Identification of transcription factors MYC and C/EBP β mediated regulatory networks in heart failure

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Abstract

Background: Heart failure is one of leading cause of death worldwide. However, the transcriptional profiling of heart failure is unclear. Moreover, the signaling pathways and transcription factors involving the heart failure developmental progress also are largely unclear.

Methods: The transcriptional profiling of heart failure was identified from integrated gene expression datasets. The enriched pathways and transcription factors were analyzed using DAVID and GSEA assay. The transcriptional networks were created by Cytoscape.

Results: Compared with the normal heart tissues, we found 90 genes were particularly differentially expressed in heart failing tissues, and those genes were associated with multiple metabolism pathways and insulin signaling pathway. Metabolism and insulin signaling pathway were both inactivated in heart failing tissues. Transcription factors MYC and C/EBP β were both negatively associated with the expression profiling of heart failing tissues in GSEA assay. Moreover, compared with normal heart tissues, MYC and C/EBP β were down regulated in heart failing tissues. Furthermore, MYC and C/EBP β mediated downstream target genes were decreased in heart failing tissues. MYC and C/EBP β were positively correlated with each other. At last, we constructed the transcription factor MYC and C/EBP β mediated regulatory networks in heart failing tissues, and identified the MYC and C/EBP β target genes which had been reported involving the failure developmental progress by literature research.

Conclusions: Our results suggested that transcription factor MYC and C/EBP β played critical roles in heart failure developmental progress. And new heart failure treatments may be developed by targeting MYC and C/EBP β .

Background

Heart failure is a rapidly growing public health issue and one of leading cause of death worldwide [1]. Serial vicious cycles of cardiomyocyte depletion, cardiac dilatation and mechanical dysfunction are culminating in heart failure [2]. Once patients have developed to the end stage of heart failure, intervention is limited to heart transplantation [3]. In order to understand the molecular mechanisms regulating heart failure, several studies have used microarrays for genome wide analysis of heart failure [4–6]. However, due to the complexity of genetic and epigenetic abnormality of heart failure, the previously reported gene expression signature in heart failing tissues vary considerably from study to study, making it difficult to reconcile their findings or reach any definite conclusions [7]. Moreover, the misregulated molecular signaling pathways and key transcription factors in heart failure are largely unknown.

Transcription factors control the transcriptional activity of multiple target genes by binding to a specific region of the DNA sequence [8, 9]. It has reported that transcription factor C/EBP β plays central roles in physiologic hypertrophy and heart failure [10]. C/EBP β could repress cardiomyocyte growth and proliferation. Reducing C/EBP β expression exaggerates the cardiac failure upon pressure overload [10].

TP53 is another major transcription factor in cardiac transcriptional network [11]. TP53 deficient hearts are resistant to the failure development upon acute pressure overload [12]. Interestingly, both C/EBP β and TP53 are involving tumor developmental progress by regulating metabolism [13, 14] and TGF β signaling pathway [15, 16].

MYC is an oncogene. High level of MYC expression is required for tumor initiation, progression and maintenance [17–19]. MYC regulates multiple critical cellular functions, for example, metabolism [20] and RNA splicing [21]. Inhibition of MYC by BET Bromodomain inhibitor is a promising anti-cancer strategy [22]. Interestingly, BET Bromodomain is a critical regulator of heart failure [23], and BET bromodomain inhibitors could suppresses the development of heart failure by the regulation of the innate inflammatory network [24]. All those results suggest the potentially significant roles of MYC in heart failure. However, the expression of MYC and MYC mediated downstream target genes are not studied in heart failing patients.

Here, by integrating the published heart failure associated expression datasets, we identify 90 genes which are particularly differentially expressed in heart failing tissues. Signaling pathways and transcription factors associated with heart failure are also determined. Our results reveal the importance of MYC and C/EBP β as new therapeutic targets in heart failing patients.

Methods

Data collection

Gene expression series matrix of heart failing tissues and normal heart tissues was downloaded from Gene Expression Omnibus (GEO) website (<https://www.ncbi.nlm.nih.gov/geo/>) with GEO number GSE5406, GSE16499 and GSE68316.

GEO data processing

All the expression datasets were processed separately. A probe was removed if it was not corresponded gene symbol, and the expression values were averaged if multiple probes corresponded to the same gene symbol using R software “plyr” package.

Gene Ontology (GO) enrichment analysis

GO enrichment of signaling pathways and transcription factors analysis was performed using The Database for Annotation, Visualization and Integrated Discovery (DAVID) website (<https://david.ncifcrf.gov>).

Gene set enrichment analysis (GSEA)

GSEA was performed using GSEA 2.0 software. Signaling pathways gene sets and transcription factor targets gene sets were downloaded from the GSEA Web site (<http://www.broad.mit.edu/gsea/index.html>).

Genes ranked by signal-to-noise ratio, and statistical significance was determined by 1,000 gene set permutations.

Heatmap presentation

Heatmaps were created by R software “pheatmap” package. “pheatmap” is a R package available in bioconductor. The clustering scale was determined by "average" method.

Spearman correlation

Spearman correlation was used to study the correlation between C/EBP β and MYC expression by the “lm” method of R software.

C/EBP β and MYC associated transcriptional network

The networks of C/EBP β and MYC downstream target genes were created by Cytoscape GeneMANIA App.

Statistical analysis

The box plots were generated from prims5.0. Statistical analysis was performed using the Student's t test. P value less than 0.05 was chosen to be statistically significant difference unless specifically notified.

Results

The transcriptomic features of heart failure.

To identify the differentially expressed genes during the development of heart failure, we analyzed the expression data of heart failing and normal heart tissues from previously published GEO datasets GSE5460, GSE16499 and GSE68316 [4-6]. Totally, 252 samples were collected, including 36 normal heart tissues and 216 heart failing tissues. First, we analyzed the globe expression profiling of each dataset. Compared with the normal heart tissues, the differentially expressed genes in heart failing tissues ($P < 0.01$) were selected for further studies. This resulted in the identification 2184 differentially expressed genes in GSE5406, 1644 differentially expressed genes in GSE16499 and 3477 differentially expressed genes in GSE68316 dataset (Fig. 1a). Among all the differentially expressed genes, only 4 genes were commonly up regulated and 86 genes were commonly down regulated in GSE5406, GSE16499 and GSE68316 datasets (Fig. 1b). In GSE16499 and GSE68316 datasets, the number of down regulated genes was for more than the up regulated genes (Fig. 1a). In GSE16499 dataset, 1407 genes were suppressed in heart failing tissues. While, only 237 genes were activated in heart failing tissues. Those results suggested that the pathological observations of depletion of cardiomyocytes and loss of mechanical functions in cardiac remodeling were induced by the suppression of heart specific genes.

Metabolism and insulin signaling pathway are inactivated in heart failing patients.

To reveal the functional relevance of the common differentially expressed genes in heart failing tissues, we performed functional signaling pathway enrichment analysis through DAVID [25] and GSEA [26] assay. Pyrimidine, purine metabolism signaling pathway and cysteine, methionine metabolism signaling pathway were highly enriched using the differentially expressed genes through DAVID analysis (Fig. 2a). Heatmap presentations showed that NME1, POLE3, POLD2, ENTPD6, PNP genes from pyrimidine, purine metabolism signaling pathway and LDHA, AHCY, AMD1 genes from cysteine, methionine metabolism signaling pathway were all down regulated in heart failing tissues in GSE5406, GSE16499 and GSE68316 datasets (Fig. 2b). Those results suggested the inactivation of those pathways in the development of heart failure.

Through GSEA analysis, we found that the insulin signaling pathway was negatively correlated with the heart failing expression profiling (Fig. 2c), suggested the inactivation of insulin signaling pathway in the development of heart failure. For example, MAP2K1 is a critical downstream gene of insulin signaling pathway [27, 28]. We showed that MAP2K1 was down regulated in heart failing tissues in GSE5406, GSE16499 and GSE68316 datasets (Fig. 2d).

The association among heart failure, inactivation of metabolism pathways and insulin resistance was well established [29, 30]. The cardiac metabolism, growth and survival in the heart were dependent on insulin signaling pathway [31, 32]. Loss of insulin signaling pathway induced cardiac energy deficiency and structural dysfunction accelerating the heart failure progress [33, 34]. And targeting the cardiac metabolism pathways and insulin-PI3K-Akt signaling pathway demonstrated therapeutic promise in preclinical models of heart disease [35-38]. All those observations confirmed our results derived from the GEO datasets. However, the detailed functions of genes involving the metabolism and insulin signaling pathways should be further studied in heart failure developmental progress.

Transcription factors MYC and C/EBP are negatively associated with in heart failing expression profiling.

Except signaling pathways, the transcription factors enriched in heart failing tissues were also identified through DAVID analysis. We found that transcription factor MYC was highly associated with the differentially expressed genes in GSE5406, GSE16499 and GSE68316 datasets (Fig. 3a). Interestingly, TP53 and E2F were all highly enriched (Fig. 3a). E2F family genes were critical regulators of cell proliferation and cell cycle progression [39, 40]. Also, E2F family genes mediated the cardiac growth and development [41, 42].

Similar results were obtained using GSEA assay. We found that transcription factor MYC was negatively associated with the heart failing expression profiling in all three GEO datasets (Fig. 3b). Additionally, we showed that transcription factor C/EBP was also negatively correlated with the heart failing expression profiling (Fig. 3c).

C/EBP is a CCAAT/enhancer-binding protein transcription factor which regulates cell growth and differentiation [43]. Previous results suggested that C/EBP β was a critical regulator of exercise induced cardiac growth and protected against pathological cardiac remodeling [10]. C/EBP β was also a master

regulator of metabolism pathways [13] and insulin resistance [44, 45]. All those reports highlighted the critical roles of C/EBP β in the development of heart failure. However, the functions of MYC in the development of heart failure are unclear.

Transcription factors MYC and C/EBP β are down regulated in heart failing tissues.

Next, we detected the expression of MYC and C/EBP β in heart failing and normal heart tissues. Previous report showed that MYC was increased in pathological hypertrophy [46]. Inhibition of MYC was a potential therapeutic approach in the treatment of hypertrophic cardiomyopathy [47]. On the contrary, we found the down regulation of MYC expression in heart failing tissues in GSE5406 and GSE16499 datasets (Fig. 4a). Similarly, we found that C/EBP β gene expression was particularly down regulated in heart failing tissues, compared with normal heart tissues in all GSE5406, GSE16499 and GSE68316 datasets (Fig. 4b).

Since MYC and C/EBP β were both down regulated in heart failure tissues, we tested the correlation between MYC and C/EBP β expression in GSE5406 and GSE16499 datasets. We found that C/EBP β expression was positively correlated with MYC expression. Heart tissues with high C/EBP β expression were also with high MYC expression (Fig. 4c). All those results emphasized the importance of MYC and C/EBP β in heart failure development.

MYC and C/EBP β target genes are down regulated in heart failing tissues.

Transcription factors are usually the master regulators of disease and regulate multiple target genes by binding to a specific region of the DNA sequence [8, 9]. In the GSEA assay, we identified 62 MYC target genes and 22 C/EBP β target genes. Consistent with the decreased expression of MYC and C/EBP β in heart failing tissues (Fig. 4a and 4b), MYC target genes were down regulated in heart failing tissues, compared with normal heart tissues (Fig. 5a). As demonstrated in the heatmap, C/EBP β target genes were also particularly down regulated in heart failing tissues in GSE16499 dataset (Fig. 5b).

Interestingly, we found that some genes, for example, EIF4A1, SYNCRIP, ARF6 and C/EBP β , were both MYC and C/EBP β downstream target genes (Fig. 5c). We showed that SYNCRIP gene expression was particularly down regulated in heart failing tissues in all GSE5406, GSE16499 and GSE68316 datasets (Fig. 5d). However, whether SYNCRIP was involving in the heart failure development was unclear.

The MYC and C/EBP β mediated transcriptional networks.

To further explore MYC and its connection to downstream target genes, the MYC mediated regulatory network was constructed using Cytoscape. As expected, as a MYC target gene, C/EBP β was connected with MYC through the transduction of multiple genes (Fig. 6a). Furthermore, through literature research, we found that 9 MYC target genes were previously reported involving the development of heart failure, including STAT3 [48], PRMT1 [49], PRKCH [50], HSPA4 [51], DDX3X [52], GYG1 [53], GADD45B [54] and PKN1 [55] (Fig. 6a, the red ones).

Similarly, the C/EBP β mediated regulatory network was constructed (Fig. 6b). Some C/EBP β target genes, for example, OSMR [56], MAP2K3 [57], CDKN1B [58], PDE4D [59] and DDAH2 [60] also have been studied in heart failure developmental progress (Fig. 6b, the red ones). All those results highlighted the importance of MYC, C/EBP β and their downstream target genes in heart failure development. The functions of other MYC and C/EBP β target genes should be further studied to reveal their connections with heart failure.

Discussion

Complex diseases like heart failure are often involving malfunctions of multiple genes. Disease related genes detected by different microarray studies are often highly inconsistent, even when there is not much technical noise [7]. As described in this paper, compared with normal heart tissues, there are 2184 differentially expressed genes in heart failing tissues in GSE5406, 1644 genes in GSE16499 and 3477 genes in GSE68316 dataset (Fig. 1a). However, only 90 genes are commonly up/down regulated in all three datasets (Fig. 1b). Interestingly, those differentially expressed genes are associated with MYC and C/EBP β transcription factors or metabolism pathways and insulin signaling pathway (Fig. 2 and 3). Those converged transcription factors or signaling pathways may have particularly significant roles in heart failure development than single gene.

Indeed, C/EBP β and MYC themselves and their target genes are all down regulated in heart failing tissues (Fig. 4 and 5). C/EBP β is a master regulator in the development of heart failure [10]. Also, some C/EBP β target genes, for example, MAP2K3 [57] and PDE4D [59] regulate the heart failure developmental progress. The functions of MYC in the regulating of heart failure development are rather complicated. Previous report suggests that inhibition of MYC is a potential therapeutic approach in the treatment of hypertrophic cardiomyopathy [47]. However, we observe the down regulation of MYC expression in heart failing tissues (Fig. 4a). MYC target genes are also decreased in heart failing tissues (Fig. 5a). Those inconsistency further emphasizes the complex transcriptional network regulated by MYC and the complex developmental progress of heart failure. So, based on our analysis, we propose that activation of MYC expression is a potential therapeutic approach in the treatment of heart failure. However, the side effects induced by activation of MYC should be further noticed.

Overall, our results provide the changed expression profiling of metabolism signaling pathway, insulin signaling pathway, transcription factors MYC and C/EBP β in the development of heart failure. Although clinical further validations are needed, our analysis suggests that transcription factor MYC and C/EBP β play critical roles in heart failure developmental progress. And new heart failure treatments may be developed by targeting MYC and C/EBP β .

Conclusion

Metabolism signaling pathway, insulin signaling pathway, transcription factors MYC and C/EBP β were inhibited in heart failure developmental progress.

Declarations

List of abbreviations

GEO: Gene Expression Omnibus; GO: Gene Ontology; DAVID: The Database for Annotation, Visualization and Integrated Discovery; GSEA: Gene set enrichment analysis;

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

HW.W and XR.W designed and performed data analysis. LP.X helped with the data analysis. HW.W and XR.W wrote the manuscript. HC reviewed the manuscript and supervised the work. All listed authors have read and approved the manuscript.

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References

1. Vos T, Flaxman AD, Naghavi M, Lozano R, Michaud C, Ezzati M, Shibuya K, Salomon JA, Abdalla S, Aboyans V *et al*: **Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries**

- 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010.** *Lancet* 2012, **380**(9859):2163-2196.
2. Hashimoto H, Olson EN, Bassel-Duby R: **Therapeutic approaches for cardiac regeneration and repair.** *Nat Rev Cardiol* 2018, **15**(10):585-600.
 3. Lietz K, Miller LW: **Improved survival of patients with end-stage heart failure listed for heart transplantation: analysis of organ procurement and transplantation network/U.S. United Network of Organ Sharing data, 1990 to 2005.** *J Am Coll Cardiol* 2007, **50**(13):1282-1290.
 4. Hannenhalli S, Putt ME, Gilmore JM, Wang J, Parmacek MS, Epstein JA, Morrissey EE, Margulies KB, Cappola TP: **Transcriptional genomics associates FOX transcription factors with human heart failure.** *Circulation* 2006, **114**(12):1269-1276.
 5. Kong SW, Hu YW, Ho JW, Ikeda S, Polster S, John R, Hall JL, Bisping E, Pieske B, dos Remedios CG *et al*: **Heart failure-associated changes in RNA splicing of sarcomere genes.** *Circ Cardiovasc Genet* 2010, **3**(2):138-146.
 6. Yang W, Li Y, He F, Wu H: **Microarray profiling of long non-coding RNA (lncRNA) associated with hypertrophic cardiomyopathy.** *BMC Cardiovasc Disord* 2015, **15**:62.
 7. Li Y, Jiang Q, Ding Z, Liu G, Yu P, Jiang G, Yu Z, Yang C, Qian J, Jiang H *et al*: **Identification of a Common Different Gene Expression Signature in Ischemic Cardiomyopathy.** *Genes (Basel)* 2018, **9**(1).
 8. Bushweller JH: **Targeting transcription factors in cancer - from undruggable to reality.** *Nat Rev Cancer* 2019, **19**(11):611-624.
 9. Liu Z, Merkurjev D, Yang F, Li W, Oh S, Friedman MJ, Song X, Zhang F, Ma Q, Ohgi KA *et al*: **Enhancer activation requires trans-recruitment of a mega transcription factor complex.** *Cell* 2014, **159**(2):358-373.
 10. Bostrom P, Mann N, Wu J, Quintero PA, Plovie ER, Panakova D, Gupta RK, Xiao C, MacRae CA, Rosenzweig A *et al*: **C/EBPbeta controls exercise-induced cardiac growth and protects against pathological cardiac remodeling.** *Cell* 2010, **143**(7):1072-1083.
 11. Das B, Young D, Vasanji A, Gupta S, Sarkar S, Sen S: **Influence of p53 in the transition of myotrophin-induced cardiac hypertrophy to heart failure.** *Cardiovasc Res* 2010, **87**(3):524-534.
 12. Nomura S, Satoh M, Fujita T, Higo T, Sumida T, Ko T, Yamaguchi T, Tobita T, Naito AT, Ito M *et al*: **Cardiomyocyte gene programs encoding morphological and functional signatures in cardiac hypertrophy and failure.** *Nat Commun* 2018, **9**(1):4435.
 13. Takagi M, Uno H, Nishi R, Sugimoto M, Hasegawa S, Piao J, Ihara N, Kanai S, Kakei S, Tamura Y *et al*: **ATM Regulates Adipocyte Differentiation and Contributes to Glucose Homeostasis.** *Cell Rep* 2015, **10**(6):957-967.
 14. Vousden KH, Ryan KM: **p53 and metabolism.** *Nat Rev Cancer* 2009, **9**(10):691-700.
 15. Gomis RR, Alarcon C, Nadal C, Van Poznak C, Massague J: **C/EBPbeta at the core of the TGFbeta cytostatic response and its evasion in metastatic breast cancer cells.** *Cancer Cell* 2006, **10**(3):203-214.

16. Lopez-Diaz FJ, Gascard P, Balakrishnan SK, Zhao J, Del Rincon SV, Spruck C, Tlsty TD, Emerson BM: **Coordinate transcriptional and translational repression of p53 by TGF-beta1 impairs the stress response.** *Mol Cell* 2013, **50**(4):552-564.
17. Gabay M, Li Y, Felsher DW: **MYC activation is a hallmark of cancer initiation and maintenance.** *Cold Spring Harb Perspect Med* 2014, **4**(6).
18. Vita M, Henriksson M: **The Myc oncoprotein as a therapeutic target for human cancer.** *Semin Cancer Biol* 2006, **16**(4):318-330.
19. Schaub FX, Dhankani V, Berger AC, Trivedi M, Richardson AB, Shaw R, Zhao W, Zhang X, Ventura A, Liu Y *et al*: **Pan-cancer Alterations of the MYC Oncogene and Its Proximal Network across the Cancer Genome Atlas.** *Cell Syst* 2018, **6**(3):282-300 e282.
20. Thai M, Thaker SK, Feng J, Du Y, Hu H, Ting Wu T, Graeber TG, Braas D, Christofk HR: **MYC-induced reprogramming of glutamine catabolism supports optimal virus replication.** *Nat Commun* 2015, **6**:8873.
21. Koh CM, Bezzi M, Low DH, Ang WX, Teo SX, Gay FP, Al-Haddawi M, Tan SY, Osato M, Sabo A *et al*: **MYC regulates the core pre-mRNA splicing machinery as an essential step in lymphomagenesis.** *Nature* 2015, **523**(7558):96-100.
22. Delmore JE, Issa GC, Lemieux ME, Rahl PB, Shi J, Jacobs HM, Kastiris E, Gilpatrick T, Paranal RM, Qi J *et al*: **BET bromodomain inhibition as a therapeutic strategy to target c-Myc.** *Cell* 2011, **146**(6):904-917.
23. Anand P, Brown JD, Lin CY, Qi J, Zhang R, Artero PC, Alaiti MA, Bullard J, Alazem K, Margulies KB *et al*: **BET bromodomains mediate transcriptional pause release in heart failure.** *Cell* 2013, **154**(3):569-582.
24. Duan Q, McMahon S, Anand P, Shah H, Thomas S, Salunga HT, Huang Y, Zhang R, Sahadevan A, Lemieux ME *et al*: **BET bromodomain inhibition suppresses innate inflammatory and profibrotic transcriptional networks in heart failure.** *Sci Transl Med* 2017, **9**(390).
25. Huang da W, Sherman BT, Lempicki RA: **Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources.** *Nat Protoc* 2009, **4**(1):44-57.
26. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES *et al*: **Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles.** *Proc Natl Acad Sci U S A* 2005, **102**(43):15545-15550.
27. Xu J, Keeton AB, Franklin JL, Li X, Venable DY, Frank SJ, Messina JL: **Insulin enhances growth hormone induction of the MEK/ERK signaling pathway.** *J Biol Chem* 2006, **281**(2):982-992.
28. Ueyama A, Ban N, Fukazawa M, Hirayama T, Takeda M, Yata T, Muramatsu H, Hoshino M, Yamamoto M, Matsuo M *et al*: **Inhibition of MEK1 Signaling Pathway in the Liver Ameliorates Insulin Resistance.** *J Diabetes Res* 2016, **2016**:8264830.
29. Bertero E, Maack C: **Metabolic remodelling in heart failure.** *Nat Rev Cardiol* 2018, **15**(8):457-470.
30. Boudina S, Bugger H, Sena S, O'Neill BT, Zaha VG, Ilkun O, Wright JJ, Mazumder PK, Palfreyman E, Tidwell TJ *et al*: **Contribution of impaired myocardial insulin signaling to mitochondrial dysfunction**

- and oxidative stress in the heart.** *Circulation* 2009, **119**(9):1272-1283.
31. Chokshi A, Drosatos K, Cheema FH, Ji R, Khawaja T, Yu S, Kato T, Khan R, Takayama H, Knoll R *et al*: **Ventricular assist device implantation corrects myocardial lipotoxicity, reverses insulin resistance, and normalizes cardiac metabolism in patients with advanced heart failure.** *Circulation* 2012, **125**(23):2844-2853.
 32. Guo CA, Guo S: **Insulin receptor substrate signaling controls cardiac energy metabolism and heart failure.** *J Endocrinol* 2017, **233**(3):R131-R143.
 33. Belke DD, Betuing S, Tuttle MJ, Graveleau C, Young ME, Pham M, Zhang D, Cooksey RC, McClain DA, Litwin SE *et al*: **Insulin signaling coordinately regulates cardiac size, metabolism, and contractile protein isoform expression.** *J Clin Invest* 2002, **109**(5):629-639.
 34. van Berlo JH, Maillet M, Molkentin JD: **Signaling effectors underlying pathologic growth and remodeling of the heart.** *J Clin Invest* 2013, **123**(1):37-45.
 35. Luo J, McMullen JR, Sobkiw CL, Zhang L, Dorfman AL, Sherwood MC, Logsdon MN, Horner JW, DePinho RA, Izumo S *et al*: **Class IA phosphoinositide 3-kinase regulates heart size and physiological cardiac hypertrophy.** *Mol Cell Biol* 2005, **25**(21):9491-9502.
 36. Oudit GY, Crackower MA, Eriksson U, Sarao R, Kozieradzki I, Sasaki T, Irie-Sasaki J, Gidrewicz D, Rybin VO, Wada T *et al*: **Phosphoinositide 3-kinase gamma-deficient mice are protected from isoproterenol-induced heart failure.** *Circulation* 2003, **108**(17):2147-2152.
 37. McMullen JR, Amirahmadi F, Woodcock EA, Schinke-Braun M, Bouwman RD, Hewitt KA, Mollica JP, Zhang L, Zhang Y, Shioi T *et al*: **Protective effects of exercise and phosphoinositide 3-kinase(p110alpha) signaling in dilated and hypertrophic cardiomyopathy.** *Proc Natl Acad Sci U S A* 2007, **104**(2):612-617.
 38. DeBosch B, Sambandam N, Weinheimer C, Courtois M, Muslin AJ: **Akt2 regulates cardiac metabolism and cardiomyocyte survival.** *J Biol Chem* 2006, **281**(43):32841-32851.
 39. Liu H, Tang X, Srivastava A, Pecot T, Daniel P, Hemmelgarn B, Reyes S, Fackler N, Bajwa A, Kladney R *et al*: **Redeployment of Myc and E2f1-3 drives Rb-deficient cell cycles.** *Nat Cell Biol* 2015, **17**(8):1036-1048.
 40. Morgunova E, Yin Y, Jolma A, Dave K, Schmierer B, Popov A, Eremina N, Nilsson L, Taipale J: **Structural insights into the DNA-binding specificity of E2F family transcription factors.** *Nat Commun* 2015, **6**:10050.
 41. King JC, Moskowitz IP, Burgon PG, Ahmad F, Stone JR, Seidman JG, Lees JA: **E2F3 plays an essential role in cardiac development and function.** *Cell Cycle* 2008, **7**(23):3775-3780.
 42. Major JL, Dewan A, Salih M, Leddy JJ, Tuana BS: **E2F6 Impairs Glycolysis and Activates BDH1 Expression Prior to Dilated Cardiomyopathy.** *PLoS One* 2017, **12**(1):e0170066.
 43. Zahnow CA: **CCAAT/enhancer-binding protein beta: its role in breast cancer and associations with receptor tyrosine kinases.** *Expert Rev Mol Med* 2009, **11**:e12.
 44. Tian J, Goldstein JL, Brown MS: **Insulin induction of SREBP-1c in rodent liver requires LXRAalpha-C/EBPbeta complex.** *Proc Natl Acad Sci U S A* 2016, **113**(29):8182-8187.

45. Wang L, Shao J, Muhlenkamp P, Liu S, Klepcyk P, Ren J, Friedman JE: **Increased insulin receptor substrate-1 and enhanced skeletal muscle insulin sensitivity in mice lacking CCAAT/enhancer-binding protein beta.** *J Biol Chem* 2000, **275**(19):14173-14181.
46. Trenerry MK, Carey KA, Ward AC, Cameron-Smith D: **STAT3 signaling is activated in human skeletal muscle following acute resistance exercise.** *J Appl Physiol (1985)* 2007, **102**(4):1483-1489.
47. Wolfram JA, Lesnefsky EJ, Hoit BD, Smith MA, Lee HG: **Therapeutic potential of c-Myc inhibition in the treatment of hypertrophic cardiomyopathy.** *Ther Adv Chronic Dis* 2011, **2**(2):133-144.
48. Patel NJ, Nassal DM, Greer-Short AD, Unudurthi SD, Scandling BW, Gratz D, Xu X, Kalyanasundaram A, Fedorov VV, Accornero F *et al*: **betaIV-Spectrin/STAT3 complex regulates fibroblast phenotype, fibrosis, and cardiac function.** *JCI Insight* 2019, **4**(20).
49. Pyun JH, Kim HJ, Jeong MH, Ahn BY, Vuong TA, Lee DI, Choi S, Koo SH, Cho H, Kang JS: **Cardiac specific PRMT1 ablation causes heart failure through CaMKII dysregulation.** *Nat Commun* 2018, **9**(1):5107.
50. Ji F, Liu Q, Feng Z, Han X, Li Z: **Genetic association between 1425G/A SNP in PRKCH and hypertrophic cardiomyopathy in a Chinese population.** *Oncotarget* 2017, **8**(70):114839-114844.
51. Mohamed BA, Barakat AZ, Zimmermann WH, Bittner RE, Muhlfeld C, Hunlich M, Engel W, Maier LS, Adham IM: **Targeted disruption of Hspa4 gene leads to cardiac hypertrophy and fibrosis.** *J Mol Cell Cardiol* 2012, **53**(4):459-468.
52. Molina-Navarro MM, Trivino JC, Martinez-Dolz L, Lago F, Gonzalez-Juanatey JR, Portoles M, Rivera M: **Functional networks of nucleocytoplasmic transport-related genes differentiate ischemic and dilated cardiomyopathies. A new therapeutic opportunity.** *PLoS One* 2014, **9**(8):e104709.
53. Hedberg-Oldfors C, Glamuzina E, Ruygrok P, Anderson LJ, Elliott P, Watkinson O, Occleshaw C, Abernathy M, Turner C, Kingston N *et al*: **Cardiomyopathy as presenting sign of glycogenin-1 deficiency-report of three cases and review of the literature.** *J Inherit Metab Dis* 2017, **40**(1):139-149.
54. Steenbergen C, Afshari CA, Petranka JG, Collins J, Martin K, Bennett L, Haugen A, Bushel P, Murphy E: **Alterations in apoptotic signaling in human idiopathic cardiomyopathic hearts in failure.** *Am J Physiol Heart Circ Physiol* 2003, **284**(1):H268-276.
55. Sakaguchi T, Takefuji M, Wettschureck N, Hamaguchi T, Amano M, Kato K, Tsuda T, Eguchi S, Ishihama S, Mori Y *et al*: **Protein Kinase N Promotes Stress-Induced Cardiac Dysfunction Through Phosphorylation of Myocardin-Related Transcription Factor A and Disruption of its Interaction with Actin.** *Circulation* 2019.
56. Hu J, Zhang L, Zhao Z, Zhang M, Lin J, Wang J, Yu W, Man W, Li C, Zhang R *et al*: **OSM mitigates post-infarction cardiac remodeling and dysfunction by up-regulating autophagy through Mst1 suppression.** *Biochim Biophys Acta Mol Basis Dis* 2017, **1863**(8):1951-1961.
57. Li Y, Li Z, Zhang C, Li P, Wu Y, Wang C, Bond Lau W, Ma XL, Du J: **Cardiac Fibroblast-Specific Activating Transcription Factor 3 Protects Against Heart Failure by Suppressing MAP2K3-p38 Signaling.** *Circulation* 2017, **135**(21):2041-2057.

58. Sun X, Momen A, Wu J, Noyan H, Li R, von Harsdorf R, Husain M: **p27 protein protects metabolically stressed cardiomyocytes from apoptosis by promoting autophagy.** *J Biol Chem* 2014, **289**(24):16924-16935.
59. Idres S, Perrin G, Domergue V, Lefebvre F, Gomez S, Varin A, Fischmeister R, Leblais V, Manoury B: **Contribution of BKCa channels to vascular tone regulation by PDE3 and PDE4 is lost in heart failure.** *Cardiovasc Res* 2019, **115**(1):130-144.
60. Zhou R, Ma P, Xiong A, Xu Y, Wang Y, Xu Q: **Protective effects of low-dose rosuvastatin on isoproterenol-induced chronic heart failure in rats by regulation of DDAH-ADMA-NO pathway.** *Cardiovasc Ther* 2017, **35**(2).

Figures

Figure 1

The transcriptomic features of heart failure (a) Un-supervised clustering heatmaps represented the differentially expressed genes ($P < 0.01$) in heart failing tissues compared to normal heart tissues in GSE5406, GSE16499 and GSE68316 datasets. Each of the columns represented one patient sample. Genes up-regulated (red), down-regulated (blue,) and moderately regulated (yellow and green) genes were delineated. Number of patient sample from each dataset was also showed. (b) Venny diagrams depicted

the overlapped differentially expressed genes in GSE5406, GSE16499 and GSE68316 datasets. Red cycle represented the up regulated genes in heart failing tissue, and green cycle represented the down regulated genes in heart failing tissue.

Figure 2

Metabolism and insulin signaling pathway are inactivated in heart failing patients. (a) Functional pathways enrichment analysis of the common differentially expressed 90 genes in heart failing tissues.

(b) Differentially expressed genes from cysteine, methionine, pyrimidine and purine metabolism signaling pathway were represented using heatmaps. (c) Enrichment plots showed the insulin signaling pathway in GSE5406, GSE16499 and GSE68316 datasets. Enrichment of NES and P values were shown. (d) Box plots showed the expression of MAP2K1 in GSE5406, GSE16499 and GSE68316 datasets. P values showed the difference of MAP2K1 expression between heart failing tissues and normal heart tissues determined by Student's t test.

Figure 3

Transcription factors MYC and C/EBP are negatively associated with in heart failing expression profiling. (a) Functional transcription factor enrichment analysis of the common differentially expressed 90 genes in heart failing tissues. (b) Enrichment plots of transcription factor MYC in GSE5406, GSE16499 and GSE68316 datasets. Enrichment of NES and P values were shown. (c) Enrichment plots of transcription factor C/EBP in GSE5406, GSE16499 and GSE68316 datasets.

Figure 4

Transcription factors MYC and C/EBP β are down regulated in heart failing tissues. (a) Box plots showed the transcription factor MYC expression. P values showed the difference of genes expression between heart failing and normal heart tissues determined by Student's t test. (b) Box plots showed the transcription factor C/EBP β expression. (c) Pearson correlation between MYC and C/EBP β expression in GSE5406 and GSE16499 datasets. Adjusted R-square and P value were shown.

Figure 5

MYC and C/EBP β target genes are down regulated in heart failing tissues. (a) Heatmap demonstrated the expression profile of MYC target genes in heart failing and normal heart tissues in GSE16499 dataset. Genes up-regulated (red), down-regulated (blue,) and moderately regulated (yellow and green) genes were delineated. (b) Heatmap demonstrated the expression profile of C/EBP β target genes in heart failing and

normal heart tissues in GSE16499 dataset. (c) Venny diagram depicted the four overlapped MYC and C/EBP β target genes. (d) Box plots showed the expression of SYNCRIP.

Figure 6

The MYC and C/EBP β mediated transcriptional networks. (a) MYC mediated regulatory network was created by cytoscape using MYC target genes. The red ones implied that those genes were previously

reported involving the development of heart failure. (b) C/EBP β mediated regulatory network was created by cytoscape using C/EBP β target genes.