (https://string-db.org/) with the nodes association confidence score > 0.4. In addition, the Cytoscape software (V3.7.1) was used to visualize and evaluate interactions and identifying the hub genes in functional networks [14].

2.4 Immune-related pathways and cells subtypes analysis

To characterize the immune cell subsets in HOCM, we’ve applied the CIBERSORT (https://cibersort.stanford.edu/) estimate software to quantify the immune cell fractions for the gene expression matrix derived from HOCM samples [15]. The advantage of CIBERSORT algorithm in the aspects
of characterizing the infiltration of various immune cell subtypes using RNA mixtures from any tissues with a systems-level insight [16]. In addition, we performed a Pearson correlation clustering analysis of hub genes and differential cell subtype infiltration values.

Meanwhile, we applied DAVID (https://david.ncifcrf.gov/home.jsp) database and DisGeNET Curated online database (http://disgenet.org/) to annotate the hub genes and discover the human diseases associated with hub genes. Subsequently, to identify the transcription factor (TF) of the hub genes, the plug-in iRegulon for Cytoscape software was applied, with the default parameters. Herein, we chose the top 3 regulators with the highest NES value to construct the regulatory network involved in HOCM.

2.5 Validation of hub genes

Additionally, total RNA from human heart IVS tissue samples was isolated and purified using TRIzol method, and reverse-transcribed using the PrimeScript RT Master Mix (TAKARA) according to the manufacturer’s instructions. Real-time PCR was performed using SYBR Premix Ex Taq II (TAKARA) and a 7500 Real-Time PCR System (Life Technologies) according to the manufacturer’s instructions. The expression level of each sample was internally normalized against that of the glyceraldehyde 3-phosphatedehydrogenase (GAPDH). The relative quantitative value was calculated using $2^{-\Delta \Delta Ct}$ method. Each experiment was performed in triplicate. The primers used in Real-time PCR were listed in Table A.1.

3. Results

3.1 Identification of DEGs in HOCM

Comparing the healthy and HOCM IVS samples 405 genes with different expression ($|\text{Log}_2 \text{FC}|>1.5$, adjusted p-value < 0.05) were identified, including 254 up regulated and 151 down regulated (Fig. 2A and Table A.2). Hierarchical clustering analysis of the top 50 DEGs for the comparison of HOCM and healthy samples is shown in Fig. 2B. The principal component analysis (PCA) showed a significant distribution for the HOCM and healthy samples in Fig. 2C.

3.2 Functional analysis of DEGs and identification of hub genes

In enrichment analysis for DEGs, we found that GO terms of top 5 BP including muscle system process, heart contraction, extracellular structure organization, heart process and positive regulation of inflammatory response were closely related to DEGs associated with HOCM (Fig. 2D and Table A.3), whereas GO terms of top 5 CC consisting of collagen-containing extracellular matrix, extracellular matrix, secretory granule lumen, cytoplasmic vesicle lumen and vesicle lumen were closely related to DEGs associated with HOCM (Fig. 2E and Table A.3). Meanwhile, GO terms of top 5 MF comprising integrin binding, extracellular matrix structural constituent, collagen binding, structural constituent of cytoskeleton and metalloendopeptidase inhibitor activity were closely related to DEGs associated with HOCM (Fig. 2F and Table A.3).

Regarding the KEGG pathway enrichment, the DEGs were significantly enriched in pathways including phagosome, pathogenic Escherichia coli infection, complement and coagulation cascades, apoptosis, AGE-
RAGE signaling pathway in diabetic complications and HIF-1 signaling pathway (Fig. 3A and Table A.4).

Interestingly, we also found that DEGs enriched 8 immune-related pathways, including NF-kappa B signaling pathway, TNF signaling pathway, IL-17 signaling pathway and Toll-like receptor signaling pathway and so on. These results are illustrated in Fig. 3A. These HOCM-related pathways were related to immunity-inflammation response. Hierarchical clustering analysis of the genes of immune-related pathways in HOCM is shown in Fig. 3B. After submitting the genes enriched in immune-related pathways to the STRING database (https://string-db.org/), 7 PPI nodes were obtained, with a confidence threshold greater than 0.4. After analyzed by Cytoscape software as an undirected method, the 7 nodes of the PPI network was considered to be central agents, which were CCL2, CXCL8, FOS, MAP2K1, NFKBIA, STAT3, and TNFRSF1A (Fig. 3C). Compared with the healthy group, Through expression levels of these 7 hub genes were lower expressed in HOCM group (all p < 0.05, Fig. 3D). In addition, CCL2 is the most differentially expressed gene.

3.3 Immunocyte infiltration and hub immunocyte detection

To characterize the immunocyte status of HOCM, we've applied the CIBERSORT algorithm to quantify the immune cell fractions for the gene expression matrix derived from cardiac samples. The overall immune infiltration landscape of HOCM tissues is shown in Fig. 4A, B and Table A.5. Monocytes (P = 1.75E-14), macrophages M2 (P = 1.88E-14), naïve B cells (P = 5.28E-03), NK cells resting (P = 2.70E-04), and T cells regulatory (Tregs) (P = 0.012) demonstrated significant differential infiltration in HOCM tissues.

3.4 Identification of the relationship between the hub gene and key immune cells subtypes in HOCM

Through correlation analysis, we found that the 5 hub genes (CCL2, MAP2K1, NFKBIA, STAT3, TNFRSF1A) in immune-related pathways had the strong correlations with the degree of infiltration of monocytes (Fig. 4C). This suggests that, to some extent, monocytes may play an important role in HOCM.

3.5 Investigating the Functional Role and TF of Hub Genes

To further understand how the hub genes were correlated with HOCM, we applied DAVID and Metascape online database to explore the biological function and associated pathways. The results of GO term enrichment in hub genes indicated that the response to lipid, response to lipopolysaccharide, and response to cytokine were mainly enriched (Table 1). The results of pathways were TNF signaling pathway, interleukin-10 (IL-10) signaling, chemokine signaling pathway, signaling by interleukins, Cadmium induces DNA synthesis and proliferation in macrophages, IL-17 signaling pathway, Toll-like receptor signaling pathway and cytokine Signaling in Immune system (Table 2). We used DisGeNET Curated online database (http://disgenet.org/) to discover the human diseases associated with hub genes, which contained cardiomyopathy, familial idiopathy, and so forth (Table 3).
Table 1
The top 3 of GO items enriched by hub genes

| Category | Name                                | p-value     | FDR     | Enriched Genes                                |
|----------|-------------------------------------|-------------|---------|-----------------------------------------------|
| GO: MF   | chemokine receptor binding          | 1.11E-06    | 120E-04 | STAT3,CXCL8,CCL2                             |
| GO: MF   | protein-containing complex binding  | 1.62E-03    | 0.020   | FOS,STAT3,NFKBIA,TNFRSF1A                     |
| GO: MF   | enzyme binding                      | 3.08E-03    | 0.026   | MAP2K1,STAT3,NFKBIA,TNFRSF1A                  |
| GO: BP   | response to lipid                   | 1.48E-09    | 1.78E-06| FOS,MAP2K1,STAT3,CXCL8,NFKBIA,CCL2,TNFRSF1A  |
| GO: BP   | response to lipopolysaccharide      | 4.13E-08    | 1.56E-05| FOS,CXCL8,NFKBIA,CCL2,TNFRSF1A                |
| GO: BP   | response to cytokine                | 5.01E-08    | 1.56E-05| FOS,STAT3,CXCL8,NFKBIA,CCL2,TNFRSF1A         |
| GO: CC   | neuron projection                   | 4.60E-04    | 0.021   | FOS,MAP2K1,CCL2,TNFRSF1A                      |
| GO: CC   | C-fiber                             | 1.83E-03    | 0.025   | CCL2                                         |
| GO: CC   | I-kappaB/NF-kappaB complex          | 2.94E-03    | 0.034   | NFKBIA                                       |
| Name                                                   | p-value     | FDR          | Enriched Genes                                      |
|--------------------------------------------------------|-------------|--------------|-----------------------------------------------------|
| TNF signaling pathway                                  | 9.269E-10   | 2.118E-07    | FOS, MAP2K1, NFKBIA, CCL2, TNFRSF1A                  |
| Interleukin-10 signaling                               | 7.347E-09   | 6.715E-07    | STAT3, CXCL8, CCL2, TNFRSF1A                         |
| Chemokine signaling pathway                            | 1.296E-08   | 8.462E-07    | MAP2K1, STAT3, CXCL8, NFKBIA, CCL2                  |
| Signaling by Interleukins                              | 3.952E-08   | 2.258E-06    | FOS, MAP2K1, STAT3, CXCL8, CCL2, TNFRSF1A           |
| Cadmium induces DNA synthesis and proliferation in macrophages | 6.076E-08   | 3.085E-06    | FOS, MAP2K1, NFKBIA                                 |
| IL-17 signaling pathway                                | 1.004E-07   | 4.588E-06    | FOS, CXCL8, NFKBIA                                  |
| Toll-like receptor signaling pathway                   | 1.578E-07   | 6.355E-06    | FOS, MAP2K1, CXCL8, NFKBIA                          |
| Cytokine Signaling in Immune system                    | 3.451E-07   | 0.00001051   | FOS, MAP2K1, STAT3, CXCL8, CCL2, TNFRSF1A           |
Table 3
The top 10 diseases enriched by hub genes

| ID      | Name                          | p-value     | FDR         | Enriched Genes                                      |
|---------|-------------------------------|-------------|-------------|-----------------------------------------------------|
| C0035126 | Reperfusion Injury            | 2.44E-08    | 3.27E-06    | FOS,STAT3,CXCL8,CCL2                                 |
| C0151744 | Myocardial Ischemia           | 2.33E-07    | 1.21E-05    | STAT3,CXCL8,NFKBIA,CCL2,TNFRSF1A                    |
| C0004153 | Atherosclerosis               | 4.91E-07    | 1.98E-05    | FOS,MAP2K1,STAT3,CXCL8,CCL2,TNFRSF1A                |
| C0010054 | Coronary Arteriosclerosis     | 3.4E-06     | 7.74E-05    | FOS,STAT3,CXCL8,NFKBIA,CCL2                         |
| C0027051 | Myocardial Infarction         | 4.43E-06    | 9.42E-05    | STAT3,CXCL8,NFKBIA,CCL2,TNFRSF1A                    |
| C1956346 | Coronary Artery Disease       | 5.46E-06    | 0.000111    | FOS,CXCL8,NFKBIA,CCL2,TNFRSF1A                      |
| C1449563 | Cardiomyopathy, Familial Idiopathic | 8.4E-06 | 0.000152    | STAT3,CXCL8,CCL2,TNFRSF1A                             |
| C0027059 | Myocarditis                   | 1.04E-05    | 0.000177    | STAT3,CXCL8,CCL2                                    |
| C0020538 | Hypertensive disease          | 2.82E-05    | 0.000306    | FOS,STAT3,CXCL8,CCL2,TNFRSF1A                       |
| C0004364 | Autoimmune Diseases          | 0.000501    | 0.002245    | STAT3,CXCL8,CCL2,TNFRSF1A                            |

Finally, we predicted TFs and found that BCL3 transcription coactivator (BCL3), activating transcription factor 1 (ATF1) and GATA binding protein 5 (GATA5), as the master regulators of the hub genes are involved in HOCM (Fig. 4D).

3.6 Human tissue for gene expression validation

To test gene expression, the expression of the hub genes was accessed by Q-PCR in human cardiac tissues (Figure. 4E). In our results, three genes show significant downregulation comparing to the control group, which are CCL2 (p < 0.0001), CXCL8 (p = 0.001), TNFRSF1A (p = 0.012) and FOS (p = 0.002), consistent with the analysis results. STAT3 (p = 0.006) shows clear upregulation comparing to the control group, inconsistent with the analysis results. MAP2K1 (p = 0.148) and NFKB1A (p = 0.237) show little difference in our assays. Therefore, the PCR results partly supported the reliability of our analysis.

4. Discussion

HOCM is a kind of HCM, characterized by LVOTO caused by the cardiac hypertrophy of IVS. Emerging evidence has revealed the central role of immune-related pathways in cardiac hypertrophy. Our research found that the infiltration of monocyte, macrophages M2, naïve B cells, NK cells resting, and Tregs in cardiac tissues were closely associated with HOCM. Through further analysis, our study also suggested that CCL2,
MAP2K1, NFKBIA, STAT3, TNFRSF1A may be the core regulatory genes of immune-related pathways and were closely correlated with the degree of infiltration of monocytes, indicating that these genes may be critical regulatory markers in HOCM.

A greater understanding of the immune system itself has accelerated the progress in defining the cell populations of the immune system that plays a role in HOCM. Monocytes are key innate immune system mediators of inflammatory responses. In our analysis, the monocytes in HOCM samples had the most significant difference compared with healthy samples. Meanwhile, we found that the monocyte had the moderate correlation with 5 hub genes. In addition, CCL2 and TNFRSF1A were validated by PCR. Monocytes can differentiate into macrophages and have a proved significant relationship with HF and myocardial infarction in both animal and human research [17, 18]. Additionally, monocytes infiltration can cause cardiac hypertrophy and remodeling [19]. Considering the vital participation of monocytes, immune regulation is a promising treatment direction for HOCM and more work is needed to reveal the detailed mechanism. Our results revealed that monocytes may play an important role in HOCM and may serve as a predictive tool in HOCM progression.

Activation of natural killer T cells can attenuate myocardial infarction-induced cardiac remodeling [20], and inhibition of T cell immune activity can ameliorate maladaptive cardiac remodeling in mouse model [21]. Meanwhile, macrophages have a much more substantial role in regulating cardiac hypertrophy and remodeling during heart injury [10]. According to the function of macrophages, it is classified into two types, type M1 (classically activated) and type M2 (alternatively activated). Type M1 macrophage can secrete TNF, IL-1, IL-12, and other chemokines to play a proinflammatory function, and the type M2 macrophage mainly secretes anti-inflammatory factors, like epidermal cell growth factor (EGF) and transforming growth factor β (TGF-β) in the late stage of inflammation [22, 23]. M1 macrophages mainly facilitate tissue destruction; M2 macrophages promote tissue remodeling and repair, and previous studies showed an increase in M2 macrophage infiltration in myocardium promotes fibrosis [23–25], so altering macrophage phenotype in the heart may be a potential direction to modify cardiac fibrosis. In consequence, our results suggested the higher relative infiltration value of M2 and NK cells may influence the pathogenesis of HOCM.

Resting naïve B cell is defined as B cell before activation and further differentiation into specific subtypes. At present, we have not found any report about the direct correlation of resting naïve B cells and cardiac hypertrophy or fibrosis. Zouggari Y et al. found that B-cell depletion can decline left ventricular fibrosis and cardiac function in the model of myocardial infarction [26]. Furthermore, adoptive transfer of activated Treg cells to cardiac hypertrophic mouse model alleviated CD4+, CD8+ T cells, and macrophages infiltration into the heart and alleviated cardiac hypertrophy and fibrosis [27]. In our research, the higher relative infiltration values of Tregs and naïve B cells may be associated with the pathogenesis of HOCM, consistent with the previous studies.

There are a few studies investigating cardiac immune-related genes in HOCM patients. Among the candidate biomarkers, Zhu et al. have reported that FOS was related to immune inflammatory responses, cardiomyocyte apoptosis, cardiac remodeling and myocardial dysfunction [28, 29]. Schunkert et al. also found FOS gene was associated with hypertrophic rat hearts. And, in vivo, the expression of MAP2K1 in the hearts of transgenic mice promoted concentric cardiac hypertrophy [30], Bueno OF et al. reviewed many
literature and got the intermediate signaling pathway consisting of MAP2K1 (MEK1) and extracellular signal-regulated kinases (ERK1/2) as important regulators of cardiac hypertrophy and myocytes survival [31].

STAT3 has a key role in inflammation that underlies cardiovascular disease and impacts on cardiac structure and function and is important for maintaining endothelial cell function and capillary integrity with aging and hypertension [32]. Besides, STAT3 also involves the cardiac hypertrophy and fibrosis in TAC mouse models [33, 34]. Duerr GD et al. suggested that in the cardiac hypertrophy group CCL2 and CXCL8 had increased expression and anti-inflammatory IL-10 had a suppression which indicated the persistent inflammatory reaction [35]. About TNFRSF1A, it is a member of TNF receptor protein and has have effects on remodeling, hypertrophy, inflammation, and apoptosis in HF [36].

Finally, the TFs analysis results show that GATA5, BCL3, and ATF1 were significantly predicted in hub genes’ regulatory network. Herein, GATA5, a zinc-finger transcription factor essential for cardiovascular development and structural remodeling[37], was the sole and potent transactivator for the β-myosin heavy chain promoter, can bind to nuclear factors induced by leukemia inhibitory factor stimulation during myocardial cell hypertrophy [38]. ATF1, a member of activating transcription factor (ATF) subfamily and basic-region leucine zipper family, is a key driver of human plaque monocytes to acquire the atheroprotective macrophage state [39]. That is to say, ATF1 can promote monocytes to differentiate into a macrophage, in accordance with our analysis.

5. Conclusion

Taken together, our research found that the infiltration of monocyte, macrophages M2, naïve B cells, NK cells resting, and Tregs in cardiac tissues were closely associated with HOCM progression. In addition, CCL2, MAP2K1, NFKBIA, STAT3, and TNFRSF1A were involved in the regulation of monocytes tissue infiltration, which was closely associated with the process of HOCM. Most of hub regulators were validated in previous researches. However, further experimental evidence concerning the mechanism is still needed.

Declarations

Ethical Approval and Consent to participate

Ethics approval and consent to participate. Clinical tissue specimens were obtained from the Guangdong Provincial People’s Hospital. This study was approved by the ethics committee of the Guangdong Provincial People’s Hospital (Grant No. GDREC2016255H).

Consent for publication

Not applicable.

Availability of supporting data

The dataset supporting the conclusions of this article is available in the GEO repository (http://www.ncbi.nlm.nih.gov/geo/, GEO accession number: GSE39461).
Competing interests

There were no conflicts of interest.

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Authors’ contributions

Xianyu Qin and Shaoxian Chen analyzed the data and drafted the manuscript. Min Wu edited the manuscript. Yueheng Wu and Jian Zhuang supervised the project, advised with regards to the project design, and edited the manuscript.

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References

1. Geske JB, Ommen SR, Gersh BJ: Hypertrophic Cardiomyopathy: Clinical Update. *JACC Heart Fail* 2018, 6(5):364-375.

2. Semsarian C, Ingles J, Maron MS, Maron BJ: New perspectives on the prevalence of hypertrophic cardiomyopathy. *J Am Coll Cardiol* 2015, 65(12):1249-1254.

3. Brock R: Functional obstruction of the left ventricle; acquired aortic subvalvar stenosis. *Guys Hosp Rep* 1957, 106(4):221-238.

4. Teare D: Asymmetrical hypertrophy of the heart in young adults. *Br Heart J* 1958, 20(1):1-8.

5. Maron MS, Olivotto I, Betocchi S, Casey SA, Lesser JR, Losi MA, Cecchi F, Maron BJ: Effect of left ventricular outflow tract obstruction on clinical outcome in hypertrophic cardiomyopathy. *N Engl J Med* 2003, 348(4):295-303.

6. Prinz C, Farr M, Hering D, Horstkotte D, Faber L: The diagnosis and treatment of hypertrophic cardiomyopathy. *Dtsch Arztebl Int* 2011, 108(13):209-215.
7. Veselka J, Anavekar NS, Charron P: Hypertrophic obstructive cardiomyopathy. *Lancet* 2017, 389(10075):1253-1267.

8. Leask A: Getting to the heart of the matter: new insights into cardiac fibrosis. *Circ Res* 2015, 116(7):1269-1276.

9. Zhang Y, Huang Z, Li H: Insights into innate immune signalling in controlling cardiac remodelling. *Cardiovasc Res* 2017, 113(13):1538-1550.

10. Frieler RA, Mortensen RM: Immune cell and other noncardiomyocyte regulation of cardiac hypertrophy and remodeling. *Circulation* 2015, 131(11):1019-1030.

11. Gersh BJ, Maron BJ, Bonow RO, Dearani JA, Fifer MA, Link MS, Naidu SS, Nishimura RA, Ommen SR, Rakowski H et al.: 2011 ACCF/AHA Guideline for the Diagnosis and Treatment of Hypertrophic Cardiomyopathy: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Developed in collaboration with the American Association for Thoracic Surgery, American Society of Echocardiography, American Society of Nuclear Cardiology, Heart Failure Society of America, Heart Rhythm Society, Society for Cardiovascular Angiography and Interventions, and Society of Thoracic Surgeons. *J Am Coll Cardiol* 2011, 58(25):e212-260.

12. Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Speed TP: Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* 2003, 4(2):249-264.

13. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK: limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res* 2015, 43(7):e47.

14. Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, Santos A, Doncheva NT, Roth A, Bork P et al.: The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. *Nucleic Acids Res* 2017, 45(D1):D362-D368.

15. Chen B, Khodadoust MS, Liu CL, Newman AM, Alizadeh AA: Profiling Tumor Infiltrating Immune Cells with CIBERSORT. *Methods in molecular biology (Clifton, NJ)* 2018, 1711:243-259.

16. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, Hoang CD, Diehn M, Alizadeh AA: Robust enumeration of cell subsets from tissue expression profiles. *Nat Methods* 2015, 12(5):453-457.

17. Dutta P, Nahrendorf M: Monocytes in myocardial infarction. *Arterioscler Thromb Vasc Biol* 2015, 35(5):1066-1070.

18. Elchinova E, Teubel I, Roura S, Fernández MA, Lupón J, Gálvez-Montón C, de Antonio M, Moliner P, Domingo M, Zamora E et al.: Circulating monocyte subsets and heart failure prognosis. *PLoS ONE* 2018, 13(9):e0204074.

19. Wang L, Zhang YL, Lin QY, Liu Y, Guan XM, Ma XL, Cao HJ, Liu Y, Bai J, Xia YL et al.: CXCL1-CXCR2 axis mediates angiotensin II-induced cardiac hypertrophy and remodelling through regulation of monocyte infiltration. *Eur Heart J* 2018, 39(20):1818-1831.

20. Sobirin MA, Kinugawa S, Takahashi M, Fukushima A, Homma T, Ono T, Hiramayashi K, Suga T, Azalia P, Takada S et al.: Activation of natural killer T cells ameliorates postinfarct cardiac remodeling and failure in mice. *Circulation research* 2012, 111(8):1037-1047.
21. Tirziu D, Giordano FJ, Simons M: **Cell communications in the heart**. *Circulation* 2010, **122**(9):928-937.

22. Derlindati E, Dei Cas A, Montanini B, Spigoni V, Curella V, Aldigeri R, Ardigò D, Zavaroni I, Bonadonna RC: Transcriptomic analysis of human polarized macrophages: more than one role of alternative activation? *PLoS ONE* 2015, **10**(3):e0119751.

23. Shivshankar P, Halade GV, Calhoun C, Escobar GP, Mehr AJ, Jimenez F, Martinez C, Bhatnagar H, Mjaatvedt CH, Lindsey ML *et al.*: Caveolin-1 deletion exacerbates cardiac interstitial fibrosis by promoting M2 macrophage activation in mice after myocardial infarction. *Journal of molecular and cellular cardiology* 2014, **76**:84-93.

24. Hu D, Dong R, Yang Y, Chen Z, Tang Y, Fu M, Wang DW, Xu X, Tu L: Human kallikrein overexpression alleviates cardiac aging by alternatively regulating macrophage polarization in aged rats. *FASEB J* 2019:jf201802371RR.

25. Carlson S, Helterline D, Asbe L, Dupras S, Minami E, Farris S, Stempnie-Otero A: Cardiac macrophages adopt profibrotic/M2 phenotype in infarcted hearts: Role of urokinase plasminogen activator. *Journal of molecular and cellular cardiology* 2017, **108**:42-49.

26. Zouggari Y, Ait-Oufella H, Bonnin P, Simon T, Sage AP, Guérin C, Vilar J, Caligiuri G, Tsiantoulas D, Laurans L *et al.*: B lymphocytes trigger monocyte mobilization and impair heart function after acute myocardial infarction. *Nat Med* 2013, **19**(10):1273-1280.

27. Kvakan H, Kleinewietfeld M, Qadri F, Park JK, Fischer R, Schwarz I, Rahn HP, Plehm R, Wellner M, Elitok S *et al.*: Regulatory T cells ameliorate angiotensin II-induced cardiac damage. *Circulation* 2009, **119**(22):2904-2912.

28. Singh MV, Cicha MZ, Meyerholz DK, Chapleau MW, Abboud FM: Dual Activation of TRIF and MyD88 Adaptor Proteins by Angiotensin II Evokes Opposing Effects on Pressure, Cardiac Hypertrophy, and Inflammatory Gene Expression. *Hypertension* 2015, **66**(3):647-656.

29. Palomer X, Capdevila-Busquets E, Botteri G, Davidson MM, Rodríguez C, Martínez-González J, Vidal F, Barroso E, Chan TO, Feldman AM *et al.*: miR-146a targets Fos expression in human cardiac cells. *Dis Model Mech* 2015, **8**(9):1081-1091.

30. Purcell NH, Wilkins BJ, York A, Saba-El-Leil MK, Meloche S, Robbins J, Molkentin JD: Genetic inhibition of cardiac ERK1/2 promotes stress-induced apoptosis and heart failure but has no effect on hypertrophy in vivo. *Proceedings of the National Academy of Sciences of the United States of America* 2007, **104**(35):14074-14079.

31. Bueno OF, Molkentin JD: Involvement of extracellular signal-regulated kinases 1/2 in cardiac hypertrophy and cell death. *Circulation research* 2002, **91**(9):776-781.

32. Zouein FA, Booz GW, Altara R: **STAT3 and Endothelial Cell-Cardiomyocyte Dialog in Cardiac Remodeling.** *Front Cardiovasc Med* 2019, **6**:50.

33. Chen X, Su J, Feng J, Cheng L, Li Q, Qiu C, Zheng Q: TRIM72 contributes to cardiac fibrosis via regulating STAT3/Notch-1 signaling. *Journal of cellular physiology* 2019.

34. Kumar S, Wang G, Zheng N, Cheng W, Ouyang K, Lin H, Liao Y, Liu J: HIMF (Hypoxia-Induced Mitogenic Factor)-IL (Interleukin)-6 Signaling Mediates Cardiomyocyte-Fibroblast Crosstalk to Promote Cardiac Hypertrophy and Fibrosis. *Hypertension* 2019, **73**(5):1058-1070.
35. Duerr GD, Heinemann JC, Dunkel S, Zimmer A, Lutz B, Lerner R, Roell W, Mellert F, Probst C, Esmailzadeh B et al: **Myocardial hypertrophy is associated with inflammation and activation of endocannabinoid system in patients with aortic valve stenosis.** *Life sciences* 2013, 92(20-21):976-983.

36. Hamid T, Gu Y, Ortines RV, Bhattacharya C, Wang G, Xuan YT, Prabhu SD: **Divergent tumor necrosis factor receptor-related remodeling responses in heart failure: role of nuclear factor-kappaB and inflammatory activation.** *Circulation* 2009, 119(10):1386-1397.

37. Pikkarainen S, Tokola H, Kerkelä R, Ruskoaho H: **GATA transcription factors in the developing and adult heart.** *Cardiovascular research* 2004, 63(2):196-207.

38. Morimoto T, Hasegawa K, Kaburagi S, Kakita T, Masutani H, Kitsis RN, Matsumori A, Sasayama S: **GATA-5 is involved in leukemia inhibitory factor-responsive transcription of the beta-myosin heavy chain gene in cardiac myocytes.** *The Journal of biological chemistry* 1999, 274(18):12811-12818.

39. Boyle JJ, Johns M, Kampfer T, Nguyen AT, Game L, Schaer DJ, Mason JC, Haskard DO: **Activating transcription factor 1 directs Mhem atheroprotective macrophages through coordinated iron handling and foam cell protection.** *Circulation research* 2012, 110(1):20-33.