Research Paper

The Pathogenesis of Atherosclerosis Based on Human Signaling Networks and Stem Cell Expression Data

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Abstract

Atherosclerosis is a common and complex disease, whose morbidity increased significantly. Here, an integrated approach was proposed to elucidate systematically the pathogenesis of atherosclerosis from a systems biology point of view. Two weighted human signaling networks were constructed based on atherosclerosis related gene expression data of stem cells. Then, 37 candidate Atherosclerosis-risk Modules were detected using four kinds of permutation tests. Five Atherosclerosis-risk Modules (three Absent Modules and two Emerging Modules) enriched in functions significantly associated with disease genes were identified and verified to be associated with the maintenance of normal biological process and the pathogenesis and development of atherosclerosis. Especially for Atherosclerosis-risk Emerging Module P96, it could distinguish between normal and disease samples by Supporting Vector Machine with the average expression value of the module as classification feature. These identified modules and their genes may act as potential atherosclerosis biomarkers. Our study would shed light on the signal transduction of atherosclerosis, and provide new insights to its pathogenesis from the perspective of stem cells.

Key words: atherosclerosis, stem cell, human signaling network, module, expression data

Introduction

Atherosclerosis is a chronic inflammatory disease associated with the lipid deposition and plaque fibrosis in the arterial wall, and characterized by marked dysfunction in lipid homeostasis and signaling pathways that control the inflammatory response [1, 2].

Stem cells, including endothelial progenitor cells (EPCs), smooth muscle progenitor cells (SMPCs), mesenchymal stem cells (MSCs), different sources of hematopoietic stem cells (HSCs), and adipose-derived stem cells (ADSCs), etc., are cells with self-renewal and differentiation potential. They have been shown to play crucial roles in pathogenesis of atherosclerosis [3]. Undifferentiated HSCs migrate from the bloodstream into diseased tissue and differentiate to macrophages, monocytes, and neutrophils in response to infection and inflammation [4, 5]. Accumulated studies have shown that HSCs are also released into the bloodstream after acute myocardial infarction and augment formation of new monocytes that participate in the progression of atherosclerosis [5]. This indicates that circulating HSCs in the bloodstream represent a pool of undifferentiated stem cells that play a role in the pathogenesis of atherosclerosis. CD34+ stem cells, one kind of HSCs, are localized in atherosclerotic aorta as well as in aortic atherosclerotic regression, and thus could be of great clinical relevance [6]. Medbury et al. examined

human endarterectomy specimens and found that the fibrous cap of the plaque contained either smooth muscle cells or CD34+ stem cells [7].

The signaling system plays a fundamental part in cells, as it essentially regulates cells and organisms under physiological and pathological states [8]. A malfunction in this system can disrupt the fine-balanced signal transduction significantly, and is believed to be involved in diseases [9]. Inflammatory pathways in stem cells are under stringent control by a variety of transcription factors and coregulatory molecules, such as NF-κB, AP1, the PPAR family, liver X receptor alpha (LXRα/Nr1h3), and their associated coactivators and corepressors [10]. However, the signaling pathways that regulate inflammation and cytokine production in stem cells for atherosclerosis remain incompletely understood. Perturbation of human signaling networks by mutations or abnormal protein expressions underlies the cause of many diseases [11]. It was suggested that diseases could be studied more effectively using systems biology approaches from the perspective of networks [12]. With the increased scope of systems biology, network modules could provide the elucidation of disease mechanisms and provide better strategies for developing multi-marker-module-based risk predictions [13].

Here, we proposed an integrated approach to elucidate systematically the pathogenesis of atherosclerosis by identifying Atherosclerosis-risk Modules based on a human signaling network and disease related gene expression data of stem cells from a systems biology point of view (Figure 1). Our approach would bring a novel understanding of disease signal transduction, and provide valuable insights to the identification of potential biomarkers and the pathogenesis of atherosclerosis.

Materials and methods

Data source

A gene expression profile GSE9820 was extracted from the GEO database (http://www.ncbi.nlm.nih.gov/geo/) [14], containing 23 samples (11 normal samples and 12 atherosclerosis samples) with RNA expressions from CD34+ stem cells. Sixty atherosclerosis genes were extracted from CTD (http://ctdbase.org/) [15], DO (http://www.disease-ontology.org/) [16] and GWAS (https://www.ebi.ac.uk/gwas/home) [17].

The human signaling network information was derived from Edwin Wang et al. (http://www.bri.nrc.ca/wang/cancerMap/HumanSignaling Network_v5.txt) [18], which contained 6287 genes and 62239 signaling relations (including activation, inhibition and physical interaction).

Construction of weighted human signaling networks

Two weighted signaling networks were generated by giving weights to edges of the human signaling network. The weight of the i-th edge E_i connecting two genes a and b was calculated as Pearson Correlation Coefficient of expression values of a and b in normal and atherosclerosis statuses, respectively.

$$Weight(E_i) = Pearson(a,b) = \frac{\sum(a_j - \bar{a})(b_j - \bar{b})}{\left(\sqrt{\sum_{j=1}^{n}(a_j - \bar{a})^2}\right)\left(\sqrt{\sum_{j=1}^{n}(b_j - \bar{b})^2}\right)}$$

where a_j and b_j are gene expression values of genes a and b of sample j, $\bar{a}$ and $\bar{b}$ are average expression values for the genes of all samples in each status, and n is the number of samples in corresponding statuses.

Thus, (i) a normal weighted signaling network where correlation coefficients for the normal status were used as edge weights and (ii) an atherosclerosis weighted signaling network where correlation coefficients for the disease status were used as edge weights, were constructed.

Detection of candidate Atherosclerosis-risk Modules

Candidate Atherosclerosis-risk Modules were detected using two steps.

First, network modules of two weighted signaling networks were mined using the online tool ClusterONE (http://www.paccanarolab.org.sci-hub.org/clusterone/), respectively. ClusterONE is a graph-clustering algorithm to identify functional modules in the network. Each module was consisted of a set of genes that were both topologically close and had highly correlated interactions. Modules contained at least four genes were selected.

Next, 4 permutation tests were performed for each network module.

Given a network module M from weighted human signaling networks with h edges E_1, Λ, E_h, the expression differential score V was evaluated by differences between Pearson correlation coefficients and those between average expression values, respectively, as follows:

$$V(M) = \begin{cases} \sum_{k=1}^{h} |S_k - S'_k|, \text{ Pearson correlation coefficient} \\ |S_u - S'_u|, \text{ Average expression values} \end{cases}$$

where

$$S_k = Pearson(X,Y) = \frac{\sum (X - \overline{X})(Y - \overline{Y})}{\left(\sqrt{\sum_{i=1}^{h}(X_i - \overline{X})^2}\right)\left(\sqrt{\sum_{i=1}^{h}(Y_i - \overline{Y})^2}\right)}$$

$$S'_k = Pearson(X',Y') = \frac{\sum (X' - \overline{X}')(Y' - \overline{Y}')}{\left(\sqrt{\sum_{i=1}^{h}(X'_i - \overline{X}')^2}\right)\left(\sqrt{\sum_{i=1}^{h}(Y'_i - \overline{Y}')^2}\right)}$$

$$S_u = Expression(X) = \frac{1}{g}\sum_{i=1}^{g} X_i$$

$$S'_u = Expression(X') = \frac{1}{g}\sum_{i=1}^{g} X'_i$$

For normal and atherosclerosis samples, X, Y and X', Y' are gene expression values, S_k and S'_k are Pearson correlation coefficients of the k-th edge , S_u and S'_u are the average expression value of genes in M, respectively. g is the number of genes in the module. For each network module, the differential score V was calculated.

To obtain the significance of each module, four permutation tests were performed. From weighted human signaling networks, 1000 degree-conserved random modules and 1000 size-conserved random modules were constructed for each module. Random differential scores V_1, Λ, V_{1000} for Pearson correlation coefficients and average expression values of random modules were calculated, respectively. Modules with expression differential scores significantly greater than the random ones (permutation test, FDR p < 0.05) were considered significant. A module significant in at least 3 of 4 permutation tests was a candidate Atherosclerosis-risk module.

Identification of Atherosclerosis-risk Modules

Candidate Atherosclerosis-risk Modules that were enriched in functions significantly associated with atherosclerosis genes were defined as Atherosclerosis-risk Modules. The functional annotation analysis was performed with atherosclerosis genes or genes in each candidate Atherosclerosis-risk Modules using the hyper-geometric test:

$$P(X = 1) = \frac{C_m^l C_{q-m}^{g-l}}{C_q^g}$$

where q denotes the number of all human genes, g denotes the number of atherosclerosis genes or genes in candidate Atherosclerosis-risk Modules, m denotes the number of genes in function j, l denotes the number of genes of Atherosclerosis-risk Modules in function j. The Bonferroni-corrected p-value < 0.05 was set as the criterion for screening Atherosclerosis-risk Modules.

Results

Atherosclerosis-risk Modules

Using four kinds of permutation tests, 37 candidate Atherosclerosis-risk Modules significantly differential between normal and atherosclerosis statuses were detected from two weighted human signaling networks. After functional enrichment analysis, 5 Atherosclerosis-risk Modules enriched in functions significantly associated with atherosclerosis genes were identified (Table 1). Among them, 3 Atherosclerosis-risk Modules (C83, C368, C377) which were identified from the normal weighted signaling network were defined as Atherosclerosis-risk Absent Modules, and the other 2 modules (P96, P20) which were identified from the atherosclerosis weighted signaling network were defined as Atherosclerosis-risk Emerging Modules.

Table 1. Atherosclerosis-risk Modules.

Atherosclerosis-risk Module	Number of genes	Genes of Atherosclerosis-risk Modules
C83	8	DDB2, ERCC5, TAF1, ERCC3, SMARCC2, SMARCD1, CIITA, SMARCA4
C368	10	CX3CR1, ARRB2, CCR1, CCL7, CCR2, CXCL16, CXCL3, CCL8, CXCL1, ADRBK2
C377	16	LYN, RGS16, INPP5D, SYK, CD79B, CD79A, BLK, PLA2G4A, CD22, LAT2, FCGR2B, PPP1R8, PTPN6, LIMS1, NCK2, PDGFB
P20	12	BUB1B, CDC23, PSMD1, CDC20, CCNB1, BUB1, CCNB2, ANAPC7, MAD2L1, UBE2E1, PSMD14, PSMA5
P96	8	IL1A, IL6, FAS, CXCL1, IL8, IL1B, ICAM1, ADIPOQ

Atherosclerosis related function analysis of Atherosclerosis-risk Modules

Atherosclerosis-risk Modules were significantly enriched in functional categories and pathways related to atherosclerosis (Table S1). Atherosclerosis-risk Module C368 was significantly enriched in functions including "GO:0005125~ cytokine activity", "GO:0006955~immune response", "GO:0006954~inflammatory response" and so on. Atherosclerosis-risk Module C377 was significantly enriched in "GO:0002684~positive regulation of immune system process" and other functions. Atherosclerosis-risk Module P96 was significantly enriched in a number of functions, such as "58.(CD40L)_immmnosurveillance".

Functions "GO:0006954~inflammatory res-ponse", "GO:0006955~immune response", "GO:00026 84~positive regulation of immune system process" and "58.(CD40L)_immmnosurveillance" were associated with the immune system and inflammatory. Atherosclerosis is an inflammatory disease with lesions filling with immune cells that can orchestrate and affect inflammatory responses.

Unstable plaques were particularly rich in activated immune cells, suggesting that they might initiate plaque activation [19]. "GO:0005125~cytokine activity" is related to the cytokines. Many cytokines are expressed in atherosclerotic plaques and all cells involved in the disease are capable of producing cytokines and responding to them. Cytokines could modulate endothelial cells (EC) permeability.

Activated ECs release a range of chemokines and other cytokines that then cause the recruitment of circulating immune cells, particularly monocytes and T lymphocytes [20, 21]. In addition, the ECs express adhesion proteins, such as intercellular adhesion molecule-1 (ICAM1) and vascular cell adhesion molecule-1 (VCAM1), which participate in the recruitment of immune cells [22, 23].
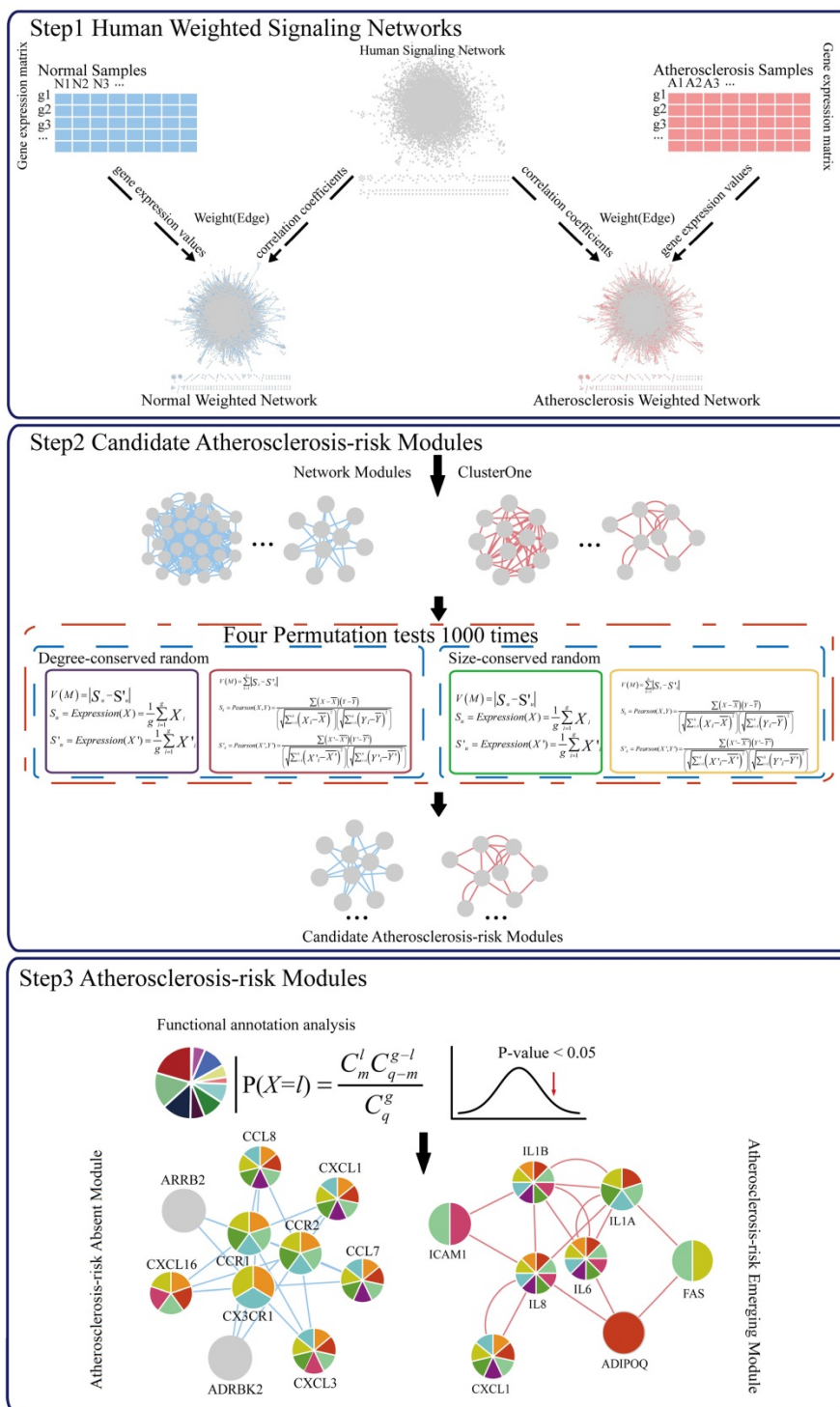

Figure 1: A schematic diagram of Atherosclerosis-risk Modules identification. Atherosclerosis-risk Modules were identified from the systems level based on weighted human signaling networks and atherosclerosis related stem cell expression profiles. A) Construction of weighted human signaling networks. B) Detection of candidate Atherosclerosis-risk Modules. C) Identification of Atherosclerosis-risk Modules.

Atherosclerosis-risk Modules, especially Atherosclerosis-risk Absent Modules, were significantly enriched in the functional categories related to the basic functions of body, such as "GO:0031328~positive regulation of cellular biosynthetic process", "GO:0051173~positive regulation of nitrogen compound metabolic process", "GO:0010604~positive regulation of macromolecule metabolic process" and "GO:0031399~regulation of protein modification process". These functions are crucial to maintain the normal biological processes, and disturbance and changes of them were associated with atherosclerosis closely [24-27].

Atherosclerosis-risk Absent Modules and Atherosclerosis-risk Emerging Modules were significantly enriched in functional categories significantly related to the disease, such as inflammatory response and cytokine activity, which suggested that these modules play important roles in the pathogenesis and development of atherosclerosis.

Analysis of genes in Atherosclerosis-risk Modules

The genes in Atherosclerosis-risk Modules were linked closely with the pathogenesis of atherosclerosis as they possessed disease associated functions. In three Atherosclerosis-risk Absent Modules, C368, C377 and C83, 80%, 68.8% and 50% of genes were verified by literature to be associated with the pathogenesis of atherosclerosis, respectively. In two Atherosclerosis-risk Emerging Modules, P96 and P20, 100% and 50% of genes were verified to be associated with the pathogenesis of atherosclerosis, respectively. It was worth noting that genes in Atherosclerosis-risk Absent Module C368 and Atherosclerosis-risk Emerging Module P96 with high verification percent were both enriched in functional categories associated with atherosclerosis (Figure 2), such as

"GO:0009611~response to wounding", "GO:0006954~inflammatory response", "GO:0006955~immune response" and "GO:0005125~cytokine activity". In addition, some categories, such as "GO:0042330~taxis", "GO:0006935~chemotaxis" and "hsa04060: Cytokine-cytokine receptor interaction", in which known atherosclerosis genes could not be significantly enriched, were significantly enriched for Atherosclerosis-risk Absent Module C368 and Atherosclerosis-risk Emerging Module P96. These categories have also been proven to be associated with atherosclerosis closely. "GO:0042330~taxis" and "GO:0006935~chemotaxis" were associated with chemotaxis, which recruited monocyte and macrophages into the subendothelial space where they phagocytized oxidized lipids, formed foam cells and initiated processes leading to advanced lesions [28, 29].

Atherosclerosis-risk Absent Module C368 was identified only from the normal weighted signaling network rather than the atherosclerosis weighted signaling network. Most genes in this module were members of chemokines. These chemokines were considered to be pro-inflammatory and could be induced during an immune response to recruit cells of the immune system to a site of infection [30]. Meanwhile, chemokines were also considered to be homeostatic and involved in controlling the migration of cells during normal processes of tissue maintenance or development and could promote wound healing [31, 32]. For instance, CCR1 was confirmed to play a protective role on atherosclerosis [29]. CX3CR1 is the only member of CX3C - chemokines Fractalkine (FKN) receptor [33]. Resent research has shown that FKN / CX3CR1 was involved in the inflammatory process and played a pro-inflammatory effect of atherosclerosis [2, 33]. Hence, absence of Atherosclerosis-risk Module C368
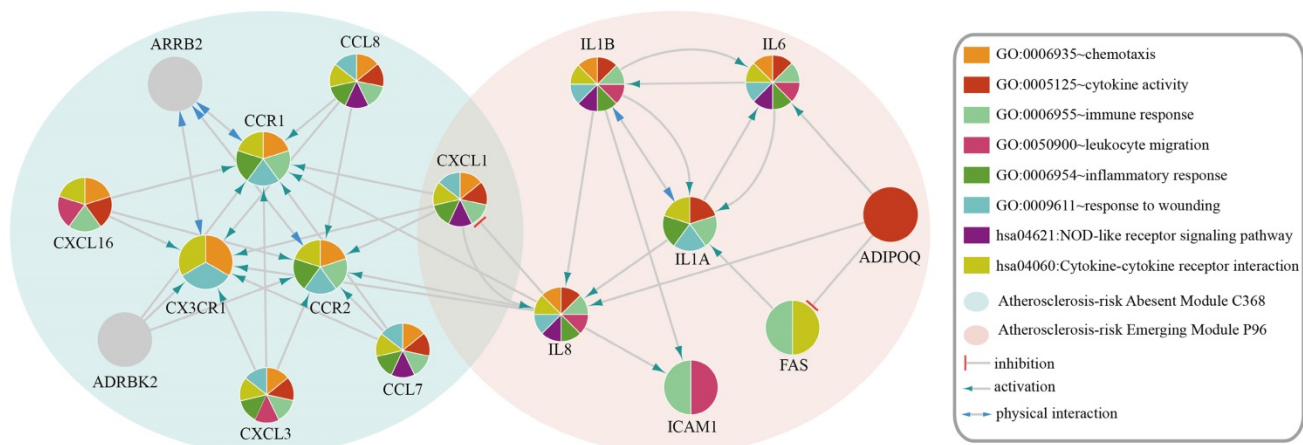

Figure 2: Functional categories of Atherosclerosis-risk Absent Module C368 and Atherosclerosis-risk Emerging Module P96. Different patterns of each circle represent different functions these modules enriched in.

in disease status suggested that the protective effect of Module C368 were suppressed, which promoted the occurrence of atherosclerosis.

Interestingly, CXCL1 was in both Atherosclerosis-risk Absent Module C368 and Emerging Module P96. CXCL1 is an antimicrobial gene encodes a member of the CXC subfamily of chemokines. This protein played a role in inflammation as a chemoattractant for neutrophils and could increase chemotactic activity [34]. The appropriate expression of CXCL1 would inhibit the formation of plaques [34, 35]. Expression difference of CXCL1 in two modules demonstrated the function changes in the process of atherosclerosis.

Moreover, Atherosclerosis-risk Emerging Module P96 could be identified only in atherosclerosis weighted signaling network, and genes in this module were mainly related to inflammatory response and chemotaxis. For instance, IL1A and IL1B belonged to interleukin family, which attracted neutrophils, and caused the release of inflammatory mediators. IL1B was involved in the pathogenesis of atherosclerosis for endothelial cells. Secreted IL1A, IL1B amplified and differentiated progenitor cells (CD34 (+)) cells via c-MSCs signal [22, 36-38]. IL6 could lead to the accumulation of inflammatory cells in atherosclerotic plaques, affect the stability of plaque and promote thrombosis and necrosis [39, 40]. IL8 is the main attractor and activator of neutrophils. IL8 also had a role in promoting artery atherosclerotic plaque pathological angiogenesis, which was one of the main factors that lead to plaque instability [22, 41]. Hence, the emergence of Atherosclerosis-risk Module P96 in disease status may facilitate the development of atherosclerosis.

Discussion

Atherosclerosis is a disease of the arteries characterized by the deposition of plaques of fatty material on their inner walls. Compelling evidence has demonstrated how risk factors, such as hypercholesterolemia, provoked inflammation and reinforced the initiation and progression of atherosclerosis [42]. Interestingly, recent studies have reported that hypercholesterolemia had an impact on stem cells to strengthen inflammation [42, 43]. Studies have shown that atherosclerotic plaques were closely associated with ectopic calcification of vascular differentiation of stem cells [44]. Moreover, perturbation of the signals stem cells secreted by mutations or abnormal protein expression underlies a cause of atherosclerosis [45, 46]. Here, we put forward an integrated approach to identify Atherosclerosis-risk Modules using atherosclerosis related stem cell expression profiles based on a human signaling network. Five Atherosclerosis-risk Modules (three Absent Modules and two Emerging Modules) were identified from two weighted human signaling networks, which were both significantly differential between normal and disease statuses and enriched in functions significantly associated with atherosclerosis genes.

Atherosclerosis-risk Absent Modules were only identified from the normal weighted signaling network, which were crucial to maintain the normal cell regulation and transport function. Absence of these modules in disease status would lead to the disorder of the signal of stem cells and the change of the internal environment so as to participate in the pathogenesis of atherosclerosis [24-27, 47, 48]. The 3 Atherosclerosis-risk Absent Modules enriched significantly in the functional categories related to the maintenance of normal biological process, including "GO:0031328~positive regulation of cellular biosynthetic process", "GO:0051173~positive regulation of nitrogen compound metabolic process" and "GO:0006935~chemotaxis". Cellular biosynthetic process is involved in many biological processes. DeBerardinis et al. have demonstrated that the disorder of cellular biosynthetic process could lead to related signal disorders and the development of diseases [49]. Qiao et al. have proved that metabolic disorders amplified macrophage chemotactic responses and accelerated the atherogenesis process [50]. The production of reactive nitrogen species was implicated in atherosclerosis principally as means of damaging low-density lipoprotein that in turn initiated the accumulation of cholesterol [51]. Meanwhile, the average expression value of Atherosclerosis-risk Absent Modules in disease status was lower than that in normal status significantly, which suggested that the absence of Atherosclerosis-risk Absent Modules might involve in the pathogenesis of atherosclerosis.

Atherosclerosis-risk Emerging Modules were only identified in atherosclerosis weighted signaling network, which were associated with its pathogenesis, especially for Atherosclerosis-risk Module P96. It was enriched significantly in functional categories related to the pathogenesis of atherosclerosis. Meanwhile, the average expression value of Atherosclerosis-risk Emerging Module P96 in atherosclerosis status was higher than that in normal status significantly. Moreover, the ability to classify samples of normal and disease statuses could reveal the association with diseases using Supporting Vector Machine (SVM) or random forest [52]. Here, SVM was applied to classify samples of different statuses (normal/atherosclerosis) of the stem cell expression profile GSE9820 with the average expression value of Atherosclerosis-risk

Emerging Module P96 as classification feature, and Leave-one-out cross-validation was performed [53]. A receiver operating characteristic (ROC) curve was plotted and the area under the curve (AUC) was 0.841. It demonstrated that Atherosclerosis-risk Emerging Module P96 had a good classification performance in distinguishing between normal and disease samples. Meanwhile, all genes of Atherosclerosis-risk Emerging Module P96 were proven to be associated with the pathogenesis of atherosclerosis proven by literature. Three genes, IL6, ICAM1 and ADIPOQ, were confirmed to be atherosclerosis genes by databases CTD, DO and GWAS. ADIPOQ, as the start point of signal transduction in Module P96, regulated many processes and affected other genes in the module. ICAM1 might influence genes outside the module since it was the end of signals. IL6 and other interleukins in this module all regulated other genes or were regulated by other genes in several ways. Hence, the emergence and activation of Atherosclerosis-risk Emerging Module or dysfunction of its genes could contribute to the development of atherosclerosis, and the genes of Atherosclerosis-risk Emerging Module P96 might be potential disease genes.

Though sample size (11 normal and 12 atherosclerosis samples) of the expression profile we used could be a limitation of our study, it was still enough to construct weighted human signaling networks since they were significantly consistent in expression (p<0.05). From these networks, candidate Atherosclerosis-risk Modules were detected and Atherosclerosis-risk Modules were further identified. Larger sample size might help to improve the results and illustrate the pathogenesis of atherosclerosis better in the future.

In summary, a novel integrated approach was proposed to identify Atherosclerosis-risk Modules from the systems level based on weighted human signaling networks and atherosclerosis related stem cell expression profiles. Five Atherosclerosis-risk Modules (three Absent Modules and two Emerging Modules) were identified and verified to be associated with the pathogenesis of atherosclerosis. Atherosclerosis-risk Emerging Module P96 could distinguish between normal and disease samples. These modules and their genes may act as potential atherosclerosis biomarkers. Our study would shed light on the signal transduction of atherosclerosis, and provide new insights to its pathogenesis from the perspective of stem cells.

Supplementary Material

Supplementary table.
http://www.ijbs.com/v14p1678s1.pdf

Competing Interests

The authors have declared that no competing interest exists.

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