

The effect pathways analysis in the abdominal aortic aneurysms

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Abstract. – BACKGROUND: Abdominal aortic aneurysm (AAA) is a relatively common disease in elderly. Currently, only surgical treatment has been available for ruptured AAA. Thus, it is impressing to elucidate the molecular cellular mechanisms of AAA in order to develop the effective medications.

AIM: This study is to explore the significant pathways and crosstalk between them in response to AAA.

METHODS: The crosstalk of pathways was analyzed based on PPI datasets and expression profiles.

RESULTS: It was showed that significant pathways included Cytokine-cytokine receptor interaction (hsa04060), B cell receptor signaling pathway, Chemokine signaling pathway (hsa04062), Cell adhesion molecules (CAMs) (hsa04514), and Hematopoietic cell lineage (hsa04640), which were in accordance with Lenk's results. Further analysis indicated that Chemokine signaling pathway (Hsa04062) and Cytokine-cytokine receptor interaction (Hsa04060) were both connected with the Cell adhesion molecules (CAMs) (Hsa04514) through the signal transduction (GO:0007165). B cell receptor signaling pathway (Hsa04662) and Cytokine-cytokine receptor interaction (Hsa04060) were both connected with the Natural killer cell mediated cytotoxicity (Hsa04650) through the apoptosis (GO:0006915) and signal transduction (GO:0007165), respectively. These crosstalks seemed to exit according to previous reports. We hope our study could provide insights for abdominal aortic aneurysm mechanism to some extent.

CONCLUSIONS: We analyzed the significant pathways related with AAA through Sp and DAVID method. The results were in accordance with previous reports.

Key Words:

Abdominal aortic aneurysms, GO enrichment analysis, Pathway crosstalk.

aortic diameter > 3 cm¹. Patients with AAA can exhibit back pain, abdominal pain, or a pulsatile abdominal mass². The risk factors include increasing age (> 65 years), male sex, cigarette smoking, hypertension, and atherosclerosis³. Importantly, AAA also displays some characters of familial associations⁴. Approximately 15% of AAA patients have a positive family history⁵. Genome-wide scans of AAA patients have suggested a role for genes located on chromosome 19q13 and 4q31⁶. Putative candidate genes in these regions consist of CCAAT enhancer binding protein (CEBPG), peptidase D (*PEPD*), and *CD22*⁷.

Currently, only surgical treatment has been available for ruptured AAA, which is not applicable for small AAA. Thus, it is impressing to elucidate the molecular cellular mechanisms of AAA in order to develop the effective medications⁸. Pathologically, AAA development is associated with a chronic inflammation, extracellular matrix (ECM) degradation, and smooth muscle cells apoptosis^{9,10}. Chronic inflammation is the defining feature of AAA with the presence of a mononuclear inflammatory cell infiltrate in aneurysmal tissue. The inflammatory cell infiltrate is the major source of ECM degrading enzymes (including matrix metalloproteinases (MMPs)¹¹, cathepsins¹², and granzyme) that result in structural proteins degradation, and eventual rupture of the vessel^{13,14}. Also, elevated release of pro-inflammatory cytokines (e.g., interleukin (IL)-1, tumor necrosis factor (TNF)- α , monocyte chemoattractant protein (MCP-1)¹⁵, regulated upon activation, normal T cell expressed and secreted (RANTES)¹⁶, and osteopontin¹⁷) from infiltrating cells lead to further exacerbation of tissue injury and induction of smooth muscle cells death. Smooth muscle cells are largely responsible for production of the aortic extracellular matrix. Additional proteases may be released from dying smooth muscle cells (SMC), further contributing to matrix degradation. There-

Introduction

Abdominal aortic aneurysm (AAA) is a relatively common disease in elderly that usually defined as a pathologic dilatation of the infrarenal

fore, this enhanced degradation of structural proteins, together with a reduced capacity to synthesize new matrix proteins, resulting in dilatation¹⁸.

However, the exact mechanism of AAA is still unclear. In this study, we utilized the gene expression profiles from microarray platforms to further mine significant pathways involved in the pathogenesis of AAA through protein-protein interaction (PPI) network construction. In addition, crosstalk between these significant pathways was also analyzed. In addition, pathway crosstalk analysis is also performed based on PPI network and co-expressed significance of a gene pair. A scoring scheme is used to define a function as the combination of statistical significance of an interaction between pathway¹⁹. We hope our study could predict more target sites for medication treatment of abdominal aortic aneurysms.

Methods

Data Sources

Firstly, we collected Kyoto encyclopedia of genes and genomes (KEGG)²⁰ datasets involved 211 pathways containing 5385 genes. And the protein-protein interaction (PPI) data from human protein reference database (HPRD)²¹ and Bio General Repository for Interaction Datasets (BIOGRID)²² database were also collected. Total 326119 unique PPI pairs in which 39240 pairs from HPRD and 379426 pairs from BIOGRID were collected.

Then we constructed an ensemble protein-protein interaction network by integrating two above existing PPI databases in human.

We extracted the gene expression profile data on Lenk et al²³, which was deposited in National center for Biotechnology Information Gene Expression Omnibus (NCBI GEO) (<http://www.ncbi.nlm.nih.gov/geo/>) database (ID: GSE7084). Full thickness aortic wall tissue specimens were collected in RNAlater (Ambion) from patients undergoing aneurysmal repair operations at the Harper University Hospital, Detroit, MI, USA. Control aortas were all collected within 24 h of death and snap-frozen in liquid nitrogen. This study was approved by the Institutional Review Board of Wayne State University, Detroit, MI, USA and the research was carried out in compliance with the Helsinki Declaration.

Limma and Bayes method²⁴ was used to measure the differential expression status of gene. Background intensities were adjusted and the

original expression datasets from all conditions were processed into expression estimates using the robust multichip average (RMA)²⁵ method with the default settings implemented in R (version 2.12.1)²⁶, and then constructed the linear model.

Pathway Crosstalk Analysis

Here the crosstalk pathways are defined as those pathways which have the overlapping genes and edges with each other. The overlapping genes mean both of the two pathways included and the overlapping edges mean both of the two pathways included the PPI interaction edges.

To determine the co-expressed significance of a gene pair in disease cases, we used the Pearson's correlation coefficient (PCC) test to calculate the p -value.

Map those p -values to the nodes and edges in the PPI network. The following formula is used to define a function as the combination of statistical significance of an interaction by a scoring scheme. The detail description could be seen in Liu et al²⁷.

$$S(e) = f[\text{diff}(x), \text{corr}(x, y), \text{diff}(y)] \\ = -2 \sum_{i=1}^k \log_e(pi)$$

The $\text{diff}(x)$ and $\text{diff}(y)$ are differential expression assessments of gene x and gene y , respectively. $\text{Cor}(x, y)$ represents their correlation between gene x and gene y . f is a general data integration method that can handle multiple data sources differing in statistical power. Where $k = 3$, p_1 and p_2 are the p -values of differential expression of two nodes, p_3 is the p -value of their co-expression.

Significant Pathways Analysis

$$Sp = \sum_{e \in P} S(e)$$

The frequency of scores that are larger than Sp was used as the significance p -value of pathway P to describe its importance.

We also used the DAVID²⁸ for the pathway enrichment analysis with the p -value < 0.05 input the differentially expressed genes (DEGs).

Then, the overlap pathways of the two enrichment analysis methods were selected as the significant.

Pathway Crosstalk Analysis

The detailed analysis of crosstalk of relationships among pathways was then investigated, especially that with overlap of two significant pathway analysis results.

To define the interaction significance between pathways, we summarized all the scores of edges $S(e)$ of all non-empty overlaps. Specifically, the interaction score between two pathways was estimated by their overlapping status of weighted pathways in the following formula:

$$C(p_i, p_j) = \sum_{e \in O_{ij}} S(e)$$

where P_i and P_j are two pathways, and O is their overlapping.

To estimate the significance of the overlapping between different pathways, we randomly sampled 10^5 times of the same size two pathways in the edges of pathway network and calculated their overlapping scores. The frequency that larger than C was regarded as the interaction significance p -value. At last, the crosstalk with the p -value < 0.001 were considered as the significant pathway crosstalk.

Significant GO Enrichment Analysis in Each Pathway

The functional enrichment among proteins in one pathway is defined as:

$$P = 1 - \sum_{i=0}^{k-1} \frac{\binom{f}{i} \binom{n-f}{m-i}}{\binom{n}{m}}$$

where n is the number of nodes in the network, f is the number of proteins annotated with a particular GO function, m is the number of proteins involved in the pathway and k is the frequency of the GO term. We identified the GO function enrichment of the pathways respectively.

Results

To investigate the significant pathways and the crosstalks among them, we firstly downloaded the GSE7084 from GEO (<http://www.ncbi.nlm.nih.gov/geo/>). The R language was used to select 329 DEGs. Based on the expression profile, PPI datasets, and KEGG pathways, we identified five significant pathways and predicted the crosstalks among them.

Significant Pathway Analysis

We adopted two methods to identify significant pathway (Sp and DAVID) in this study. Our results showed that total 33 pathways (Table I) were detected with the p -value < 0.01 using Sp method. However, only 8 significant pathways were identified by DAVID method. Among them, 5 overlap significant pathways were overlapped, including Cytokine-cytokine receptor interaction (hsa04060) with the p -value = 6.56E-04, B cell receptor signaling pathway (hsa04662) with the p -value = 0.004, Chemokine signaling pathway (hsa04062) with the p -value = 0.01, Cell adhesion molecules (CAMs) (hsa04514) with the p -value = 0.02, Hematopoietic cell lineage (hsa04640) with the p -value = 0.03 (Table II). This indicated these five pathways may play an important role in AAA development.

Crosstalk of GO Relationships Among Pathways

We considered the pathway crosstalk between these 5 significant pathways and other significant pathways (only selected by the PPI-network approach) detected by the overlapping score. We found 35 significant pathways were crosstalk to these 5 significant pathways.

For detail analysis the crosstalk between the significant pathways, we applied the hypergeometric test to find the significant gene ontology (GO) terms in each pathway with the p -value < 0.05 . The results of the top five GO terms in part of the pathways were used to construct the connection among pathways. From the result of Figure 1, we could find Chemokine signaling pathway (Hsa04062) and Cytokine-cytokine receptor interaction (Hsa04060) both connected with the Cell adhesion molecules (CAMs) (Hsa04514) through the signal transduction (GO:0007165), through the crosstalk's p -value < 0.01 .

We also find that significant pathways B cell receptor signaling pathway (Hsa04662), and Cytokine-cytokine receptor interaction (Hsa04060) both connected with the Natural killer cell mediated cytotoxicity (Hsa04650) through the apoptosis (GO:0006915) and signal transduction (GO:0007165), respectively.

Discussion

Although multiple etiological factors have been suggested to contribute to AAA, its pathobiology is incompletely understood. In this study,

Table I. Significant pathway analysis using the Sp method

ID	Node	Edge	Size	Score	p-value	Description
hsa04730	16	152	70	2787.696	0	Long-term depression
hsa04666	18	75	95	1029.815	0	Fc gamma R-mediated phagocytosis
hsa05131	19	216	64	2781.431	0	Shigellosis
hsa04662	20	147	75	2597.308	0	B cell receptor signaling pathway
hsa04640	27	132	88	2000.915	0	Hematopoietic cell lineage
hsa00562	36	284	57	3253.212	0	Inositol phosphate metabolism
hsa05416	41	975	75	11527.43	0	Viral myocarditis
hsa04621	43	314	62	3885.179	0	NOD-like receptor signaling pathway
hsa04622	44	569	71	5974.66	0	RIG-I-like receptor signaling pathway
hsa04630	46	120	155	1405.723	0	Jak-STAT signaling pathway
hsa04270	57	339	116	3870.355	0	Vascular smooth muscle contraction
hsa04650	60	436	137	6612.15	0	Natural killer cell mediated cytotoxicity
hsa05140	64	1693	74	17856.84	0	Leishmaniasis
hsa04514	73	501	136	5289.345	0	Cell adhesion molecules (CAMs)
hsa04062	128	814	189	9957.9	0	Chemokine signaling pathway
hsa04060	114	453	265	4819.683	1.00E-05	Cytokine-cytokine receptor interaction
hsa04620	26	85	102	1011.067	5.00E-05	Toll-like receptor signaling pathway
hsa05200	183	1237	328	12374.77	5.00E-05	Pathways in cancer
hsa04142	56	287	121	3048.751	0.00013	Lysosome
hsa05215	26	162	89	1778.884	0.00016	Prostate cancer
hsa04144	131	846	205	8536.507	0.00018	Endocytosis
hsa04614	10	23	17	305.1303	0.00035	Renin-angiotensin system
hsa05010	27	183	168	1940.464	0.00149	Alzheimer's disease
hsa04370	21	110	76	1201.09	0.00192	VEGF signaling pathway
hsa04510	23	37	201	443.5307	0.00193	Focal adhesion
hsa00520	12	19	45	243.7392	0.00234	Amino sugar and nucleotide sugar metabolism
hsa04660	31	156	108	1643.683	0.00456	T cell receptor signaling pathway
hsa00360	3	9	17	120.7839	0.00515	Phenylalanine metabolism
hsa04020	91	489	178	4900.779	0.00589	Calcium signaling pathway
hsa00563	3	5	25	70.32994	0.00625	Glycosylphosphatidylinositol(GPI)-anchor biosynthesis
hsa04962	20	144	44	1515.968	0.00625	Vasopressin-regulated water reabsorption
hsa04350	55	453	85	4543.9	0.00679	TGF-beta signaling pathway
hsa00620	2	3	40	41.71999	0.00937	Pyruvate metabolism

we found five significant pathways associated with AAA development, including Cytokine-cytokine receptor interaction (hsa04060), B cell receptor signaling pathway, Chemokine signaling pathway (hsa04062), Cell adhesion molecules (CAMs) (hsa04514), and Hematopoietic cell lineage (hsa04640), which was in accordance with Lenk's results²³. Further, we found that Chemokine signaling pathway (Hsa04062) and Cytokine-cy-

tokine receptor interaction (Hsa04060) were both connected with the Cell adhesion molecules (CAMs) (Hsa04514) through the signal transduction (GO:0007165).

Cytokine-cytokine receptor interaction and Chemokine signaling pathway were involved in chronic inflammation of AAA. Several pro-inflammatory cytokines were up-regulated after abdominal aortic aneurysm repair, such as inter-

Table II. Significant pathway analysis using the DAVID method.

ID	Description	Count	p-value	FDR (Benjamini)
hsa04060	Cytokine-cytokine receptor interaction	15	6.56E-04	0.070247
hsa04662	B cell receptor signaling pathway	7	0.003698	0.185866
hsa05340	Primary immunodeficiency	5	0.004957	0.167939
hsa04062	Chemokine signaling pathway	10	0.012186	0.2884
hsa04514	Cell adhesion molecules (CAMs)	8	0.016194	0.304036
hsa04664	Fc epsilon RI signaling pathway	6	0.019563	0.306157
hsa04640	Hematopoietic cell lineage	6	0.028491	0.367667
hsa05414	Dilated cardiomyopathy	6	0.036651	0.404341

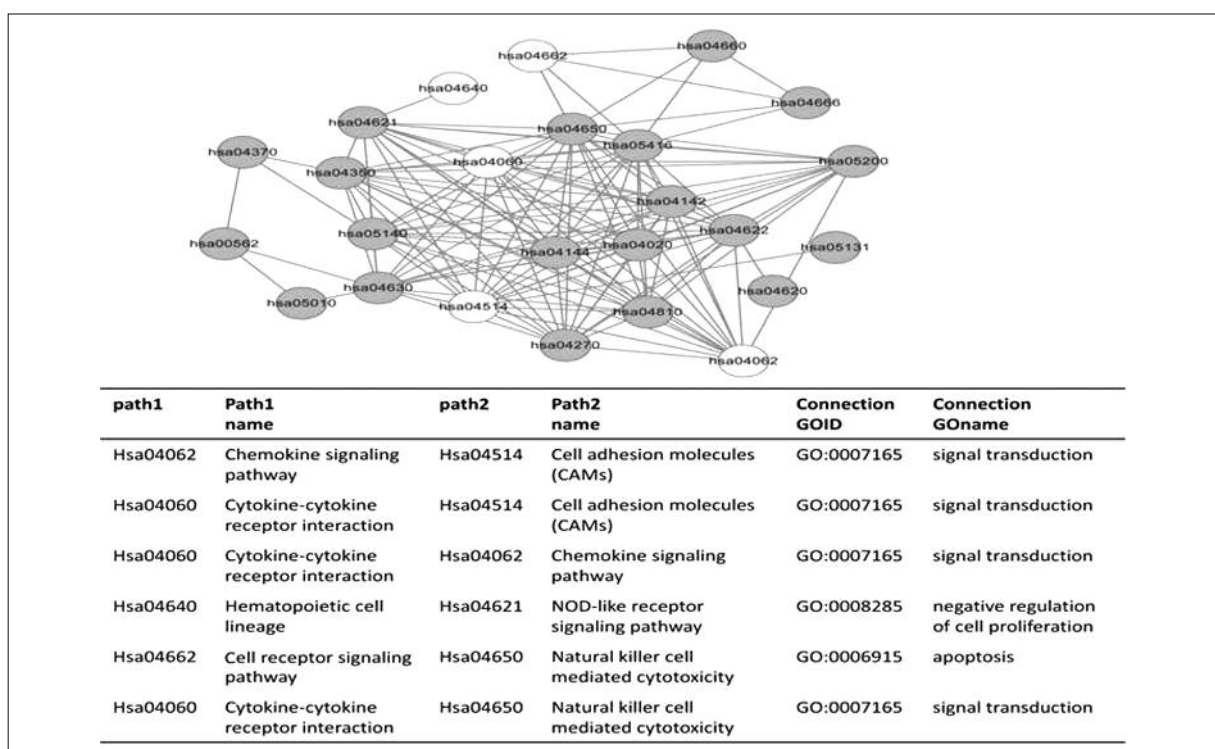


Figure 1. Significantly enriched GO biological processes were identified in every pathway. The edge of each pair of pathways represented the connection with the same GO terms. The solid lines mean the crosstalk’s *p*-value < 0.01. The table in the Figure 1 only list part of results of connections between significant pathways.

leukin (IL)-6, IL-8, IL-1 β , and tumor necrosis factor (TNF)- α ^{29,30}. Recent studies have shown up-regulation of IL-8 production and its receptor CXCR2 within the aortic wall. IL-8 and CXCR2 were mainly expressed in CD68-positive macrophages that accumulated in atheromatous plaques³¹. In addition, CXCR4/CXCL12 interaction is also found participating in the recruitment and retention of inflammatory lymphocytes that infiltrate the aorta in AAA³². Chemokines represent a superfamily of cytokines that function as potent chemoattractants and activators of specific leukocytes. Members of the chemokine family were also highly expressed within AAA samples, such as monocyte chemoattractant protein (MCP)-1³³, MCP-2, epithelial neutrophil-activating peptide-78 (ENA-78), growth related oncogene (GRO), and (RANTES)¹⁵. Multiple genes of the CC chemokine receptor (CCR) family, specifically CCR2, CCR6, CCR7, and CCR9 as well as the CC ligand (CCL) family, specifically CCL3, CCL5, CCL24, CCL25, and CCL28, were detectable in male elastase-perfused aortas³⁴.

Further, the interactions of cytokines and cell adhesion molecules (CAM) may be important in the pathogenesis of abdominal aortic aneurysms.

Study has indicated that cytokines, such as IL-1 β , TNF-alpha treatment resulted in a significant increase in the expression of intracellular adhesion molecule-1 (ICAM-1) and soluble (sICAM) on aortic endothelial cell³⁵.

In addition, we also found B cell receptor signaling pathway (Hsa04662) and Cytokine-cytokine receptor interaction (Hsa04060) were both connected with the Natural killer cell mediated cytotoxicity (Hsa04650) through the apoptosis (GO:0006915) and signal transduction (GO:0007165), respectively.

B cell is an important component of adaptive immunity. They produce and secrete millions of different antibody molecules, each of which recognizes a different (foreign) antigen. The B cell receptor (BCR) is an integral membrane protein complex that is composed of two immunoglobulin (Ig) heavy chains, two Ig light chains and two heterodimers of Ig-alpha and Ig-beta. After BCR ligation by antigen, three main protein tyrosine kinases (PTKs) – the SRC-family kinase LYN, SYK and the TEC-family kinase BTK – are activated. Phosphatidylinositol 3-kinase (PI3K) and phospholipase C-gamma 2 (PLC-gamma 2) are important downstream effectors of BCR signal-

ing. Ultimately, this signaling results in B cell proliferation, differentiation and Ig production as well as other processes. B cells were found predominant in the inflamed adventitia of AAA³⁶. Both BCR-associated transmembrane protein, CD19 and CD22 are suggested as molecular makers for B cells. The mean intensity of expression for CD19 was found lower in AAA than that in peripheral blood B cells³⁷. CD22 revealed protein expression in B lymphocytes present in the aneurysmal aortic wall³⁸. The absence of CD22 expression lowers the signaling threshold for BCR-crosslinking and can thus lead to an increased induction of apoptosis in the B cell³⁹. In addition, SHP-1 was a negative regulator in B cell receptor signaling to inhibit activation of B cells through its association with FCγRIIB1. And SHP-1 has also been implicated in inhibition of NK cell cytotoxicity through its association with the killer inhibitory receptors (KIRs), members of the Ig family that bind to human MHC class I molecules⁴⁰. Therefore, SHP-1 may be a link between B cell receptor signaling and NK cell pathway. Among innate immune cells in AAA patients, natural killer (NK) cells were elevated in the circulation and demonstrated increased cytotoxicity against aortic SMCs⁴¹. Infiltrating NK cells in AAA produce pro-inflammatory cytokines (IFN-γ, TNF-α) that can cause or exacerbate aortic tissue injury⁴². Fractalkine (CX3CL1) promotes adhesion and extravasation of leucocytes through interactions with fractalkine receptor (CX3CR1) expressed on CD56⁺/CD16⁺ NK cells⁴³.

Conflict of Interest

None declared.

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