The ubiquitin-proteasome activity of macrophages involved in the rupture of atherosclerotic plaque based on bioinformatics analysis

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Abstract

Background

Macrophages have been demonstrated to promote the rupture of the atherosclerotic lesion, a primary cause of ischemic events, by modulating the formation of plaque necrosis. We aimed to search the key factors of genetic macrophages in concerns of carotid ruptured plaques.

Methods

The database of the gene expressed GSE41571 is retrieved from the Gene Expression Omnibus (GEO) to differentially obtain the measured expression genes (DEGs) by tendering the tool usage of GEO2R. The enriched pathways of ontology at DEGs performances with DAVID interacted the constructions of protein to protein networking by the analysis of STRING and Cytoscape software. And also the identified PPI networks in the significance of hub genes module plugged into respective Cytohubba and MCODE Cytoscape.

Result

A total of 1481 DEGs in macrophages from ruptured and stable carotid atherosclerotic plaques are noticed, by including 568 up-regulation genes and 913 down-regulation genes. The analyzed GO shows a DEGs are mainly involved in protein ubiquitination, endocytosis, mRNA processing, and mitotic cell cycle. KEGG pathway enrichment analysis revealed that some major protein catabolism pathways, including the ubiquitin-proteasome, autophagy, and endocytosis systems, play key roles in the progressive carotid plaque clotting. The genes of the hub in the PPI network included POLR2E , PPP2R1A , HSP90AA1 , and CBL . The modules were mainly associated with ubiquitin-mediated proteolysis, endocytosis, spliceosome, proteasome.

Conclusion

Our findings offer novel molecular insights into the mechanisms by which macrophages drive formation and vulnerability of plaques. The candidate DEGs and potential biological pathways might be used as diagnostic targets, and facilitate the development of novel macrophage-targeting therapies for unstable atherosclerosis.

1. Introduction

The term atherosclerosis (AS) is defined as a chronic immuno-inflammatory disorder that involved in the accumulated lipid-rich plaques in the vasculature, which also involved immune cell including macrophages and T lymphocytes migrate into the lesion area and the subendothelium of extracellular matrix [1, 2]. AS and its thrombotic complications play important roles in the pathology of most cardiovascular disease. Now the treatment mainly focuses on the control of hyperlipidemia, hypertension and the use of ACE inhibitors. Even we reduce the cardiovascular risk by the combination of medical

treatments and surgical intervention; it is still necessary for AS and thrombotic complications to be optionally select the additional therapeutic criteria. In addition, the rupture of atherosclerosis plaques forms an acute thrombus subsequently into a formation of MI or stroke[3]. Thus, it is necessary to precisely understand the molecular ruptured mechanism in atherosclerosis so that it could provide the target on early diagnosis with better therapies of atherosclerosis plaques.

Many studies have indicated that macrophages play key roles at all stages of atherosclerotic plaques formation [4, 5]. In early atherosclerosis lesions, the circulating monocytes were recruited and accumulate into the atherosclerotic lesion regions due to endothelial adhesion molecule expression, then differentiate into macrophages [6, 7]. Macrophages actively participate in lipoprotein ingestion in atherosclerotic lesions and subsequently become lipid-laden foam cells contribute to form an early sub-endothelial lesion. Likely, the limited macrophages presented a reduced efflux in cholesterol as a protective characteristic against the development of clotting factor[8]. Recent studies indicated that macrophages might associate to the initiation and development of plaque by secreting cytokines to maintain the inflammatory response that participating in plaque stabilization particularly by anti-inflammatory macrophages in the local microenvironment[9, 10]. Moreover, advanced lesions, macrophages proliferation might take on a more important role. At this stage, macrophages could further secret matrix-degrading proteases responsible for necrotic core formation, lead to intraplaque hemorrhage in plaques [11, 12]. In addition, the increase of macrophage apoptosis or necrosis in plaques make the lesion unstable and a fibrous cap was prone to be thinner leading to the secreting ruptured plaque by matrix metalloproteinase's [13]. In the advancement of macrophages lesions, and the apoptotic smooth cells in muscles might further aggravate within the expansion of core at plaque instability in thinning of the fibrous cap[14, 15]. Importantly, the fibrous cap was destabilized by the MMPs and matrix-degrading enzymes, making the advanced plaques were more likely to rupture leading to more severe outcomes, including ischemia and stroke infarcts [16]. However, the mechanistic biochemically at the rupture of atherosclerotic plaque caused by macrophage remains unclear. Meanwhile, the ability of migration of macrophages in the process of atherosclerotic plaque decreased that further progress into the resolved failure of inflammation into a complicated formation of sclerotic plaque [17].

Therefore, it is more meaningful to differentially identify the genes expressions (DEGs) at the stable with ruptured plaques, including the biological processes and pathways involved, to provide a foundation for illuminate molecular mechanisms, help to screen cellular or condition-specific biomarker for diagnosis or prevention of atherosclerosis plaques rupture and its complications. Recently, atherosclerotic gene expression profiling studies have been performed by high-throughput microarray technology and suggested that hundreds of DEGs are including the variety of guidelines in both biological processes, and biomolecular functioning, especially complex network during the process of atherosclerosis [18]. In the analytics of bioinformatics surely proved it possible to observe the altered expressions in genes between stable and ruptured atherosclerosis plaques. Our present study was designed to the compared expression of mRNAs in stable and ruptured atherosclerotic plaques from GSE41571 datasets, which included both the 5 and 6 stable ruptured human atherosclerotic clots by dissecting through micro-laser, and to study

their diagnostic or treatment values as a biomarker of AS. The original gene expression profiles (GSE41571) data is taken from Gene Expressed Omnibus (GEO). And in the ontology of subsequent genes at the annotative pathways of enrichment, the interaction of (PPI) is noticed separately in the performances of DEGs by using bioinformatics method.

We aimed to provide a better understanding of the molecular mechanism in the process of carotid atherosclerotic plaques and identify more key genes for novel diagnosis and therapeutic targets for the prevention of AS plaques rupture.

2. Materials And Methods

2.1 Datasets based on microarray acquisition

The association of detailed atherosclerotic plaque data at microarray data sets of GSE41571 is selected from the (GEO) website (http://www.ncbi.nlm.nih.gov/geo). GSE41571 retrieved on the GPL570 (Affymetrix HG-U133 + 2.0 GeneChip arrays). The macrophages were isolated from the stability between ruptured 5 and 6 human atherosclerotic plaques dissected by micro-laser, and genome-wide expression analyses were performed.

2.2 Pretreatment and differential analysis

To identify the ruptured and atherosclerotic stability in means of DEGs, we used online software GEO2R. Further, we investigated by the method of Benjamini-Hochberg to calculate adjustments of P-value, with the false rate of discovery P-value (FDP) [19]. Moreover, the sets of FDR with < 0.05 and $|\log_2$Fold Changes (FC)$| \geq 1$ threshold the sets of selected DEGs.

2.3 Pathways of Enrichment Analysis in DEGs and Gene Ontology

In the identification of DEGs, the performed KEGG and Go enriched pathway analyzes the DAVID (Database for Annotation, Visualization and Integration Discovery, from http://david.abcc.ncifcrf.gov/) [20]. Whereas, the significantly selected cut-off functional terms include FDR < 0.01. Additionally, in the biological process, the cellular component (CC) functionally alter the revealing enrich analysis.

2.4 Protein-Protein Interaction (PPI) Integrated Network and Module Analysis.

In the present study, the retrieval interacting the genes (STRING 10.5; https://string-db.org/) the search tool in the database builds the networking in PPI among DEGs to reveal the interactions between proteins. The validation based on experiments interacts a combined score of > 0.7 with its significant STRING selection. [21]. The visualized assisting biological networks on using Cytoscape3.6.1 software (http://cytoscape.org/) might also integrate the majority of free scales, and hub genes to plug-in Cytohubba its connectivity degree ≥ 25 [22].

After we obtained the results from the analysis of using the STRING database, we detected the molecular complexity plug-in (MCODE) to modulate its screening in Cytoscape[23]. And also degree value was counted and the close connection between nodes was observed. MCODE algorithm could calculate the score of each module, and the higher score meant that the interaction relations inside the module were more closed. Modules inferred using the default settings that the degree cutoff is setting with 2, and node score cutoff at 0.2, with k-core 2, on max. Depth at 100. On significant MCODE score ≥ 10 on node selection of ≥ 10. Lastly, the functional GO and KEGG enriched the analyzed pathway with its modulating performances on the threshold at $P < 0.05$.

3. Results

3.1 Identified differentially expressed genes

After data pretreatment, the maximum with 1481 DEGs is analyzed on GSE41571 data-sets, comprising the 586 genes at up-regulation and 913 genes down-regulation. There were great differences between both the ruptured and stable atherosclerotic plaque based on their expressed gene profiles. Total 1481 genes were aggregated into pools of DEGs for further analysis.

3.2 Both functional and pathway enrichment analysis

The insight of further gained identified functional DEGs in different stages of atherosclerotic plaques; the GO functional enrichment analysis was performed using DAVID, on categorizing three keywords including both the biological process (BP), molecular function (MF) and cellular components (CC). The first top ten in GO terms of three categories are listed in Table 1–3. And the results showed that DEGs mainly took part in CC including membrane-bounded and intracellular organelle (GO.0043227, GO.0043229, GO.0070013). The MF enrichment analysis refers to the DEGs that include mainly in binding the (GO.0005488), protein binding (GO.0005515), and macromolecular complex binding (GO.0044877). The BP enriched analysis observed that the upregulated genes take part in the regulated biological process (GO.0050789), cellular component organization (GO.0016043), biological regulation (GO.0065007). The pathways of KEGG enrichment analysis showed that enrichment in lysosome are by DEGs (4142), cell cycle (4110), viral myocarditis (5416), phagosome (4145), focal adhesion (4510), Hippo signaling pathway (4390), endocytosis (4144), PI3K-Akt signaling pathway(4511) and platelet activation(4611). The enriched KEGG pathways of the DEGs with its significant are shown in Table 4.

3.3 PPI network construction on modules selection

The analysis based on the STRING database, the PPI networks of 1481 DEGs consisted of 1349 nods and 2851 edges were constructed using Cytoscape. Whereas, in the PPI network, the indication of each node in its gene represents its own interactions. And also in the degrees of linked edges in the specific number of node calculate a higher degree of defined hub genes, which functionally assume is biological importance. In our results, 36 of DEGs were considered as hub genes selection with a degree ≥ 25, including 15 up-regulated and 22 down-regulated genes, the highest degree genes including RNA

polymerase II subunit E (POLR2E), protein phosphatase 2 scaffold subunit Aalpha (PPP2R1A), the 90 alpha family class A member of 1(HSP90AA1), with heat shock protein and Cbl proto-oncogene (CBL), which already connective the degrees with respective of 57, 53, 51 and 41. The top 20 hub genes were shown in Table 5.

The significant modules from PPI network were obtained to use the plug-in MCODE in Cytoscape, with top given 5 modules are shown in Fig. 1. Module A was often best descriptor with 24 nodes and 276 edges (MCODE score = 24). Module B was another better descriptor by 20 nodes and with 186 edges (MCODE score = 19.579). The functional interrelated annotation analyzed the involved genes in the modules of A-E were performed, and the results were shown in Table 6–8. We found that BP terms were mainly enriched in protein ubiquitination, endocytosis, mRNA processing, ribosome biogenesis, and mitotic cell cycle process. The MF terms were mainly enriched in ubiquitin-protein transferase activity, RNA binding. The CC terms were mainly enriched in ubiquitin ligase complex, spliceosomal complex, nucleolus, cytosol, and clathrin-coated vesicle. The pathway KEGG enrichment revealed that genes in modules A-E mainly associate with Ubiquitin-mediated proteolysis, endocytosis, spliceosome, proteasome (all KEGG pathways were listed in Table 9).

4. Discussion

The profiling of gene expressed analysis widely explores the abnormal diseased linked expressed genes that also enable the targeted therapeutic strategies. Identification of the factors related to plaque rupture also evolves the basic further prevention of severe clinical outcomes. In this study, the potential key genes in macrophages were identified from different atherosclerotic plaques based on the bioinformatics analysis. The accumulated macrophages in the atherosclerotic lesion progression within the subendothelium are considered as the initial step. However, the mechanism and progress of atherosclerotic plaques rupture were recently unknown, along with the further investigation are needed to precede research.

According to our results, the observed functional GO enriched analysis significantly showed the DEGs related to membrane-bounded and intracellular organelle, protein binding, regulation of biological process and cellular component organization, and so on. The biological process of lysosome, endocytosis, and phagosome took part in the cellular metabolism for the renewed organelles. The specific lysosomal mechanisms such as autophagy differently play an important role in the stage of AS. Furthermore, the quick clearance of macrophages apoptosis play an important role within the reduced advanced AS, to prevent the progress of inflammation and plaque necrosis to delay the ruptured plaque [24]. KEGG pathways enrichment analysis respective to its identified 18 (from up-regulated genes) and 4 (from down-regulated genes) significantly enriched pathways, including lysosome, endocytosis, cell cycle, phagosome, focal adhesion and platelet activation in atherosclerotic plaques. Our results indicate that lysosomal mechanisms such as endocytosis, phagosome, and autophagy may illustrate its contribution towards the development of advanced staging in atherosclerosis. In particular, up-regulated DEGs enriched into several signal pathways such as the PI3K-Akt signaling pathway, PPAR signaling pathway,

chemokine-signaling pathway, and NF-kappa B signaling pathway. Down-regulated DEGs have only constructed the Hippo signaling pathway, which this pathway was down-regulated in ruptured plaques. Our results suggested that the intracellular signaling pathways were specifically activated in late or ruptured plaques. The enriched KEGG pathways of the DEGs with its significance are shown in Table 4.

PPI network analysis identified 36 hub genes, including POLR2E, PPP2R1A, HSP90AA1, CBL, USE2D1, CCNB1, ANAPC4/5, FYN, ACTR2, and SOCS3, suggest a crucial stable role within its efficacy in genes that play more stability of atherosclerosis plagues. GO functional enrichment analysis showed, hub genes are associated in mediated ubiquitin proteolysis, protein binding, cytosol, endocytosis, cellular macromolecule metabolic process. The top 20 hub genes were shown in Table 5. The CBL, USE2D1, ANAPC4/5, FYN, and SOCS3 all involved in ubiquitin-mediated proteolysis. POLR2E encodes a DNA-directed RNA polymerase II subunit RPABC1. Gene Ontology (GO) annotations related to this gene include directed-DNA polymerase activity of 5 – 3 RNA and also RNA polymerase I activity. The participated gene expression related to its pathways showed the regulation of promoter clearance in RNA polymerase II transcription. PPP2R1A gene constantly encodes the subunits of regulatory protein phosphatase 2, which implicate the contrary role of cell partitions and growth. PPP2R1A in the reduced phosphorylation of P38 MAPK serves as a suppressor in its effect of CTLA4 signaling to inhibit AKT towards the T cytotoxic lymphocytes [25–27]. Forman reported the nitric oxide production and reactive oxygen species (ROS) in macrophages could be suppressed by PPP2R1A [28]. In addition, HSP90AA1 was a key member of heat shock protein 90 families. Interestingly, several members of heat shock proteins 90 family like HSP70 and HSP90 also proposed potential biomarkers in cardiovascular risk factors [29]. Diseases associated with HSP90AA1 included hypersensitivity vasculitis and hypersensitivity reaction type III disease. In our result, we found as a key gene of Akt Signaling pathway, the expression of HSP90AA1 was significant down-regulation in rupture plague. An important paralog of HSP90AA1 was HSP90AB1, which was observed to down-regulate the ischemic tissue in the heart. The expression of decreased HSP90AB1 might suggest a defensive act against the mechanical injury of myocardial cells in ischemia, which finally leads to decreased inflammation and atherosclerosis [30]. A previous report has suggested that the Src kinases FYN was involved in regulating collagen-induced platelet activation [31]. PIK3CG protein belonged to the member protein of pI3/pI4-kinase and was an extracellular signaling critical modulator; including the cell-cell adhesion mediated by E-cadherin that importantly act proper maintenance with its integrity of structural epithelia[32]. In a combined meta-analysis of carotid plaques confirmed the rs17398575 SNP in PIK3CG gene that might induce an 18% chances plaque formation, showed PIK3CG had important clinical significance for higher chances of aggregation in platelet leading to acute coronary syndromes [33]. In our study, the results showed that PIK3CG were up-regulated in stable atherosclerotic plaques samples, compared with ruptured atherosclerotic plaques. This suggested PIK3CG helpfully remained an outstanding method in preference to stable the plaques.

On the other hand, 5 modules through served networking of PPI obtained a plug-in MCODE in Cytoscape. The above majority of hub genes were also involved in the construction of modules A-E. Moreover, the enrichment pathway analysis expressed that genes in modules are particularly connected with ubiquitin-

mediated proteolysis, endocytosis, spliceosome, and proteasome. Although the inducement in both the stages of progression of atherosclerosis though the ubiquitin-proteasome pathway should be demonstrated [34]. However, our study still did not provide enough evidence based on absolute potential in ubiquitin-proteasome activity towards developed atherosclerotic plaques. Herrmann, et al. discussed the coronary risk factors in the enhancement of ubiquitin expression [35]. Many other studies have demonstrated that the dysregulation of ubiquitin–proteasome pathways results in the accumulation of defective proteins in blood vessels, leading to vascular endothelial cell dysfunction and apoptosis. In this study, we found that ubiquitin-proteasome activity reduced ruptured plaques. At note, hub genes ANAPC4, ANAPC5, CUL2, CUL5, UBA6, UBE2H and SOCS3 from module A were all involved in the ubiquitin-proteasome pathway. As a key node, genes with PPI networking relatively stay at a higher degree. For examples, the complexity of CUL5 and SOCS3 with the SOCS/BC-box/eloBC/cul5/RING E3 ligase involvement, systematically in the ubiquitin play a role of protein degradation [36]. The hub gene Cbl, a B-lineage gene as a casitas lymphoma encode a CBL protein that involved an E3 protein ligase by signaling cell, protein ubiquitin with endocytosis. Moreover, a hub gene PLCG1 (1-Phosphatidylinositol-4,5-bisphosphate phosphodiesterase gamma-1, PLCγ1) encoding the membranous enzyme (phospholipase C family), that catalyzes the phospholipid PIP2 (1-phosphatidyl – 1D-myo-inositol 4,5-bisphosphate) into secondary trans-messengers IP3 (myoinositol 1,4,5-triphosphate) additionally with DAG (diacylglycerol). DAG and IP3 could activate protein kinase C (PKC) on releasing Ras realizing proteins (RasGRPs) which signal molecules on initiating the mitogen-protein kinase (MAPK) and NF-κB pathways. Although, altered expression of Cbl and PLCG1 suggested that relation signal pathway and activity were abnormal in ruptured atherosclerotic plaques. Our results showed that ubiquitin-proteasome activity might obviously refer to the stability of atherosclerosis plagues. On worth noticing it, of the above genes, except for SOCS3, were down-regulated expressed in macrophages, suggested that ubiquitin-proteasome activity might be suppressed in advanced stages or ruptured plagues. We speculated that the above key genes might involve in the stability of plagues via regulation of ubiquitin-proteasome activity.

As we mentioned, heterogeneous macrophages play different phenotypes and functions environmentally depend on the active intracellular signal pathways[9]. In this study, the factor of nuclear kappa B (NF-κB), chemokine and also PI3K-Akt signaling pathway were significantly activated in rupture plagues in this study. Especially the NF-κB signaling pathway, acting as a central transcription signaling pathway to regulate the inflammatory response. Evidence shows that NF-κB was activated by the degradation of its inhibitory NF-κB proteins through the ubiquitin-proteasome pathway [37].

The indication of fewer studies functionally relates the activity of ubiquitin-proteasome and NF-κB, suggesting that they play significant roles in atherosclerosis plaques. The accumulating data have suggested that increased activity within the atherosclerotic lesions, activate the system of ubiquitin into inflammatory cells relying on NF-κB dependence way, further promoting plaque rupture. The interrelation between different signaling pathways showed that cross-talk arises through hub genes participating in different signaling pathways. It was suggested that the genes might target an intervention. Our conclusion suggests a potential mechanism which ubiquitin-proteasome activated by NF-κB signaling

pathway, may mediate the inflammatory activity of macrophages that may enhance the erosion of unstable plaque lesion, further leading to plaques rupture.

Together, our result suggests that as major protein catabolism pathways, an ubiquitin-proteasome signaling pathway was distributed into atherosclerosis plaques and contributes the pathogenesis of atherosclerosis plaques. Therefore, the hub proteins and significant modules included the degradation ubiquitin proteins, the cellular endocytosis and the regulation of cell cycle may play critical roles in promoting atherosclerotic plaque rupture, and promise for the prediction of carotid atheroma plaque stability. Macrophage play a key role in the AS pathogenesis, that attracts the targeted therapy of AS. Some research has shown that it could be possible in the way inhibiting both the monocyte and macrophages recruiting its growing lesions, which may polarize the ability and plasticity to use in good pro- anti-inflammatory phenotypes [38].

5. Conclusion

The provided data comprehensively conclude the provided basis bioinformatics analyses for our better understanding of the latent mechanistic molecular process of AS. In atherosclerotic plaques, DEGs of macrophages such as HSP90AA1, CBL, USE2D1, CCNB1, ANAPC4/5, FYN, ACTR2 and SOCS3, pathways associate a ubiquitin–proteasome pathway, to biologically proceed the related terms to lysosome, endocytosis and phagosome, would hopefully reveal new targets for the prevent and treatment of atherosclerotic plaque rupture.

Declarations

Conflicts of Interest

The authors declare that there are no conflicts of interest in this work.

Authors' contributions

Jing Wan and Ya Li contributed to the design of the study. Ya Li, Wei Zhang, Yulin Ma and Mingying Lin performed the implementation and analysis of the project. Min Zhao performed the interpretation of the results and wrote the manuscript. All the authors read and approved the final manuscript.

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Tables

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Figures

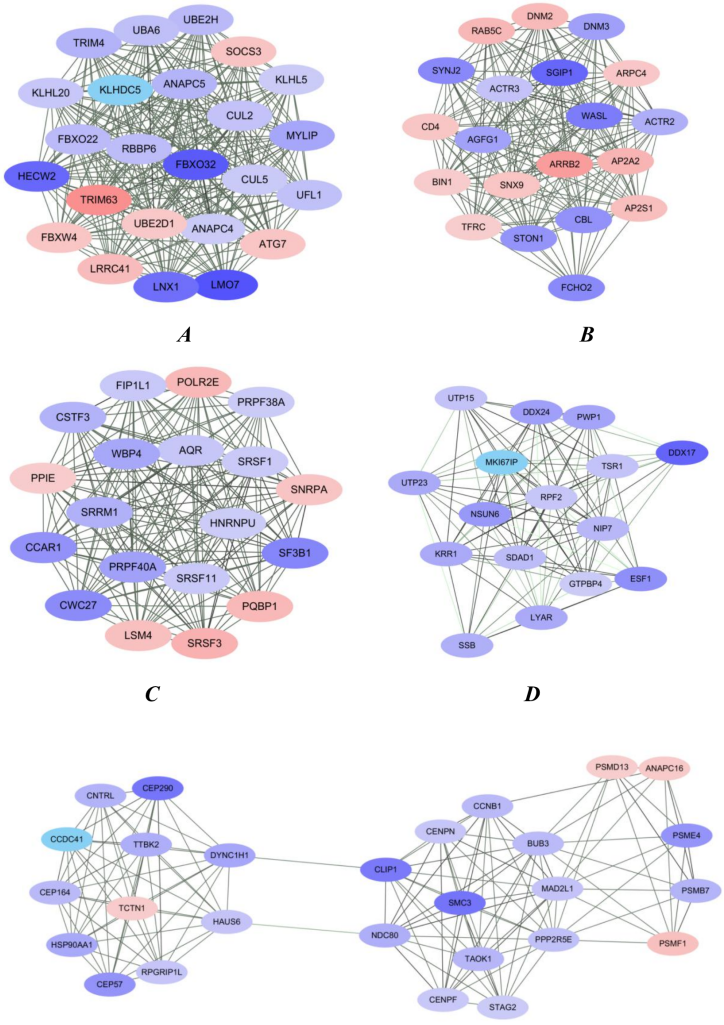

Figure 1 The significant modules from the protein-protein interaction network by Cytoccape (MCODE) analysis.

Notes: The node stands for the protein (gene); Red, up-regulated DEGs; blue, down-regulated DEGs. The width of edge was determined according to the combined score of the protein–protein interaction relationship.

Figure 1

The significant modules from the protein-protein interaction network by Cytoccape (MCODE) analysis.

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