Bioinformatics analysis of key genes and signaling pathways associated with myocardial infarction following telomerase activation

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Abstract. The present study aimed to identify key genes and signaling pathways associated with myocardial infarction (MI) following telomerase activation, and investigate the possible underlying molecular mechanisms involved in this process. Array data of GSE62973 was downloaded, including 11 samples from the Gene Expression Omnibus database. Differentially expressed genes (DEGs) were analyzed in infarct vs. control, infarct + telomerase vs. control, and infarct + telomerase vs. infarct with the Linear Models for Microarray and RNA-Seq Data package. Gene Ontology annotation and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis were performed for upregulated and downregulated genes by the Database for Annotation, Visualization and Integrated Discovery. Sub network modules of 3 protein-protein interaction (PPI) networks were analyzed by Clustering with Overlapping Neighbourhood Expansion, and genes associated with telomerase were analyzed. Proto-oncogene tyrosine-protein kinase Src (*Src*) and proto-oncogene tyrosine-protein kinase Fyn (*Fyn*) were the hub nodes of the greatest degree in the PPI network for the infarct + telomerase vs. control comparison group and infarct + telomerase vs. infarct comparison group, respectively. Olfactory receptor gene family associated genes, including olfactory receptor 10 were significantly enriched in the sub network modules of the 3 comparison groups. In addition, olfactory transduction was a significantly enriched pathway by downregulation of DEGs in the infarct vs. control comparison group, and was additionally a significantly enriched pathway by upregulated DEGs in infarct + telomerase vs. infarct comparison group. Olfactory transduction was a significant pathway enriched by genes associated with telomerase. Telomerase activation may serve an important role in MI, in part, via the regulation of *Src*, *Fyn* and olfactory receptor family associated genes.

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Introduction

Myocardial infarction (MI), additionally known as a heart attack, is a primary cause of disability and death worldwide (1). Chest pain or discomfort is the most common symptom, and this disease may be recognized by certain clinical features and imaging, or may be defined by pathology (2). In 2013, there were ~8.6 million cases of MI worldwide, and ~1 million individuals are affected by MI in the United States annually (3,4). Every 6th man and every 7th woman in Europe will succumb as a result of MI (5,6). In addition, diabetes, high blood pressure, smoking, excessive alcohol intake, poor diet and lack of exercise are risk factors of MI (7,8). Therefore, the need for the development of novel and effective therapeutic strategies for the treatment of MI is imperative.

MI may induce alterations of left ventricular architecture, and aging is a primary risk factor that results in heart alterations and cardiovascular disease (9-11). Additionally, short telomeres have been reported to be risk predictors for age-associated disease, including heart disease (12). A previous study revealed that telomerase activation may elongate telomeres and delay ageing and associated diseases, including MI (13). Certain other studies suggested that telomerase expression may stimulate cardiomyocyte proliferation and contribute to functional heart recovery following MI (14,15). Weischer *et al* (16) demonstrated that short telomere length is associated with a modest increased risk of MI by studying 19,838 individuals for up to 19 years. In addition, one study indicated that telomerase has beneficial effects on heart function (17). High expression levels of telomerase via telomerase reverse transcriptase production may reduce the magnitude of heart attacks (18). Harrington *et al* (19) suggested that telomerase limits the damage resulting from heart attacks. Therefore, telomerase may serve important roles in MI. However, the underlying

molecular mechanism of telomerase activation on MI remains to be fully understood.

In the present study, the array data of GSE62973 was downloaded and differentially expressed genes (DEGs) were analyzed in samples of mice that were injected with an adeno-associated virus that expressed telomerase, an adeno associated-virus with an empty expression cassette, or no virus, prior to induction of myocardial infarction. In addition, functional enrichment analysis was performed. A protein-protein interaction (PPI) network was generated and significant modules were analyzed. Subsequently, genes associated with telomerase were identified. The present study aimed to identify key genes and pathways associated with MI following telomerase activation, and investigate the possible underlying molecular mechanism of this process.

Materials and methods

Microarray data. The array data of GSE62973 deposited by Bär *et al* (13) was downloaded from the Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo) database. An adeno-associated virus that expressed telomerase or an empty expression cassette were used to infect mice in this data, and the MI model was subsequently established. The model was used to study the effect of telomerase activation in disease. A total of 4 myocardial infarction samples that were treated with an adeno-associated virus that expressed telomerase (infarct+telomerase), 4 myocardial infarction samples treated with an adeno-associated virus with an empty expression cassette (infarct) and 3 myocardial infarction samples that were not infected with viruses (control) were included in the present study. The raw data was downloaded for subsequent analysis, which were based on the platform of GPL10787 (Agilent-028005 SurePrint G3 Mouse GE 8x60K Microarray).

Data pre-processing. The raw data was pre-processed using an Agilent signal-channel chip provided by the Linear Models for Microarray and RNA-Seq Data (Limma; http://www.bioconductor.org/packages/release/bioc/html/limma.html) in the R package (20). Background correction, normalized expression intensity and condensed microarray data were included in the pre-processing protocol. Subsequently, combined with an annotation file of the platform, the probe identity was transformed to a gene symbol, and probes without corresponding gene symbols were eliminated. If a number of probes mapped to one gene symbol, then the mean value was set as final expression value of this gene.

DEGs analysis. The DEGs were analyzed in infarct vs. control, infarct + telomerase vs. control and infarct + telomerase vs. infarct by using the Limma package. The P-values of DEGs from the Limma package were calculated by Student's unpaired t-test (21). $|\log_2 FC| \geq 0.5$ and $P<0.05$ were used as cut-off criteria, and were considered to indicate a statistically significant difference.

Functional enrichment analysis. Gene ontology (GO) is used for gene annotation, and molecular function (MF), biological process (BP) and cellular component (CC) were included in this tool (22). Kyoto Encyclopedia of Genes and Genomes (KEGG) may be used to place associated gene sets into their pathways (23). Database for Annotation, Visualization and Integrated Discovery (DAVID; https://david.ncifcrf.gov/) is an integrated data-mining environment and is used for gene list analysis (24).

GO annotation and KEGG pathway enrichment analysis were performed for upregulated and downregulated genes by DAVID. Gene counts ≥2 and $P<0.05$ were set to determine significant enrichment.

PPI network analysis. The Search Tool for the Retrieval of Interacting Genes (25) database may be used to predict interactions between proteins. Neighbourhood, gene fusion, co-occurrence, co-expression experiments, databases and textmining were the source of the prediction method of this database. The input gene sets were DEGs in 3 comparison groups, and the species was mouse. PPI score=0.4, and protein nodes that interacted with each other were DEGs. PPI networks were generated using Cytoscape software version 3.4.0 (National Institutes of Health, Bethesda, MD, USA) (26).

Key nodes in the PPI network were obtained by calculating the degree values of nodes. Degree values represented the number of other nodes that interacted with the node. The greater the degree values, the more likely that the nodes were key nodes in the network.

Module analysis. Sub-network modules of 3 PPI networks were analyzed using Clustering with Overlapping Neighborhood Expansion (ClusterONE) version 1.0, in Cytoscape version 3.4.0 (National Institutes of Health) (27). The overlap protein complex may be analyzed and significant sub-network modules may be screened using ClusterONE software. $P<0.0003$ was set to indicate significant modules. Nodes in one module were more likely to take part in the same biological process. Subsequently, the KEGG pathways enriched by DEGs in different modules were analyzed using the DAVID online tool.

Analysis of genes associated with telomerase. The DEGs in 3 comparison groups were combined, and then the alterations in expression levels of these DEGs in 3 sample groups were observed. The present study screened 2 types of genes: Genes that were upregulated in the control and in the infarct + telomerase groups compared with in the infarct group (gene expression decreased in disease and increased following telomerase treatment), and genes that were downregulated in the control in the infarct + telomerase groups compared with in the infarct group (gene expression increased in disease and decreased following telomerase treatment). Average expression values of genes in the control, infarct and infarct + telomerase groups were calculated, and subsequently genes meeting the aforementioned conditions were screened. Combined with DAVID, KEGG pathways enriched by obtained genes were analyzed.

Results

DEGs analysis. DEGs in 3 comparison groups are presented in Table I. More DEGs were obtained in the infarct + telomerase vs. control groups (862 DEGs) and infarct + telomerase vs. infarct groups (816 DEGs) compared with the infarct

Table I. DEGs in 3 comparison groups.

Group	Upregulated gene count	Downregulated gene count	Total
Infarct vs. control	78	173	251
Infarct + telomerase vs. control	467	395	862
Infarct + telomerase vs. infarct	555	261	816

DEGs, differentially expressed genes.

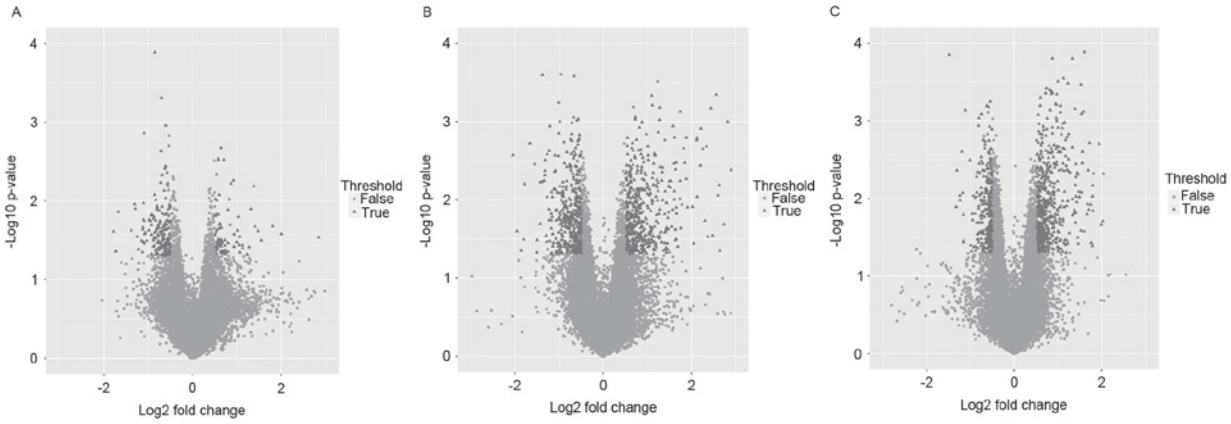

Figure 1. Volcano plots of gene expression distribution in 3 comparison groups. (A) Infarct vs. control comparison group; (B) infarct + telomerase vs. control comparison group; (C) infarct + telomerase vs. infarct comparison group. Significant differentially expressed genes were marked as True, whereas non-significant genes were denoted as False.

vs. control groups (251 DEGs), and it was suggested that the expression levels of genes were significantly altered in infarct + telomerase samples compared with control samples and infarct samples. The volcano plots of the gene expression distribution in the 3 groups were demonstrated in Fig. 1.

Functional enrichment analysis. GO and KEGG pathway analysis for upregulated and downregulated DEGs were performed, respectively. Olfactory transduction was the significantly enriched pathway associated with downregulated DEGs in the infarct vs. control comparison group (Table IIA). However, there were no significantly enriched pathways associated with upregulated DEGs in this comparison group. Extracellular matrix (ECM)-receptor interaction and valine, leucine and isoleucine degradation were significantly enriched pathways by upregulated and downregulated DEGs in infarct + telomerase vs. control comparison group, respectively (Table IIB). Olfactory transduction and homologous recombination were significantly enriched pathways by upregulated and downregulated DEGs in infarct + telomerase vs. infarct comparison group, respectively (Table IIC).

PPI network analysis. A total of 81 nodes and 133 protein pairs were included in the PPI network for the infarct vs. control comparison group (Fig. 2A). A total of 481 nodes and 1,606 protein pairs were included in the PPI network for infarct + telomerase vs. control comparison group, and proto-oncogene tyrosine-protein kinase Src (*Src)* was the hub node with greatest degree (Fig. 2B). A total of 81 nodes and 133 protein pairs were included in the PPI network for infarct + telomerase vs. infarct comparison group, and proto-oncogene tyrosine-protein kinase Fyn (*Fyn)* was the hub node with the greatest degree (Fig. 2C). Nodes with greater degree values in 3 networks are presented in Table III.

Module analysis. Sub-network modules obtained from 3 PPI networks were demonstrated in Fig. 3. A single significant sub-network module was obtained in the infarct vs. control comparison group (Fig. 3A, P=2.750E-6). A total of four significant sub-network modules were obtained in the infarct + telomerase vs. control comparison group (Fig. 3B, cluster 1: P=9.860E-8; cluster 2: P=5.186E-7; cluster 3: P=1.388E-6; cluster 4: P=2.081E-4) and infarct + telomerase vs. infarct comparison group (Fig. 3C, cluster 1: P<0.0001; cluster 2: P=5.279E-7; cluster 3: P=2.280E-5; cluster 4: P=4.260E-5). Olfactory receptor gene family associated genes including olfactory receptor 10 (*Or10)*, olfactory receptor 444 (*Or444*) and olfactory receptor 414 (*Or414*) were significantly enriched in the sub-network modules of the 3 groups. The KEGG pathway was significantly enriched by these sub-network modules, as presented in Fig. 4.

Analysis of genes associated with telomerase. A total of 1,520 DEGs were obtained following combination and removal of duplications. These genes were significantly differentially expressed in ≥1 comparison groups. Expression alterations of 509 genes revealed up-down-up trends in control, infarct, infarct + telomerase groups. Expression alterations of

Table II. GO and KEGG analysis for DEGs.

	Category	Term	Count	P-value
A, infarct vs. control				
Upregulated	MF	GO:0019904~protein domain specific binding	4	1.61×10^{-2}
		GO:0032403~protein complex binding	3	1.98×10^{-2}
	CC	GO:0005576~extracellular region	13	4.93×10^{-3}
Downregulated	BP	GO:0007186~G-protein coupled receptor protein signaling pathway	25	7.68×10^{-4}
		GO:0007166~cell surface receptor linked signal transduction	30	8.89×10^{-4}
	CC	GO:0016021~integral to membrane	55	1.47×10^{-3}
		GO:0031226~intrinsic to plasma membrane	12	1.50×10^{-3}
	MF	GO:0004984~olfactory receptor activity	17	4.14×10^{-3}
		GO:0016503~pheromone receptor activity	5	1.38×10^{-2}
	PATHWAY	mmu04740:Olfactory transduction	12	6.80×10^{-3}
		mmu04010:MAPK signaling pathway	5	4.74×10^{-2}
B, Infarct + telomerase vs. control				
Upregulated	BP	GO:0001568~blood vessel development	20	2.47×10^{-6}
		GO:0001944~vasculature development	20	3.53×10^{-6}
	CC	GO:0005576~extracellular region	97	1.14×10^{-16}
		GO:0044421~extracellular region part	54	5.98×10^{-12}
	MF	GO:0005201~extracellular matrix structural constituent	8	2.84×10^{-6}
		GO:0005509~calcium ion binding	38	3.13×10^{-5}
	PATHWAY	mmu04512:ECM-receptor interaction	14	4.50×10^{-8}
		mmu04510:Focal adhesion	16	5.04×10^{-5}
Downregulated	BP	GO:0006811~ion transport	22	3.21×10^{-3}
		GO:0009083~branched chain family amino acid catabolic process	3	6.20×10^{-3}
	CC	GO:0001673~male germ cell nucleus	3	3.17×10^{-2}
		GO:0005739~mitochondrion	30	3.89×10^{-2}
	MF	GO:0005244~voltage-gated ion channel activity	11	6.43×10^{-4}
		GO:0022832~voltage-gated channel activity	11	6.43×10^{-4}
	PATHWAY	mmu00280:Valine, leucine and isoleucine degradation	6	7.37×10^{-4}
		mmu00380:Tryptophan metabolism	5	3.50×10^{-3}
C, Infarct + telomerase vs. infarct				
Upregulated	BP	GO:0007186~G-protein coupled receptor protein signaling pathway	68	4.03×10^{-6}
		GO:0007166~cell surface receptor linked signal transduction	82	1.18×10^{-5}
	CC	GO:0031224~intrinsic to membrane	189	4.74×10^{-7}
		GO:0016021~integral to membrane	179	7.71×10^{-6}
	MF	GO:0004867~serine-type endopeptidase inhibitor activity	14	8.09×10^{-7}
		GO:0030414~peptidase inhibitor activity	17	8.74×10^{-7}
	PATHWAY	mmu00590:Arachidonic acid metabolism	8	1.35×10^{-3}
		mmu04740:Olfactory transduction	31	7.75×10^{-3}

Table II. Continued.

	Category	Term	Count	P-value
C, Infarct + telomerase vs. Infarct				
Downregulated	BP	GO:0042110~T cell activation	6	3.90×10^{-3}
		GO:0001775~cell activation	8	6.73×10^{-3}
	CC	GO:0005657~replication fork	3	1.68×10^{-2}
		GO:0005694~chromosome	8	3.81×10^{-2}
	MF	GO:0003677~DNA binding	32	3.05×10^{-4}
		GO:0008190~eukaryotic initiation factor 4E binding	2	3.65×10^{-2}
	PATHWAY	mmu03440:Homologous recombination	4	1.23×10^{-3}
		mmu03430:Mismatch repair	3	1.31×10^{-2}

GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes; BP, biological process; CC, cellular component; MF, molecular function; Term, identification number and names of GO or KEGG terms; Counts, the number of genes enriched in GO or KEGG terms; MAPK, mitogen activated protein kinase; ECM, extracellular matrix.

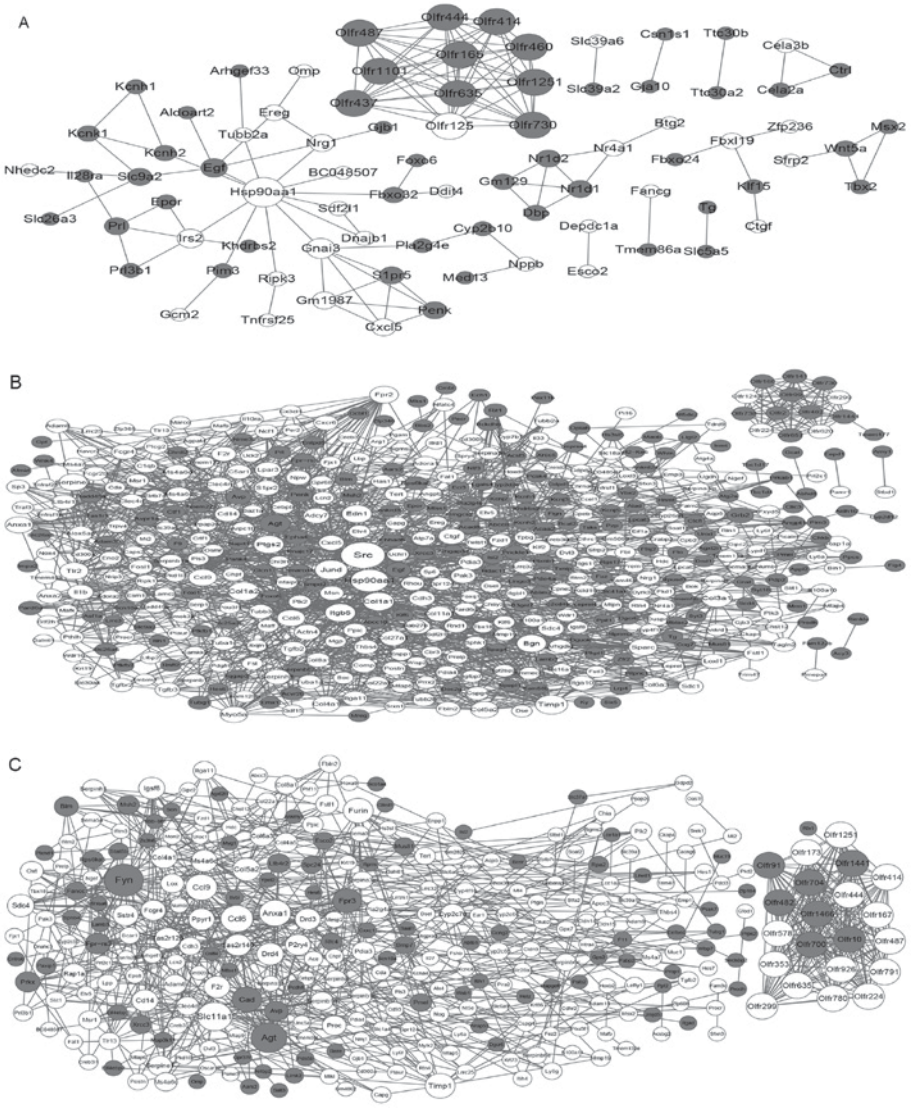

Figure 2. Protein-protein interaction networks for 3 comparison groups. (A) infarct vs. control, (B) infarct + telomerase vs. control and (C) infarct + telomerase vs. infarct. Dark grey nodes, upregulated genes; clear nodes, downregulated genes. The greater the degree value of the nodes, the larger the nodes.

Table III. Nodes with greater degree values in 3 networks.

PPI type	Degree	Node
Infarct vs. control	12	Hsp90aa1
	10	Olfr1251,Olfr635,Olfr460,Olfr1101,Olfr444,Olfr437,Olfr125, Olfr165,Olfr414,Olfr487,Olfr730
	6	Gnai3
	5	Irs2,Egf
Infarct + telomerase vs. control	81	Src
	60	Hsp90aa1
	42	Jund
	41	Timp1
	38	Col1a2
	37	Col1a1
	35	Itgb5,Agt
	34	Col3a1,Ptgs2,Edn1
	32	Bgn
	30	Tlr2
	29	Serpine1,Fpr2
	28	Sparc,Ccl9
	27	Ccl6
	26	Anxa1,Col5a2
Infarct + telomerase vs. infarct	30	Fyn
	26	Agt
	24	Slc11a1
	21	Ccl6,Olfr1441
	20	Olfr10,Olfr578,Olfr1466,Olfr482,Olfr224,Olfr704,Olfr487, Olfr926,Anxa1,Olfr173,Olfr1251,Olfr414,Olfr353,Olfr167, Olfr299,Olfr780,Olfr635,Olfr91,Olfr700,Olfr444,Ccl9, Olfr791
	18	Furin,Cad,Timp1
	17	Fpr3
	15	Col5a2

PPI, protein-protein interaction; Hsp90aa1, heat shock protein 90 α family class A member 1; Olfr, olfactory receptor; Gnai3, G-protein subunit α i3; Irs2, insulin receptor 2; Egf, epidermal growth factor; Src, SRC proto-oncogene non-receptor tyrosine kinase; Jund, JunD proto-oncogene AP-1 transcription factor subunit; Timp1, tissue inhibitor of metalloproteinase 1; Col, collagen; Itgb, integrin β; Agt, angiotensin; Ptgs2, prostaglandin-endoperoxide synthase 2; Edn1, endothelin 1; Bgn, biglycan; Tlr2, toll like receptor 2; Fpr2, formyl peptide receptor 2; Sparc, secreted protein acidic and cysteine rich; Ccl, chemokine (C-C motif) ligand; Anxa1, annexin A1; Fyn, FYN proto-oncogene Src family tyrosine kinase; Slc11a1, solute carrier family 11 member 1; Cad, carbamoyl-phosphate synthetase 2 aspartate transcarbamylase and dihydroorotase.

266 genes revealed down-up-down trends in control, infarct, infarct + telomerase groups. The expression alteration trends of these genes were in accordance with the control in MI following addition of telomerase using infarct as a reference, and suggested that telomerase may affect the expression of these genes. Signaling pathways significantly enriched by these genes are listed in Table IV. Olfactory transduction was a significant pathway enriched by genes associated with telomerase.

Discussion

The present study revealed that *Src* and *Fyn* were the hub nodes of the greatest degrees in the PPI network for the infarct + telomerase vs. control comparison group and infarct + telomerase vs. infarct comparison group, respectively. Olfactory receptor gene family associated genes, including *Or10*, *Or444* and *Or414* were significantly enriched in the sub-network modules of 3 comparison groups. In addition, olfactory transduction was a significantly enriched pathway with downregulated DEGs in the infarct vs. control comparison group, and was a significantly enriched pathway with upregulated DEGs in the infarct + telomerase vs. infarct comparison group. Olfactory transduction was a significant signaling pathway enriched by genes associated with telomerase.

In the present study, *Src* was the hub node of the greatest degree in the PPI network for the infarct + telomerase vs. control

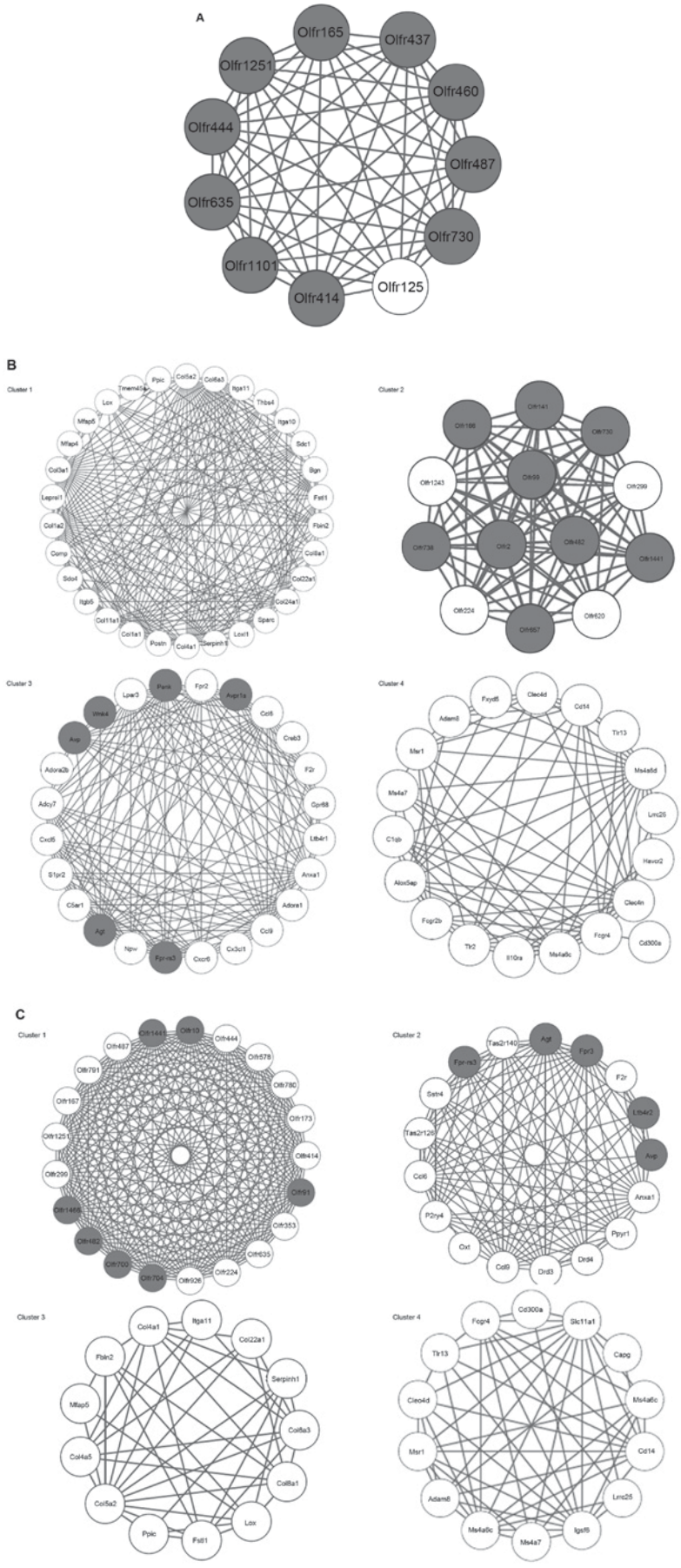

Figure 3. Significant sub-network modules obtained from 3 protein-protein interaction networks. (A) Significant sub-network module obtained in the infarct vs. control comparison group. (B) A total of 4 (clusters 1-4) significant sub-network modules were obtained in the infarct + telomerase vs. control comparison group. (C) A total of 4 (clusters 1-4) significant sub-network modules were obtained in the infarct + telomerase vs. infarct comparison group. Dark grey nodes, upregulated genes; clear nodes, downregulated genes.

Table IV. Pathways significantly enriched by genes that revealed expression alterations in up-down-up and down-up-down trends.

Expression direction	Term	Count	P-value
Up-down-up	mmu04740:Olfactory transduction	34	8.79×10^{-5}
	mmu00590:Arachidonic acid metabolism	9	1.06×10^{-4}
	mmu00830:Retinol metabolism	6	7.20×10^{-3}
	mmu00591:Linoleic acid metabolism	5	8.97×10^{-3}
	mmu00980:Metabolism of xenobiotics by cytochrome P450	5	3.02×10^{-2}
	mmu00982:Drug metabolism	5	4.51×10^{-2}
Down-up-down	mmu03430:Mismatch repair	3	1.10×10^{-2}

KEGG, Kyoto Encyclopedia of Genes and Genomes; Term represents the identification number and the names of KEGG term; Counts represents the number of genes enriched in KEGG terms.

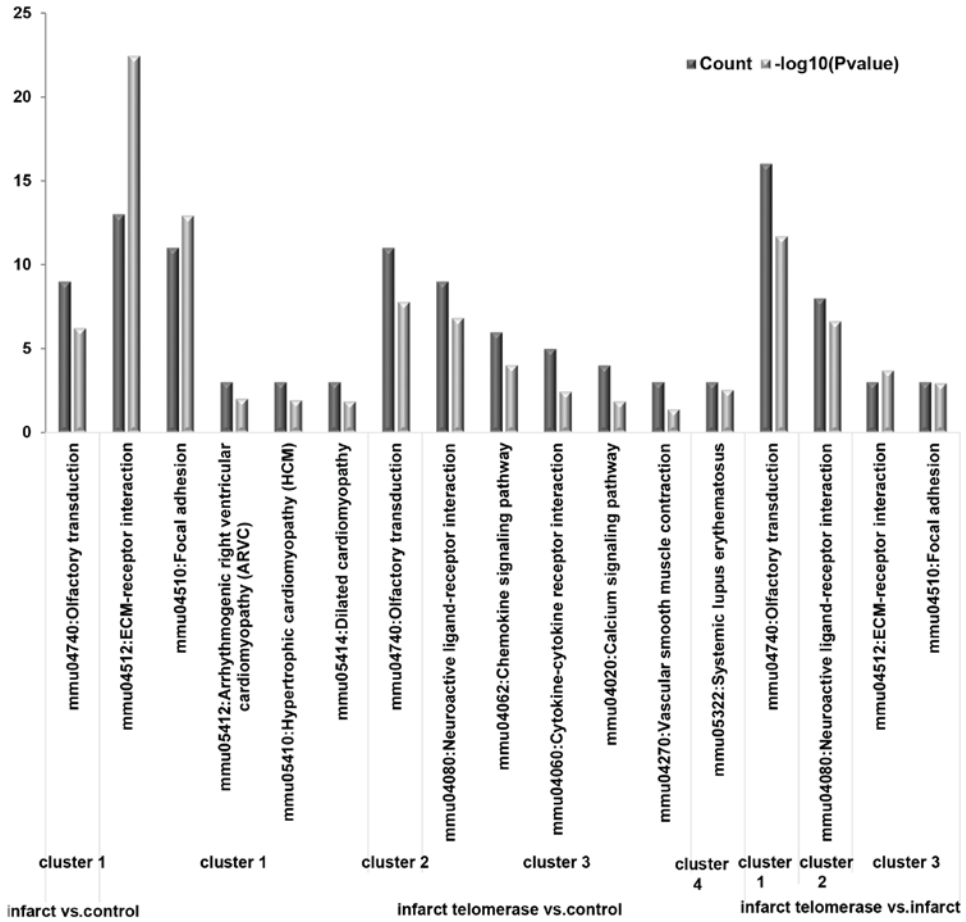

Figure 4. Kyoto Encyclopedia of Genes and Genomes pathways that were significantly enriched by the sub-network modules.

comparison group. *Src* inhibition may stabilize a kinase insert domain receptor/cadherin complex and may reduce edema and tissue injury following MI (28). Cellular-*Src* blockade lowers the induction of arrhythmia and improves conduction velocity following MI (29). Furthermore, *Src* protein tyrosine kinases serve preconditioning roles against MI (30). In addition, hydrogen sulfide may recruit macrophage migration via the integrin β1-Src-focal adhesion kinase/protein tyrosine kinase 2-Ras-related C3 botulinum toxin substrate 1 (Rac1) pathway in MI (31). Therefore, the results of the present study were in accordance with previous reports (28-31) and suggested that *Src* may serve significant roles in MI following telomerase activation.

In addition, *Fyn* was the hub node of the greatest degree in the PPI network for the infarct + telomerase vs. infarct comparison group in the present study. Activation of nuclear factor erythroid 2-related factor 2 via the Rac1/glycogen synthase kinase-3β/Fyn signaling pathway may prevent angiotensin

II-induced cardiomyopathy (32), and inhibition of nephrin activation by c-maf inducing protein via the C-Src tyrosine kinase-CREB-binding protein-Fyn axis, serves a key role in angiotensin II-induced podocyte damage (33). Pre-treatment with angiotensin II may limit MI in isolated rabbit hearts (34). Additional angiotensin II receptor blocker treatment has minimal impact on the development of coronary atherosclerosis in patients with acute MI compared with an angiotensin-converting enzyme inhibitor alone (35). Although there may be little direct research on the roles of *Fyn* in MI in previous studies, it may be hypothesized from the results of the present study that *Fyn* serves an important role in MI following telomerase activation.

In addition, in the present study, the olfactory receptor gene family associated genes, including *Or10, Or444* and *Or414*, were significantly enriched in the sub-network modules of 3 comparison groups. A previous study revealed that the olfactory receptor 10J5 gene was expressed in the human aorta, coronary artery and umbilical vein endothelial cells, and served functional roles in angiogenesis (36). Drutel *et al* (37) suggested that olfactory receptor genes may serve roles in cardiac progression. Certain studies have suggested that olfactory receptors are involved in olfactory signal recognition and muscle regeneration (38,39). In addition, reduced blood flow to a part of the heart, due to thrombosis, etc., results in damage to the heart muscle, and subsequently, MI occurs (2). Therefore, the olfactory receptor gene family associated genes may indirectly serve important roles in heart disease, including MI.

In the present study, olfactory transduction was a significantly enriched pathway by downregulated DEGs in the infarct vs. control comparison group, and was a significantly enriched pathway by upregulated DEGs in the infarct + telomerase vs. infarct comparison group, and olfactory transduction was the significant pathway enriched by genes associated with telomerase. Li *et al* (40) demonstrated that olfactory transduction was a significant pathway enriched with dysregulated genes in coronary artery disease. Additionally, olfactory receptor gene family associated genes may serve important roles in heart disease, including MI. Therefore, it may be hypothesized that the olfactory transduction pathway may be involved in the development of MI.

In conclusion, *Src*, *Fyn* and olfactory receptor gene family associated genes serve significant roles in MI. These genes and their associated signaling pathways were differentially expressed in MI following telomerase activation in the present study. Therefore, telomerase activation may serve important roles in MI partly via *Src*, *Fyn* and olfactory receptor gene family associated genes. However, a limitation of the present study is that as of yet, there is no experimental verification to conclude this, and further experiments are required in the future to verify these findings.

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