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 (hsa04662), 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 (Hsa04662) 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.

Introduction

Abdominal aortic aneurysm (AAA) is a relatively common disease in elderly that usually defined as a pathologic dilatation of the infrarenal 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 (CEBP-G), peptidase D (PEPD), and CD227. 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 apoptosis9,10. 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 vessel13,14. 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-
Therefore, this enhanced degradation of structural proteins, together with a reduced capacity to synthesize new matrix proteins, resulting in dilatation.

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 pathway. 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) datasets involved 211 pathways containing 5385 genes. And the protein-protein interaction (PPI) data from human protein reference database (HPRD) and Bio General Repository for Interaction Datasets (BIOGRID) 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 al., 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 RNA later (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 method 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) method with the default settings implemented in R (version 2.12.1), 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 al.

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

The dif(x) and dif(y) are differential expression assessments of gene x and gene y, respectively. 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, p1 and p2 are the p-values of differential expression of two nodes, p3 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 DAVID 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:

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

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 form 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 (hsa04662) with the $p$-value $= 0.001$, 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,
we found five significant pathways associated with AAA development, including Cytokine-cytokine receptor interaction (hsa04060), B cell receptor signaling pathway, Chemokine signaling pathway (hsa04662), Cell adhesion molecules (CAMs) (hsa04514), and Hematopoietic cell lineage (hsa04640), which was in accordance with Lenk’s results\(^23\). Further, we found that Chemokine signaling pathway (hsa04620) and Cytokine-cytokine 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-

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leukin (IL)-6, IL-8, IL-1β, and tumor necrosis factor (TNF)-α. 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 plaques. In addition, CXCR4/CXCL12 interaction is also found participating in the recruitment and retention of inflammatory lymphocytes that infiltrate the aorta in AAA. 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)-1, MCP-2, epithelial neutrophil-activating peptide-78 (ENA-78), growth related oncogene (GRO), and (RANTES). 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 aortas.

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-1β, TNF-alpha treatment resulted in a significant increase in the expression of intracellular adhesion molecule-1 (ICAM-1) and soluble (sICAM) on aortic endothelial cell. Additionally, 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-

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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.
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 AAA. 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 cells. CD22 revealed protein expression in B lymphocytes present in the aneurysmal aortic wall. 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 cell. In addition, SHP-1 was a negative regulator in B cell receptor signaling to inhibit activation of B cells through its association with FcgRIIB1. And 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 SMCs. Infiltrating NK cells in AAA produce pro-inflammatory cytokines (IFN-γ, TNF-α) that can cause or exacerbate aortic tissue injury. Fractalkine (CX3CL1) promotes adhesion and extravasation of leucocytes through interactions with fractalkine receptor (CX3CR1) expressed on CD56+/CD16+ NK cells.

Conflict of Interest
None declared.

References


