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Bioinformatics analysis of hereditary disease gene set to identify key modulators of myocardial remodeling during heart regeneration in zebrafish

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Abstract

Unlike mammals, adult zebrafish hearts retain a remarkable capacity to regenerate after injury. Since regeneration shares many common molecular pathways with embryonic development, we investigated myocardial remodeling genes and pathways by performing a comparative transcriptomic analysis of zebrafish heart regeneration using a set of known human hereditary heart disease genes related to myocardial hypertrophy during development. We cross-matched human *hypertrophic cardiomyopathy*-associated genes with a time-course microarray dataset of adult zebrafish heart regeneration. Genes in the expression profiles that were highly elevated in the early phases of myocardial repair and remodeling after injury in zebrafish were identified. These genes were further analyzed with web-based bioinformatics tools to construct a regulatory network revealing potential transcription factors and their upstream receptors. *In silico* functional analysis of these genes showed that they are involved in cardiomyocyte proliferation and differentiation, angiogenesis, and inflammation-related pathways. The regulatory network indicated that β-2-microglobulin-mediated signaling may play an important role in myocardial remodeling after injury. This novel cross-species bioinformatics approach to uncover key modulators of zebrafish heart regeneration through human hereditary disease genomic

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analysis could greatly facilitate the understanding of the evolutionarily conserved cardiac remodeling process.

1 Introduction

Myocardial infarction (MI) continues to be the leading cause of death from cardiovascular diseases worldwide. Patients who survive an MI have significantly elevated risks of recurrent MIs, heart failure and death[1]. After injury, the human heart is unable to regenerate itself, and the damaged myocardial tissues are replaced by fibrous scar tissue. Loss of myocardial cells results in decreased contractile function and unfavorable ventricular remodeling, which eventually leads to congestive heart failure and poor clinical outcomes[2],[3]. Additional research to develop new strategies to facilitate cardiomyocyte replacement and functional recovery is urgently needed. However, the current understanding of heart regeneration remains limited.

Unlike that of mammals, the zebrafish heart has an astonishing ability to regenerate itself with minimal scarring after 20% ventricular resection[4], and the zebrafish has thus become a popular model organism for vertebrate regeneration research. Evidence suggests that this robust regenerative response is mediated by complex developmental pathways, including the TGF-β, PDGF and FGF signaling pathways[5]. Studies have also indicated that preexisting cardiomyocytes surrounding the injured area undergo dedifferentiation and subsequent proliferation to replace the initial fibrotic scar tissue and eventually restore cardiac function[6, 7]. Surprisingly, neonatal mouse hearts also exhibit such regenerative capabilities through the proliferation of new cardiomyocytes from preexisting cardiomyocytes[8], a process similar to that in adult zebrafish. These observations imply that zebrafish heart regeneration shares common or conserved signaling pathways with mammalian heart development. However, many pathways involved in the complex process of heart regeneration have yet to be elucidated.

To examine the molecular interactions that occur in the myocardial remodeling process during heart regeneration, we developed a novel human hereditary disease gene-based approach to identify significant cardiac regeneration genes using time-course microarray data obtained during zebrafish heart regeneration. A recent study supports zebrafish as a relevant model for studying cardiomyopathy [9]. This approach was based on the hypothesis that during regeneration, injured tissue may undergo a process called embryonic recall[10]. In other words, the damaged heart must undergo cell division and differentiation/dedifferentiation, processes that are likely to be regulated by genes and networks related to critical developmental pathways. Moreover, there is evidence suggesting overlap between the pathways regulating cell division and those regulating cardiac hypertrophy[11]. On the basis of such correlations, causal genes for human hypertrophic cardiomyopathy (HCM), which cause abnormal myocardial cell growth during the developmental process, were exploited to search for myocardial remodeling genes involved in the regeneration process in zebrafish hearts[12, 13]. With these genes, we then systematically analyzed temporal expression profiles to identify key modulators and potential driver pathways. Our study establishes a new bioinformatics approach that could facilitate and guide future studies of heart regeneration given the conservation among developmental and regenerative processes.

2 Methods

2.1 Gene expression microarray dataset for zebrafish heart regeneration

Time-course microarray data following wound healing in the zebrafish heart have been published previously (NCBI GEO Dataset: GSE72348)[14]. Briefly, gene expression data for adult zebrafish hearts subjected to ventricular resection were obtained for 0, 0.25, 1, 3, 6, 10, 15, 21, 28 dpa (days post amputation). Each time point comprised two biological replicates.

2.2 Human hereditary disease databases and human-zebrafish homologous gene analysis

HCM genes were obtained from the HUGO Gene Nomenclature Committee (HGNC) (www.genenames.org), GeneCards (www.genecards.org) and the Genetic Association Database (GAD; geneticassociationdb.nih.gov). These databases provide detailed descriptions of the relationship between HCM and various genes along with the relevant publications.

Homologous gene analysis was performed based on public data from the NCBI (www.ncbi.nlm.nih.gov) website. We mapped human and zebrafish genes that were conserved in both species using online tools from the UniGene (www.ncbi.nlm.nih.gov/unigene), GeneCards, and HomoloGene (www.ncbi.nlm.nih.gov/homologene) databases. A Basic Local Alignment Search Tool (BLAST) analysis was performed for those genes without hits in the databases. All identified zebrafish homologues of HCM-associated genes were included in the analysis of gene expression profile.

2.3 Microarray data analysis with pattern-matching programs

To identify specific genes involved in the myocardial remodeling process during heart regeneration, we examined expression profiles related to certain phases of tissue regeneration that are shared by cardiac hypertrophy, which occurs after cardiac injury in zebrafish. Previous studies have already identified the characteristic gene expression profiles of fibroplasia and granulation tissue formation during the wound healing process[15, 16]. Therefore, we selected two of the specific profiles: the early-phase and acute response profiles. The early-phase profile matches to the expression pattern of fibroplasias and granulation tissue formation, and the acute response profile contains the regulatory genes switching on the genes expression of the early-phase response profile.

Genes involved in the early phase or acute response stage of the heart regeneration process were identified using the Short Time-series Expression Miner (STEM) program (www.cs.cmu.edu/~jernst/stem). Briefly, STEM clustered the genes according to their temporal gene expression patterns in the time-course microarray data into one single profile with the closest pattern[17]. Molecular pathways that were overrepresented in the profiles of genes upregulated after ventricular amputation were identified and analyzed with the PANTHER (Protein Analysis THrough Evolutionary Relationships) system, which classifies proteins based on their molecular functions, biological processes and signaling pathways (www.pantherdb.org). Subsequently, the transcription factors (TFs), TF binding sites (TFBSs) of myocardial remodeling genes, and upstream TF receptors were identified with oPOSSUM (opossum.cisreg.ca/oPOSSUM3) and QIAGEN GeneGlobe (www.qiagen.com/geneglobe). A summary flowchart of the informatics analyses in this study is shown in Figure 1A.

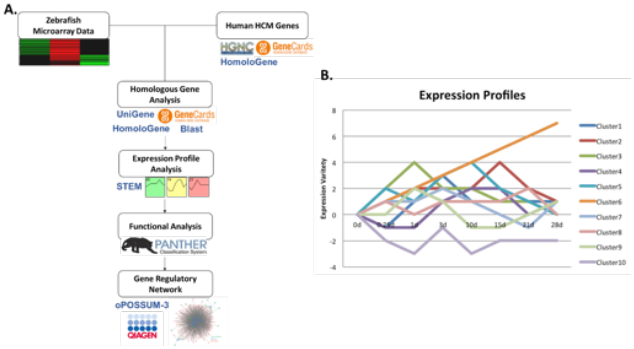

Figure 1: Expression profiles of human hypertrophic cardiomyopathy (HCM) genes that are reactivated during heart remodeling. (A) The expression profiles were obtained by database mining and array matching. The human HCM genes, 337 in total were mined from the GeneCards and GAD databases. By combining the DNA microarray data with zebrafish homologues of HCM genes, which were mapped via NCBI BLAST, we were able to obtain rough expression profiles related to heart remodeling. (B) A simplified diagram of the HCM expression profiles. Analogous expression profiles are classified as a cluster, and here, the profiles can be roughly divided into ten clusters arranged by profile numbers.

3 Results

3.1 Expression profiles of human hypertrophic cardiomyopathy genes reactivated during heart regeneration

To define the transcriptome of zebrafish heart regeneration and to identify key genes and pathways active in primary processes such as cardiomyocyte differentiation, we screened the zebrafish heart regeneration microarray dataset with human HCM genes. Previous studies have noted that several driver pathways involved in the zebrafish heart development process are reactivated during heart regeneration after injury[18, 19]. Therefore, HCM, a hereditary disease that is caused by abnormal myocardial development, was exploited to discover transcripts that may not have been otherwise recognized. Accordingly, we mined human HCM genes from the online databases HGNC, GeneCards and GAD using hypertrophic cardiomyopathy as keywords, and obtained a total of 337 HCM-associated genes that were extracted from the relevant research.

All zebrafish homologues of the human HCM-associated genes were mapped with either online HomoloGene and zfin database or the NCBI BLAST analysis. By cross-matching zebrafish homologues of HCM-associated genes with the zebrafish heart regeneration microarray dataset, we obtained complete gene expression profiles arranged in various clusters of coexpression. The genes in these clusters represented candidate zebrafish homologues for human HCM genes that might be reactivated during heart regeneration (Fig. 1B).

3.2 Identification of the myocardial remodeling genes involved in heart regeneration

The temporal patterns of profile numbers 40, 14, and 45 corresponded with the early-phase response profile which showed elevated expression at 1 dpa, continued to be expressed during the

remodeling process, and then showed decreased expression at approximately 20-28 dpa. These profiles yielded 48 genes among the matched expression clusters with positive correlations (Table 1; Fig. 2A). Functional analysis showed that, consistent with published findings these genes were mainly enriched in pathways such as the TGF-β, EGFR, gonadotropin releasing hormone receptor (GnHR), integrin signaling, and inflammation-related pathways (Table 2). Notably, the TGF-β signaling pathway has been verified to play an essential role in cell proliferation and differentiation during the zebrafish regeneration process[7, 20].

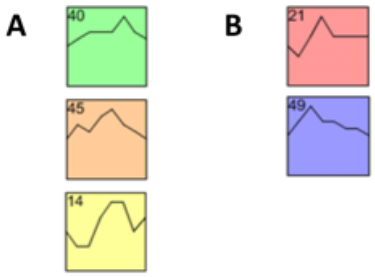

Figure 2: Identification of the myocardial remodeling genes involved in heart regeneration.
(A) Profiles matched by STEM that correspond to the early-phase response profile. There is a total of 48 cardiomyocyte remodeling genes involved in heart regeneration from profiles 40, 14 and 45. **P*<0.01. (B) Profiles matched by STEM that correspond to the acute response profile that regulate the genes in Figure 2A. There is a total of 105 regulatory gene candidates from profiles 21 and 49. **P*<0.01.

(A) Myocardial remodeling genes (corresponding to Fig. 2A)

ACTA1	MYLK2
CACNA1C	MYOM1
CACNA2D2	NDUFAF1
CACNG7	NDUFS4
CASQ2	NDUFV2
CHKA	NRAP
COX5A	PDHA1
CS	PLN
CSRP1	PRKAG2
EGFR	PVALB
FAM188A	SGCB
FKBP1B	SLC17A5
FLT1	SLC25A3
HRAS	TNNC1
HSPB8	TNNI1
IGFBP3	TNNT2
IRX4	TPM1
ITGA5	TPM4
ITGA8	
MAP1LC3A	
MAPK10	
MAPK14	
MEF2A	
MEF2C	
MPP2	
MSTN	
MYBPC1	

(B) Regulatory genes (corresponding to Fig. 2B)

ACTB	FASLG	MTO1	STAT3
ACTN1	FHOD3	MYL7	SURF1
ACTN2	FKTN	MYL9	TAZ
AGK	FXN	MYO6	TCP1
AGL	GATA4	NKX2-5	TGFB3
ANXA5	GLA	NOS2	TGM2
APEH	GNAI1	NPPA	TIMP2
AR	GRB2	NR3C2	TLN1
AUH	HTR2A	PCNA	TNC
B3GAT3	IFNA2	PDLIM3	TP53
BIRC5	ILK	PMM2	TP63
CACNA2D1	INS	POMC	TPM3
CACNB4	ITGA3	PRKAB1	TTN
CACNG2	ITGAV	PRKAB2	VCL
CACNG6	ITGB1	PRKACA	VDAC1
CAMK2G	ITGB5	PRKAG1	
CAV1	JAK2	PTEN	
CAV3	JUN	PTPN11	
CCL2	KRAS	PYGB	
CD36	LAMP2	REN	
CIB1	LARP7	RYR1	
COL1A1	LMNA	SCO2	
COL1A2	MAP2K1	SGCD	
COX15	MAP2K2	SGCG	
CTNNB1	MAPK7	SLC22A5	
DAG1	MAPK8	SLC6A4	
DES	MCM10	SLC9A1	

MYBPC3	DMD	MMP2	SMC1A
MYH6	ERBB2	MRPL3	SOS1
MYH7	ESR1	MRPL44	SRI

Table 1: The myocardial remodeling genes identified by STEM.

A

Pathway	Genes
TGF-β	HRAS, MAPK10, MAPK14, MSTN
EGFR	HRAS, MAPK10, MAPK14, MSTN
GnRH	CACNA1C, EGFR, HRAS, MAPK14, PRKAG2
Integrin	HRAS, ITGA5, ITGA8, MAPK10
Cytoskeletal regulation	ACTA1, MYH6, MYH7, MYLK2
Inflammation	ACTA1, MYH7, MYHR, MYLK2
nAChRs	ACTA1, CACNA1C, MYH6, MYH7

B

Pathway	Genes
Angiogenesis	BIRC5, CTNNB1, JUN, KRAS, MAP2K1, MAP2K2, MAPK8, PTPN11, SOS1, STAT3
CCKR	CTNNB1, ITGAV, ITGB1, JAK2, JUN, MAP2K1, MAP2K2, MAPK7, MAPK8, PRKACA, PTEN, PTPN11, RYR1, SOS1, STAT3
GnRH	ANXA5, AR, CAV1, CTNNB1, GATA4, GNAI1, ITGB1, JUN, MAP2K1, MAP2K2, MAPK8, PRKAB1, PRKAB2, PRKAG1, SOS1, STAT3, VCL
Inflammation	ACTB, CAMK2G, CCL2, GNAI1, ITGB1, JAK2, JUN, KRAS, PRKACA, PTEN, SOS1, STAT3,
Integrin	ACTB, ACTN1, ACTN2, CAV1, COL1A1, COL1A2, ILK, ITGA3, ITGAV, ITGB1, ITGB5, KRAS, MAP2K1, MAP2K2, MAPK8, SOS1, TLN1, VCL
PDGF	JAK2, JUN, KRAS, MAP2K1, MAP2K2, MAPK7, MAPK8, SOS1, STAT3

Table 2: Representative myocardial remodeling genes involved in heart regeneration and their related pathways from PANTHER. (A) Analysis of the early-phase response genes from PANTHER. The first column includes the related pathways, and the second column includes the genes participating in each pathway. (B) Analysis of the acute response genes from PANTHER.

The acute response profile contained regulatory genes that trigger the expression of genes in the early-phase profile. A total of 109 genes identified in the acute response stage from STEM profile numbers 21 and 49 (Table 1; Fig. 2B) were involved in the PDGF and angiogenesis-related pathways as well as in the GnRH, integrin signaling and inflammation-related pathways identified for the early-phase profile (Table 2).

3.3 Construction of the gene regulatory network for myocardial recovery

To further investigate the myocardial remodeling process in the early phase and the acute response stage of regeneration, we constructed a regulatory network using the TF binding site-detecting tool oPOSSUM-3. Unlike other TF-detecting tools, oPOSSUM analyzes whole sets of genes to identify the TFs of coexpressed genes. Moreover, the TFs are scored based on a comparison between the number of detected TFBS nucleotides in the target genes and the oPOSSUM background dataset to provide the significance of the TF results. Accordingly, we obtained 116 TFs that were potential regulatory TFs of our previously selected genes in the early-phase and acute response profiles and ranked them by Z-score (Table 3). A higher Z-score indicates an increased rate of occurrence of a given TFBS in the foreground sequence set of co-expressed genes using a simple binominal

distribution model[21, 22]. Again, the transcriptome analysis yielded key molecules involved in both development and heart regeneration, including MEF2A and Nkx2-5[16, 23]. These results indicate that the human HCM disease gene-based search approach can effectively uncover myocardial remodeling-related targets that are important during heart regeneration and thus support the use of our methods to facilitate heart regeneration research.

TF	Z-score	TF	Z-score	TF	Z-score	TF	Z-score
MEF2A	26.217	RORA_2	10.544	SP1	5.369	RXRA::VDR	0.09
TBP	26.18	RORA_1	10.529	Gfi	5.334	ZNF354C	-0.089
Nkx2-5	23.37	CEBPA	10.524	REL	5.162	ZEB1	-0.111
SRF	21.782	PBX1	10.176	MZF1_5-13	4.903	ELK1	-0.476
FOXI1	21.658	ESR1	10.038	STAT1	4.822	E2F1	-0.522
Pdx1	21.17	Tal1::Gata1	9.912	NR3C1	4.65	RXR::RAR_DR5	-0.566
HOXA5	18.841	NFE2L2	9.355	RELA	4.603	Pax4	-0.942
Nobox	18.697	Lhx3	9.105	HNF4A	4.575	NHLH1	-1.103
Gata1	18.503	FEV	9.062	Sox2	3.805	REST	-1.529
SRY	17.239	Pax6	8.642	YY1	3.547	TP53	-1.675
Prrx2	16.632	FOXD1	8.58	IRF2	3.325	Tcfcp2l1	-1.882
FOXA1	16.631	SPI1	8.204	Nr2e3	3.239	Stat3	-1.971
NKX3-1	16.148	Hand1::Tcfe2a	8.063	znf143	3.132	MAX	-2.119
Nkx3-2	15.967	NR4A2	7.624	HLF	3.008	Zfx	-3.243
Sox5	15.465	HNF1B	7.57	T	2.894	MYC::MAX	-3.378
ARID3A	15.381	NR2F1	7.481	CREB1	2.772	Egr1	-3.598
Foxa2	15.204	Spz1	7.383	Myf	2.723	USF1	-3.804
FOXO3	14.329	PPARG	7.214	Zfp423	2.632	HIF1A::ARNT	-3.852
AP1	13.836	NF-kappaB	6.977	Myb	2.494	ELK4	-3.886
Pou5f1	13.793	MIZF	6.915	NFKB1	2.461	Arnt	-4.08
Sox17	12.885	Arnt::Ahr	6.696	EWSR1-FLI1	2.336	NR1H2::RXRA	-4.313
SPIB	12.661	CTCF	6.641	IRF1	2.157	NFYA	-5.702
Foxd3	12.404	NFIL3	6.28	Klf4	2.092	Pax5	-7.311
ELF5	11.948	NFATC2	6.247	INSM1	2.045	GABPA	-7.369
SOX9	11.732	TEAD1	6.146	TAL1::TCF3	1.422	Myc	-8.078
PPARG::RXRA	11.716	RREB1	6.099	TLX1::NFIC	1.015	Mycn	-10.174
RUNX1	11.153	ESR2	5.932	MZF1_1-4	0.495		
Foxq1	10.654	EBF1	5.664	Ar	0.48		
FOXF2	10.599	Esrrb	5.551	Evi1	0.401		
HNF1A	10.565	Ddit3::Cebpa	5.536	PLAG1	0.362		

Table 3: Transcription factors identified by oPOSSUM.

3.4 β-2-Microglobulin signaling pathways may play a significant role in regulating myocardial remodeling during heart regeneration

We next identified the upstream receptor signaling pathways that lead to the activation of these TFs using QIAGEN GeneGlobe. The QIAGEN GeneGlobe database provides interaction networks for target gene inputs that include upstream or downstream proteins and chemicals, and these results

can be further filtered by directionality and molecular type. Through this analysis, several upstream receptors of our TFs were revealed and were ranked by the TF Z-scores (Table 4).

Receptors	Z-score	Receptors	Z-score	Receptors	Z-score	Receptors	Z-score
B2M	26.217	MUC1	14.329	ITGB3	11.153	CD36	7.214
FAS	21.782	CTLA4	14.329	CD28	10.544	LEPR	7.214
IL1R1	21.782	IL6ST	14.329	TLR9	10.524	ICAM3	7.214
TNFRSF12A	21.782	IGF1R	14.329	PRLR	10.038	KLRK1	7.214
PTCH1	21.17	TNFRSF11A	13.793	LGR4	10.038	BAFFR	6.977
KIT	18.503	Pdgfr	13.793	TNFRSF1A	9.355	BCR	6.977
EPOR	18.503	Notch	13.793	Fgfr	8.642	TCR	6.977
BTNL2	18.503	GPC1	12.885	TLR4	8.204	TLRs	6.977
CAV1	16.631	CD247	11.948	TREM1	7.624	IL-1R	6.977
LRP5	16.631	SFRP1	11.732	NCR2	7.624	GFR	6.977

Table 4: Upstream receptors that lead to the activation of target TFs.

Our data showed that a β-2-microglobulin (B2M)-related signaling pathway was likely to be a potential modulator of zebrafish myocardial remodeling after ventricular resection. B2M has been found to mediate cell apoptosis and survival by regulating the expression of hormone and growth factors[24]. A gene regulatory network for myocardial recovery was constructed (Fig. 3) including these genes, TFs and the associated upstream receptor proteins identified by QIAGEN GeneGlobe. Only the top 20 ranked TFs with upstream receptors are shown.

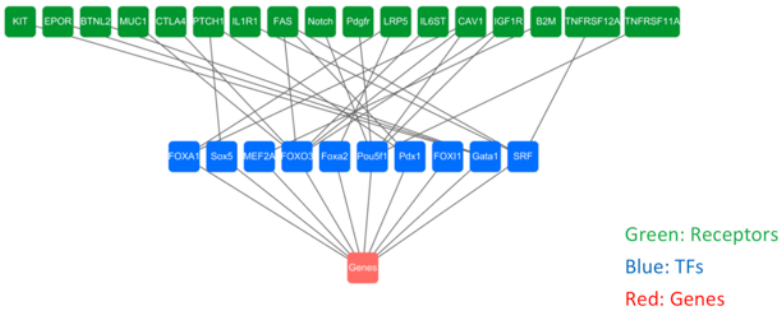

Figure 3: The gene regulatory network of myocardial recovery.

Discussion

In this study, we used a human developmental hereditary disease (i.e., hypertrophic cardiomyopathy) gene set and a zebrafish heart regeneration microarray dataset to investigate the myocardial remodeling process during heart regeneration. HCM genes were crossed-matched with zebrafish heart regeneration transcriptome profiles, and HCM expression profiles were used to search for myocardial remodeling modulators. The TFs and upstream receptors of the identified myocardial remodeling genes were further analyzed with bioinformatics databases and tools to enable the identification of the modulators.

The same zebrafish microarray dataset has previously been used to reveal organ-specific regenerative strategies[14] that include the heart-specific pathways also observed in our acute and early-phase response profiles derived from the selected myocardial remodeling genes. In this study,

gene regulatory network analysis further revealed that B2M signaling pathways may play an important role in myocardial remodeling during heart regeneration (Fig. 4).

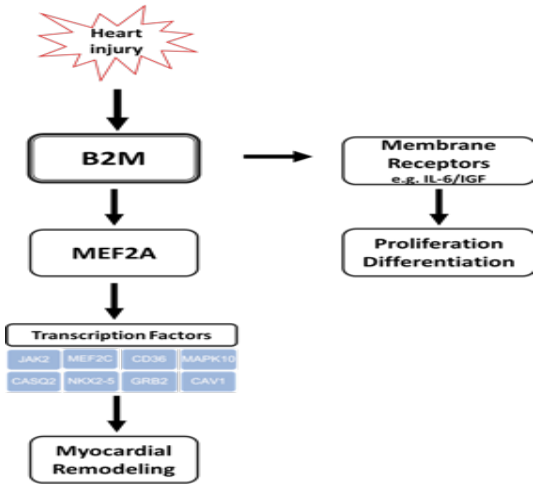

Figure 4: Proposed gene regulatory network of B2M myocardial remodeling during regeneration. After myocardial injury, related signals bind to B2M, promoting a cascade of interacting kinase proteins or molecules that activate other membrane receptors or TFs, such as MEF2A, and then further regulate downstream genes to remodel the injured myocardium.

B2M composes the light chains of major histocompatibility complex class I (MHC1) molecules and is an active part of the immune system[25]. MHC1 has been reported to regulate neuronal synapses during development via the MHC1-MEF2 signaling pathway, as verified by the reduction in MEF2 activity in B2M RNAi knockdown mice[26]. MEF2 is an essential cardiac TF that promotes the generation of cardiomyocytes from cardiac or skin fibroblasts[27, 28]. MEF2 homologues in zebrafish are also essential in cardiomyocyte differentiation, and their role is believed to be conserved throughout vertebrate hearts[29]. Moreover, B2M activates the epithelial-to-mesenchymal transition (EMT) and promotes the growth and migration of mesenchymal stem cells in cancer[30, 31]. Thus, although the mechanism by which B2M mediates myocardial remodeling is not fully understood, the potentially novel functional role of B2M in heart regeneration is worth examining in future studies.

Multiple molecules identified by our approach have already been shown to be important in heart repair, including erythropoietin (EPO) and c-kit (KIT). EPO is a growth hormone that binds to the transmembrane receptor EPOR, and EPO-responsive cardiomyogenic cells can contribute to heart repair in MI mice[32]. Additionally, c-kit-positive cardiac stem cells have been found to be necessary and sufficient for functional cardiac regeneration and repair[33]. Interestingly, another receptor at the top of our target list, caveolin 1 (CAV1*)*, was recently demonstrated in a transcriptomic analysis of epicardial lineage cells to be an essential factor in injury-induced cardiomyocyte proliferation and heart regeneration[34]. Similarly, a different transcriptomic analysis study revealed that specific enhancer regulatory elements (e.g. leptin receptor LEPR) can direct the regeneration-induced activation of gene expression in zebrafish and neonatal mouse tissues[35]. These studies provide evidences that our method is sufficient to predict candidate targets with regulatory roles in heart regeneration.

This study described a new systematic approach to identify conserved modulators of heart regeneration based on findings from developmental biology. The zebrafish has become a new model

to study the genetic basis of cardiomyopathy because of availability of both embryonic and adult models and well conserved diploid genome among vertebrates[36]. This human hereditary disease gene-based method enables the construction of gene regulatory networks and the identification of growth factors that play important roles during heart regeneration. This type of analysis can be expanded to different cardiovascular diseases to increase our understanding of complex molecular processes and to prioritize homologues for each disease-associated gene. For example, it would be worthwhile to apply familial arrhythmia-associated genes to explore potential pathways involved in the synchronization of cardiac contraction. However, this approach has several limitations. Since the interactions in the regulatory network are obtained mainly from online databases, empirical experiments are still necessary to validate the findings. Moreover, the selection of different processes and time frames during regeneration may bypass the unique characteristics and pathogeneses of human diseases. In addition, the use of RNA extracted from the whole heart makes it difficult to identify cell or tissue-specific gene regulation that occurs during the process.

Currently, heart regeneration is studied through three main approaches[37]. The first mainstream approach focuses on stem cells and attempts to target cells or tissue grafts to the injured area. The second approach intends to reprogram scar tissue into new functional myocardium through the use of TFs or miRNA. The final approach focuses on stimulating existing cardiomyocytes or resident cardiac cells with growth factors or small molecules to initiate heart regeneration. In investigating heart regeneration, our human disease gene-based method can be applied to complement the second and third approaches. The TFs or miRNA optimal for scar reprogramming could be identified by analyzing scar tissue formation-related disease genes and a heart regeneration microarray dataset, while growth factors or mediator molecules could be identified based on a gene regulatory network constructed from relevant cardiovascular diseases. With the rapid advancement of bioinformatics databases and tools, this human disease gene-based method can thus potentially be very useful in studies on the mechanisms of heart regeneration and in the field of regenerative cardiac medicine.

In conclusion, our results encourage follow-up studies using human disease gene-based analysis to identify key modulators involved in post-MI recovery. Applying our search approach to genes involved in different diseases may enable more effective investigation and may facilitate the understanding of the specific process involved in heart regeneration.

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