European Review for Medical and Pharmacological Sciences 2013; 17: 1374-1381

Gene expression analysis of ruptured and un-ruptured saccular intracranial aneurysm

L. CHEN, J.Q. WAN, J.P. ZHOU, Y.L. FAN, J.Y. JIANG

Department of Neurosurgery, Renji Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai, China

Abstract. – BACKGROUND: Although Saccular intracranial aneurysm (sIA) is the most common type abnormality of all intracranial aneurysms, the biological mechanisms of sIA are not fully understood.

METHODS: We downloaded microarray datasets from Gene Expression Omnibus (GEO) database which includes 11 ruptured intracranial aneurysm samples and 8 unruptured intracranial aneurysm samples. Significant Analysis of Microarray (SAM) was employed to identify the differentially expressed genes (DEGs) between ruptured and unruptured intracranial aneurysms.

RESULTS: We found 2129 genes differentially expressed in rupture sIA, of which 1062 genes up-regulated and 1057 genes down-regulated. Functional analysis demonstrated these genes were significantly associated with inflammatory response, wounding response and defense response. Protein-protein interaction (PPI) analysis revealed that these genes may play important roles in the pathogenesis of sIAs. Results suggested that four transcription factors (TFs) could cooperated with each other, together with several microRNAs play roles in the pathonegensis of ruptured sIAs.

CONCLUSIONS: All of above results indicate the existence of DEGs between ruptured and unruptured sIAs, which regulating the pathogenesis of ruptured sIAs. TFs and microRNAs may also play key roles in ruptured sIAs. This research hints a new thought to the therapy of ruptured sIAs.

Key Words:
sIA, DEGs, Functional analysis, PPI analysis, TFs and microRNAs.

Introduction

Saccular intracranial aneurysm (sIA) is the most common type disease of all intracranial aneurysms. Aneurysm rupture causes life-threatening hemorrhage to the subarachnoid space. The mortality of aneurysmal subarachnoid hemorrhage (SAH) is around 30%-40% despite modern neurosurgical intensive care but almost half of survivors are left disabled[1,2]. Overall risk of rupture was estimated to be around 1.2% in Western population[3] and the annual rupture risk has been even higher in Japanese series, up to 2.3%, according to an earlier study[4]. Due to the sinister outcome of aneurysmal SAH, many un-ruptured sIAs are treated prophylactically before they rupture[5].

Early studies based on Japanese and Finnish series using immunostaining techniques described loss of endothelium, loss of mural cells (vascular smooth muscle cells, myofibroblasts, and fibroblasts), break-down of the collagen matrix and partial hyalinization of the wall in association with rupture[6,7].

Studies comparing the gene expression of ruptured and unruptured sIAs with genome-wide microarrays have been published[8-11]. Most studies at the transcriptome level are in accordance with the histopathological series that associated with endothelial dysfunction, loss of mural cells, inflammatory cell infiltration and degradation of the matrix with sIA wall rupture.

The aneurysm wall ruptures when the matrix of the sIA wall has degenerated sufficiently to become too fragile to resist the hemodynamic pressure of the cerebral arteries. Under normal circumstances, degeneration or injury of the artery wall induces the mural smooth muscle cells to proliferate and synthesize a new matrix[12] which is likely to increase the tensile strength of the sIA wall and hence protect from sIA rupture. Loss of mural cells was suggested as a key event that leads to the degeneration and eventual rupture of the sIA wall[13].

Materials and Methods

Microarray Dataset

Microarray dataset of 19 saccular intracranial aneurysm samples, including 11 ruptured and 8 unruptured, was downloaded from GEO (Gene Expression Omnibus) database. All patients were of Finnish ethnicity. The samples were immedi-

Corresponding Author: Jieqing Wan, MD; e-mail: jieqingwan@126.com

ately snap-frozen in liquid nitrogen and stored in the Helsinki Neurosurgery sIA Tissue Bank. Gene expression profiling was performed using Affymetrix Human Genome U133 Plus 2.0 Array. This dataset was originally generated and analyzed by Kurki et al[11].

Microarray Data Analysis

Gene expression signal intensities were calculated by *R*obust Multi-array Average (RMA)[14] using EntrezGene-based center custom Chip Description File (CDF) developed by University of Michigan (version 16)[15]. Custom CDF was applied to deal with inaccurate original Affymetrix probe set definitions described by Dai et al[15]. To identify differentially expressed genes (DEGs) between ruptured and unruptured sIAs, Significant Analysis of Microarray (SAM) was applied[16]. SAM assigns a score to each gene on the basis of change in gene expression relative to the standard deviation of repeated measurements across samples and genes with scores greater than an adjustable threshold were considered as differentially expressed. 1000 permutations were performed to estimate the percentage of genes identified by chance, namely, the False Discovery Rate (FDR). Up-regulated genes in ruptured sIA were denoted as RUPTURE_UP while down-regulated genes as RUPTURE _DN.

Functional Analysis

It was suggested that formation of an aneurysm is a separate process from the rupture of an existing aneurysm, and that the pathobiology leading to formation of an aneurysm or its rupture is not entirely the same[13]. We hypothesized that DEGs between ruptured and unruptured sIAs could participate in processes which contributed to the biology of sIA rupture. To uncover such biological processes, DEGs were submitted to DAVID[17] to perform functional annotation clustering[18] which identifies annotation terms that significantly enriched in the query gene list and uses a fuzzy clustering algorithm to cluster redundant/similar/hierarchical terms into functional annotation groups. GO (Gene Ontology) biological process and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway annotation were used as knowledge source. We also hypothesized that activation/repression of pathways/processes that represented by global shifts in mRNA levels may be missed by DAVID analysis whose reasonable number of genes ranges from hundreds to thousands (e.g., 100-2,000 genes). Thus, we also performed Gene Set Enrichment Analysis (GSEA)[5] which ranks the all genes by the degree of differential expression and determines whether members of a gene set tend to occur toward the top (or bottom) of the list. GSEA was also featured by the large panel of curated molecular signatures which represent expression signatures of genetic and chemical perturbations. Before GSEA analysis, gene expression signal intensities were transformed into linear scale as recommended. Gene set size filters (min=15, max=500) were used. 1000 permutations were performed to estimate the False Discovery Rate (FDR). Only gene sets with a FDR < 25% were considered as significantly enriched.

Protein-Protein Interaction (PPI) Analysis

Protein products of gene expression signatures could cooperate with each other by physical interactions and involve in similar processes or functions. While functional analysis could uncover biological processes (or molecular functions) that were "described" by gene expression signatures, protein-protein interaction (PPI) analysis could reveal further detail of molecular interactions of such processes and aid in discovery of cross-talk between pathways and potential drug targets.

STRING (Search Tool for the Retrieval of Interacting Genes/Proteins)[19] is an online database resource for retrieval of interacting genes which provides uniquely comprehensive coverage of both experimental and predicted PPI information. Interactions in STRING are provided with a confidence score.

In this analysis we searched STRING to find experiment-validated PPI among RUPTURE_UP and RUPTURE_DN genes. A confidence score threshold of 0.4 was applied as default.

In Silico Transcription Factors and microRNA Analysis

DEGs between ruptured and unruptured sIAs could be activated, or repressed, by transcription factors (TFs). Targets of such TFs may significantly enriched in RUPTURE_UP or RUPTURE_DN genes. To discover such TFs, we used an online tool TfactS[20] which is aiming at detecting regulated transcription factors. TfactS provided a catalog containing transcription factors associated with 2720 target genes and 6401 experimentally validated regulations. When it was available, a distinction between transcriptional activation and inhibition was included for each

regulation. TfactS compared the number of target genes for a particular TF in the query gene lists and in the catalog and used right-sided Fisher's test to test the statistical significance of overlap. We queried TfactS with RUPTURE_UP and RUPTURE_DN genes using a sign-sensitive mode, thus only TF-target pairs with evidences of activation or inhibition were considered.

Global mRNA expression profile could be correlated with differentially expressed microRNA. We used Sylamer[21], which has been used to identify differentially expressed microRNAs in malignant germ cell tumors[22], to predict abnormal microRNA between ruptured and unruptured sIAs. Sylamer takes a ranked gene list, sorted from up-regulated to down-regulated, as input. The ranked gene list was then divided into multiple cutoffs and at each cutoff Sylamer tests whether a particular word (a microRNA target in 3' untranslated regions) is significantly enriched or depleted in the top of the list when compared to the rest of the list. Significance was calculated using hypergeometric (default setting) or binomial statistics. Resultant words were then mapped to microRNA mature sequences to identify potential microRNAs that could play a regulatory role.

Results

Discover Differentially Expressed Genes (DEGs)

A total of 2119 DEGs were identified by SAM, of which 1062 genes up-regulated in ruptured sIAs and 1057 genes down-regulated in ruptured sIAs. The estimated False Discovery Rate was 2.5%. The five up-regulated genes that chosen to be validated by RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction) in the original studies were all found (Table I).

Functional Analysis Characterized Ruptured sIAs

A total of 144 functional annotation clusters were found by DAVID among RUPTURE_UP genes with a highest enrichment score of 11.57 while 107 clusters in RUPTURE_DN with highest enrichment score of 6.16. GSEA (Gene Set Enrichment Analysis) found no significant enrichment of any gene sets in unruptured sIAs after multiple comparison correction but 183 gene sets significantly up-regulated in ruptured sIAs. The top DAVID annotation cluster enriched in RUPTURE_UP genes consisted of inflammatory response, response to wounding and defense response. Inflammatory response and response to wounding were also identified by GSEA as significantly up-regulated biological processes in ruptured sIAs. Notably, Ras signaling was found by GSEA although both tools use GO biological processes as knowledge source.

Protein-Protein Interaction Network of RUPTURE_UP and RUPTURE_DN Genes

STRING identified 485 experiment-validated protein-protein interactions among 357 RUPTURE_UP genes. The most connected gene/protein was GRB2 (Growth factor receptor-bound protein 2) with 31 interacted partners. 97 interactions among 128 RUPTURE_DN genes were found and the most connected gene/protein was PPP2R2B (Protein phosphatase 2, regulatory submit B, beta) with 7 interacted partners only.

Transcription Factors and microRNA Prediction

TfactS predicted four possible activated TFs (Figure 1) in ruptured sIAs whose validated target genes significantly enriched among RUPTURE_UP and RUPTURE_DN genes. Among them NFKB1 (Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1) and

Table I. Comparison of H-FABP, NT-pro BNP, and cTnI levels.

		Concentration (x ± s)			Positive rate n (%)		
	N	FABP H-(ng/ml)	NT-proBNP (ng/ml)	cTnI (ng/ml)	H-FABP	BNP	cTnI
Group I	22	35.73 ± 10.45	378.14 ± 10.45	0.84 ± 0.20	18 (81.8)	20 (90.9)	13 (59.1)
Group II	25	4.15 ± 5.22	142.53 ± 5.22	0.53 ± 0.11	9 (36.0)	7 (28.0)	3 (12.0)
Control	25	1.23 ± 0.15	0.74 ± 0.15	0.18 ± 0.04	0 (0.0)	0 (0.0)	0 (0.0)
		H = 78.44	H = 143.67	H = 30.47	x^2 = 33.46	x^2 = 47.49	x^2 = 4 5.62
p		< 0.001	< 0.001	< 0.001	< 0.005	< 0.005	< 0.005

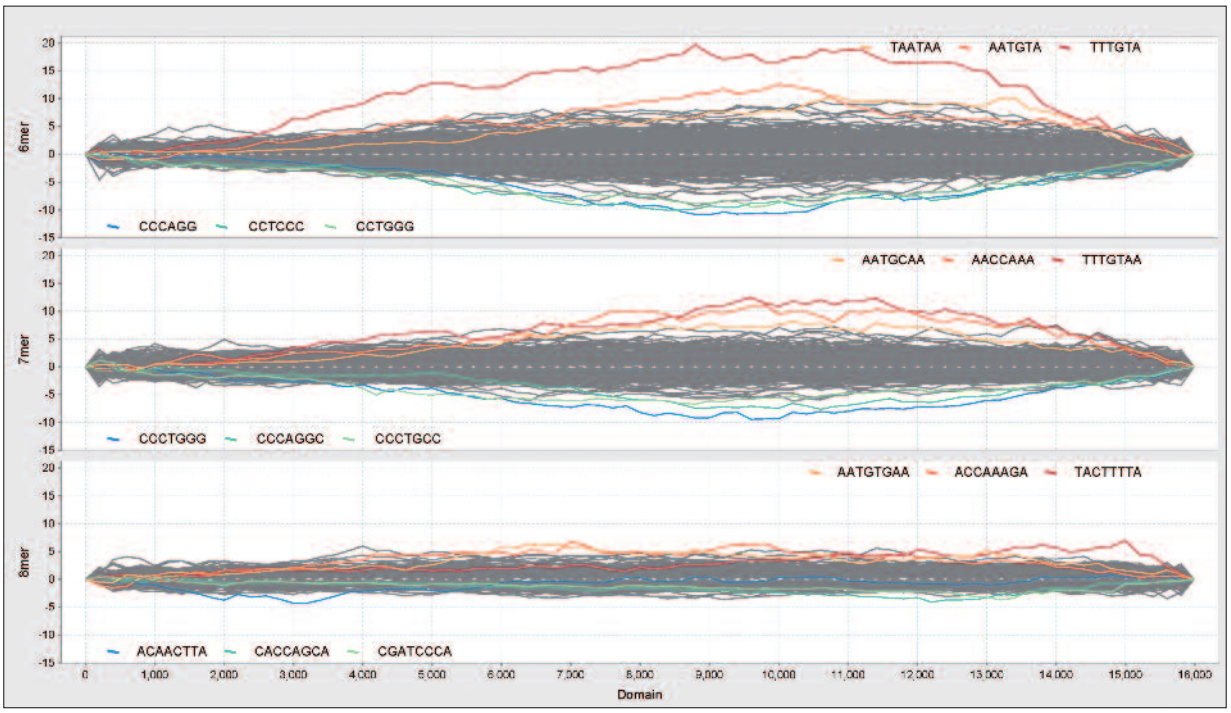

Figure 1. Interactions among four potential activated TFs. Only HIF1A was a RUPTURE_UP gene and all interactions were validated by experiments.

HIF1A (hypoxia inducible factor1, alpha subunit) were already predicted by the original studies[11] while SP1 (Specificity Protein 1) and JUN (jun oncogene) were predicted by this present study. Only HIF1A was among RUPTURE_UP genes and other TFs may play a regulatory role without significant change in mRNA level.

Sylarray reported words that significantly overrepresented in the 3'UTR (3'Untranslated Regions) for genes up-regulated in ruptured sIAs, or genes up-regulated in unruptured sIAs (Figure 2). These words were mapped to seed region for known microRNA. For 7mer, target genes for miR-33a-5p, miR-659-3p and miR-524-5p were predicted to be overrepresented in genes up-regulated in ruptured sIAs, thus these microRNAs may be inactivated in ruptured sIAs. Similarly, miR-661, miR-1207-5p and miR-1915-3p were predicted to be activated in ruptured sIAs.

Discussion

Literature Search of Differentially Expressed Genes (DEGs)

Among RUPTURE_UP genes, matrix metalloproteinase 9 (MMP9) was known to have a role in vessel wall degeneration and the progression of cerebral aneurysms [23]. Orai1 is up-regulated in vascular smooth muscle cells (VSMC) during vascular injury and is required for NFAT (nuclear factor of activated T cells) activity, VSMC prolif-

Table II. Comparison of the positive rate of H-FABP, NT-proBNP, and cTnI in group I with different classes of heart function.

	n	Positive rate n (%)		
		H-FABP	NT-pro BNP	cTnI
Class II	8	6 (75.0)	6 (75.0)	3 (37.5)
Class III	9	8 (88.9)	9 (100.0)	7 (77.8)
Class IV	5	5 (100.0)	5 (100.0)	5 (100.0)
x^2		1.72	3.85	6.18
p		> 0.05	< 0.05	< 0.05

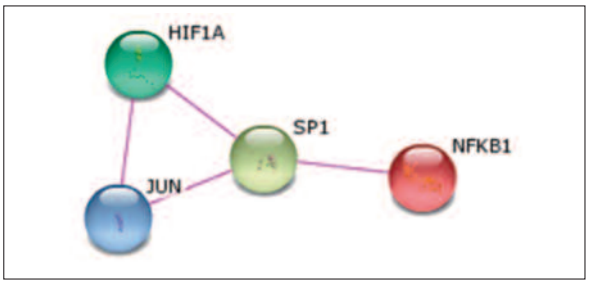

Figure 2. Sylamer result. The x axes represent the sorted gene list from most up-regulated in ruptured sIAs (*left*) to most down-regulated (*right*). The y axes show the hypergeometric significance for each word at each leading bin. Positive values indicate enrichment (–log10 (*p*-value)) and negative values, depletion (log10 (*p*-value)). For a peak occurring on the positive Y-axis, W is overrepresented in the 3'UTRs for the genes to the left of that peak, and W is underrepresented in the genes to the right. For a peak occurring on the negative Y-axis, W is overrepresented in the 3'UTRs for the genes to the right of that peak, and W is underrepresented in the genes to the left. Gray lines show the background profiles.

eration, and neointima formation following balloon injury of rat carotids[24]. CD44 expression was up-regulated by Low Molecular Weight (LMW) heparin markedly in the injured femoral arteries and *in vitro* LMW heparin decreased mouse VSMC growth capacity and up-regulated its CD44 expression simultaneously in a dose-dependent and time-dependent manner[25].

Among RUPTURE_DN genes, Myocd (Myocardin) was interacted by Epc1 (enhancer of polycomb homolog 1) which induces VSMC differentiation. Epc1, not a RUPTURE_DN gene, was proposed as a novel negative regulator of neointima formation after carotid injury[26]. Down-regulated TRPC1 (Transducin Repeat-containing Protein 1) expression during hypertension is associated with increased vascular contractility in rat[27]. Cell-autonomous vascular smooth muscle cell PTH1R (parathyroid hormone 1 receptor) activity inhibits arteriosclerotic Wnt/beta-catenin signaling and reduces vascular oxidative stress, thus limiting aortic type I collagen and calcium accrual in diabetic LDLR (LDL receptor)-deficient mice[28].

DAVID and GSEA Revealed Biological Processes Related with sIA Rupture

The highest scored RUPTURE_UP annotation cluster identified by DAVID consisted of inflammatory response which was also found by GSEA analysis. Frosen et al suggested that Inflammation could perhaps be a reaction to the ongoing degenerative processes, rather than the cause of rupture[13]. T cell infiltration was also revealed by DAVID and GSEA analysis. T cells were found in the sIA wall[6,29], but no study of the type or activity of T cells in the sIA wall has been published so far according to Frosen et al[13].

GSEA found that gene expression signatures reflecting activation status of Ras pathway[30,31] were up-regulated in ruptured sIAs. Gene ontology biological process of Ras signaling was also found to be up-regulated in sIA by GSEA which was missed by DAVID analysis. These results may provide clues for the role of Ras signaling in sIA rupture. Findings by Osuka et al indicated that Ras/MEK/ERK (Ras/member of kinases/extracellular signal-regulated kinase) signaling is activated in the chronic subdural hematoma (CSDH) outer membranes and suggested the possibility that the Ras/MEK/ERK pathway might be activated by VEGF (vascular endothelial growth factor) and play a critical role in the angiogenesis of CSDHs (chronic subdural hematomas).

GSEA analysis also captured the up-regulation of MAPK (mitogen-activated protein kinase) pathway in ruptured sIAs. MAPKs are involved in vascular wall remodeling, and Laaksamo et al[32] suggested that JNK (Jun N-terminal kinases) activity and expression are involved in IA growth and possibly rupture and p38 expression in IA growth. Their study also found that the levels of MMP9 (matrix metallopeptidase 9) was correlated with p54 JNK phosphorylation in unruptured IAs and its expression was increased to 4.3-fold in ruptured IAs.

Interestingly, KEGG pathway of non-small cell lung cancer was found to be significantly up-regulated in ruptured sIA by GSEA which was reminiscent of a previous observation that carriers of the sIA disease have an increased risk of developing lung cancer[33].

Signaling Proteins Play Key Roles in RUPTURE_UP Protein-Protein Interaction Network

PPI identified by STRING in RUPTURE_UP genes were much more complex than in RUPTURE_DN although the number of genes of RUPTURE_UP is approximate to RUPTURE_DN. This may be due to the fact that sIA rupture is a complex pathological process and much more molecular interactions would be involved.

The most connected gene/protein in RUPTURE_UP was GRB2, which is a simple adapter known to be involved in a variety of growth factor receptor signaling. Recently, it was reported

that GRB2 exerts control of FGFR2 (fibroblast growth factor receptor 2) kinase activity prior to growth factor binding[34]. GRB2 participates in various signaling pathways including Ras signaling and MAPK pathway according to GO. Thus, PPI analysis revealed further details of perturbed biological processes that discovered by functional analysis. The second most connected gene/protein SYK (spleen thyrosine kinase), a member of the family of non-receptor type Tyr protein kinases, may participate in both blood vessel morphogenesis and activation of JUN kinase activity. The third most connected gene/protein JAK1 (Janus kinase 1) is a large, widely expressed membrane-associated phosphoprotein which involved in the interferon-alpha/beta and -gamma signal transduction pathways.

Four Predicted TFs Could Possibly Act in Cooperation

Interestingly, STRING found experiment-validated interactions among the four activated TFs predicted by TfactS. Sp1/NF-kappaB pathway was unveiled as a modulator of DNA methyltransferase activity in human cancer[35,36]. Koshiji et al [32] found that anti-Sp1 immunoprecipitation captured Myc (myelocytomatosis oncogene) only in normoxic extract but HIF-1α instead in hypoxic extract. Moreover, knockdown of HIF-1α levels by specific siRNA restored Myc binding in hypoxia[37]. Physical interactions between c-Jun and Sp1 were demonstrated *in vitro* by using GST-Sp1 (glutathione S-transferase-specificity protein 1) hybrid proteins expressed in bacteria and in vitro transcribed-translated c-Jun. *In vivo*, functional interactions between c-Jun and Sp1 were demonstrated using a GAL4-based transactivation assay[38]. Alfranca et al showed that c-Jun functionally cooperates with HIF-1 (Hypoxia-Inducible Factor 1) transcriptional activity in different cell types which act in a phosphorylation-dependent way[39]. Yu et al[32] reported that c-Jun associates with HIF-1α via its oxygen-dependent degradation domain, masks the sites for ubiquitination and, thus, protects HIF-1 from proteasome-executing degradation all of which together resulted in the stabilization and accumulation of HIF-1α.

It was reported[32] that abdominal aortic aneurysm in mCd59ab-/-ApoE-/- mice had significantly higher levels of phosphorylated c-Jun. Xu et al[32] reported that hypoxia increased the nuclear localization of Sp1 which turned to increase expression of cyclooxygenase-2 and they suggested a mechanistic link exists between vascular cellular hypoxia and mediators of inflammation associated with aortic aneurysm and heart failure. Saito et al[32] reported that the blockade of endothelial NF-κB signaling prevented abdominal aortic aneurysm formation in an experimental model and suggested its important role in vascular remodeling and aneurysm formation.

Several microRNAs were Predicted to Play Roles

Although a few microRNAs (miRNAs) have been proposed to play roles in aneurysm development, such as miR-21 (miRNA-21), miR-26, miR-143/145 and miR-29 (reviewed by Boon et al[40]), whether microRNA plays a role in intracranial aneurysms, and especially sIA rupture, remains unknown. Among results of Sylamer analysis, miR-524-5p, a brain-specific miRNA, was associated with the pathological grade and overall survival of gliomas. Restored expression of miR-524-5p in glioma suppressed cell proliferation and invasion both *in vitro* and *in vivo*[41]. miR-661, which was predicted to be activated in ruptured sIAs, was found required for efficient invasion by destabilizing two of its predicted mRNA targets, the cell-cell adhesion protein Nectin-1 and the lipid transferase StarD10 in breast cancer cells conditionally expressing the epithelial Mesenchymal transition (EMT) master regulator SNAI1 (snail homolog 1)[42]. Although no articles related to miR-1915-3p was found, previous studies reported that up-regulation of miR-1915 in colorectal carcinoma cells could reduce Bcl-2 (B cell lymphoma 2) protein level and the luciferase activity of a Bcl-2 3'-untranslated region-based reporter, and also sensitized these cells to some anticancer drugs[43].

Conclusions

We found 2129 genes differentially expressed in rupture sIA, of which 1062 genes up-regulated and 1057 genes down-regulated. Functional analysis demonstrated these genes were significantly associated with inflammatory response, wounding response and defense response. Protein-protein interaction (PPI) analysis revealed that these genes may play important roles in the pathogenesis of sIAs. Results suggested that four transcription factors (TFs) could cooperated with each other, together with several microRNAs play roles in the pathonegensis of ruptured sIAs. All of above results indicate the existence of

DEGs between ruptured and unruptured sIAs, which regulating the pathogenesis of ruptured sIAs. TFs and microRNAs may also play key roles in ruptured sIAs. This research hints a new thought to the therapy of ruptured sIAs.

Conflict of Interest

None to declare.

References

1) Nieuwkamp DJ, Setz LE, Algra A, Linn FH, de Rooij NK, Rinkel GJ. Changes in case fatality of aneurysmal subarachnoid haemorrhage over time, according to age, sex, and region: a meta-analysis. Lancet Neurol 2009; 8: 635-642.
2) Stegmayr B, Eriksson M, Asplund K. Declining mortality from subarachnoid hemorrhage: changes in incidence and case fatality from 1985 through 2000. Stroke 2004; 35: 2059-2063.
3) Wermer MJ, van der Schaaf IC, Algra A, Rinkel GJ. Risk of rupture of unruptured intracranial aneurysms in relation to patient and aneurysm characteristics: An updated meta-analysis. Stroke 2007; 38: 1404-1410.
4) Yasui N, Suzuki A, Nishimura H, Suzuki K, Abe T. Long-term follow-up study of unruptured intracranial aneurysms. Neurosurgery 1997; 40: 1155-1159; discussion 1159-1160.
5) Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005; 102: 15545-15550.
6) Frosen J, Piippo A, Paetau A, Kangasniemi M, Niemela M, Hernesniemi J, Jaaskelainen J. Remodeling of saccular cerebral artery aneurysm wall is associated with rupture: histological analysis of 24 unruptured and 42 ruptured cases. Stroke 2004; 35: 2287-2293.
7) Kataoka K, Taneda M, Asai T, Kinoshita A, Ito M, Kuroda R. Structural fragility and inflammatory response of ruptured cerebral aneurysms. A comparative study between ruptured and unruptured cerebral aneurysms. Stroke 1999; 30: 1396-1401.
8) Krischek B, Kasuya H, Tajima A, Akagawa H, Sasaki T, Yoneyama T, Ujiie H, Kubo O, Bonin M, Takakura K, Hori T, Inoue I. Network-based gene expression analysis of intracranial aneurysm tissue reveals role of antigen presenting cells. Neuroscience 2008; 154: 1398-1407.
9) Marchese E, Vignati A, Albanese A, Nucci CG, Sabatino G, Tirpakova B, Lofrese G, Zelano G, Maira G. Comparative evaluation of genome-wide gene expression profiles in ruptured and unruptured human intracranial aneurysms. J Biol Regul Homeost Agents 2010; 24: 185-195.
10) Pera J, Korostynski M, Krzyszkowski T, Czopek J, Slowik A, Dziedzic T, Piechota M, Stachura K, Moskala M, Przewlocki R, Szczudlik A. Gene expression profiles in human ruptured and unruptured intracranial aneurysms: What is the role of inflammation? Stroke 2010; 41: 224-231.
11) Kurki MI, Hakkinen SK, Frosen J, Tulamo R, von und zu Fraunberg M, Wong G, Tromp G, Niemela M, Hernesniemi J, Jaaskelainen JE, Yla-Herttuala S. Upregulated signaling pathways in ruptured human saccular intracranial aneurysm wall: an emerging regulative role of Toll-like receptor signaling and nuclear factor-κB, hypoxia-inducible factor-1A, and ETS transcription factors. Neurosurgery 2011; 68: 1667-1675; discussion 1675-1666.
12) Newby AC, Zaltsman AB. Molecular mechanisms in intimal hyperplasia. J Pathol 2000; 190: 300-309.
13) Frosen J, Tulamo R, Paetau A, Laaksamo E, Korja M, Laakso A, Niemela M, Hernesniemi J. Saccular intracranial aneurysm: pathology and mechanisms. Acta Neuropathol 2012; 123: 773-786.
14) Bolstad BM, Irizarry RA, Åstrand M, Speed TP. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics 2003; 19: 185-193.
15) Dai M, Wang P, Boyd AD, Kostov G, Athey B, Jones EG, Bunney WE, Myers RM, Speed TP, Akil H, Watson SJ, Meng F. Evolving gene/transcript definitions significantly alter the interpretation of genechip data. Nucleic Acids Res 2005; 33: e175.
16) Tusher VG, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response. Proc Natl Acad Sci U S A 2001; 98: 5116-5121.
17) Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using david bioinformatics resources. Nat Protoc 2009; 4: 44-57.
18) Huang da W, Sherman BT, Tan Q, Collins JR, Alvord WG, Roayaei J, Stephens R, Baseler MW, Lane HC, Lempicki RA. The david gene functional classification tool: a novel biological module-centric algorithm to functionally analyze large gene lists. Genome Biol 2007; 8: R183.
19) Szklarczyk D, Franceschini A, Kuhn M, Simonovic M, Roth A, Minguez P, Doerks T, Stark M, Muller J, Bork P, Jensen LJ, von Mering C. The string database in 2011: functional interaction networks of proteins, globally integrated and scored. Nucleic Acids Res 2011; 39: D561-568.
20) Essaghir A, Toffalini F, Knoops L, Kallin A, van Helden J, Demoulin JB. Transcription factor regulation can be accurately predicted from the presence of target gene signatures in microarray gene expression data. Nucleic Acids Res 2010; 38: e120.
21) van Dongen S, Abreu-Goodger C, Enright AJ. Detecting microrna binding and sirna off-target effects from expression data. Nat Methods 2008; 5: 1023-1025.
22) Palmer RD, Murray MJ, Saini HK, van Dongen S, Abreu-Goodger C, Muralidhar B, Pett MR, Thorn-

ton CM, Nicholson JC, Enright AJ, Coleman N. Malignant germ cell tumors display common microrna profiles resulting in global changes in expression of messenger rna targets. Cancer Res 2010; 70: 2911-2923.

23) Aoki T, Kataoka H, Morimoto M, Nozaki K, Hashimoto N. Macrophage-derived matrix metalloproteinase-2 and -9 promote the progression of cerebral aneurysms in rats. Stroke 2007; 38: 162-169.

24) Zhang W, Halligan KE, Zhang X, Bisaillon JM, Gonzalez-Cobos JC, Motiani RK, Hu G, Vincent PA, Zhou J, Barroso M, Singer HA, Matrougui K, Trebak M. Orai1-mediated i (CRAC) is essential for neointima formation after vascular injury. Circ Res 2011; 109: 534-542.

25) Zhao G, Shaik RS, Zhao H, Beagle J, Kuo S, Hales CA. Low molecular weight (LMW) heparin inhibits injury-induced femoral artery remodeling in mouse via upregulating CD44 expression. J Vasc Surg 2011; 53: 1359-1367 e1353.

26) Joung H, Kwon JS, Kim JR, Shin S, Kang W, Ahn Y, Kook H, Kee HJ. Enhancer of polycomb1 lessens neointima formation by potentiation of myocardin-induced smooth muscle differentiation. Atherosclerosis 2012; 222: 84-91.

27) Noorani MM, Noel RC, Marrelli SP. Upregulated TRCP3 and Downregulated TRCP1 channel expression during hypertension is associated with increased vascular contractility in rat. Front Physiol 2011; 2: 42.

28) Cheng SL, Shao JS, Halstead LR, Distelhorst K, Sierra O, Towler DA. Activation of vascular smooth muscle parathyroid hormone receptor inhibits Wnt/beta-catenin signaling and aortic fibrosis in diabetic arteriosclerosis. Circ Res 2010; 107: 271-282.

29) Chyatte D, Bruno G, Desai S, Todor DR. Inflammation and intracranial aneurysms. Neurosurgery 1999; 45: 1137-1146; discussion 1146-1137.

30) Wang J, Wu J, Sun D, Zhang P, Wang L. Pathway crosstalk analysis based on protein-protein network analysis in prostate cancer. Eur Rev Med Pharmacol Sci 2012; 16: 1235-1242.

31) Bild AH, Yao G, Chang JT, Wang Q, Potti A, Chasse D, Joshi MB, Harpole D, Lancaster JM, Berchuck A, Olson JA, Jr., Marks JR, Dressman HK, West M, Nevins JR. Oncogenic pathway signatures in human cancers as a guide to targeted therapies. Nature 2006; 439: 353-357.

32) Laaksamo E, Tulamo R, Baumann M, Dashti R, Hernesniemi J, Juvela S, Niemela M, Laakso A. Involvement of mitogen-activated protein kinase signaling in growth and rupture of human intracranial aneurysms. Stroke 2008; 39: 886-892.

33) Huttunen T, Riihinen A, Pukkala E, von und zu Fraunberg M, Koivisto T, Ronkainen A, Rinne J, Hernesniemi J, Sankila R, Jaaskelainen JE. Increased relative risk of lung cancer in 2,904 patients with saccular intracranial aneurysm disease in eastern finland. Neuroepidemiology 2012; 38: 93-99.

34) Lin CC, Melo FA, Ghosh R, Suen KM, Stagg LJ, Kirkpatrick J, Arold ST, Ahmed Z, Ladbury JE. Inhibition of basal FGF receptor signaling by dimeric Grb2. Cell 2012; 149: 1514-1524.

35) Liu S, Liu Z, Xie Z, Pang J, Yu J, Lehmann E, Huynh L, Vukosavljevic T, Takeki M, Klisovic RB, Baiocchi RA, Blum W, Porcu P, Garzon R, Byrd JC, Perrotti D, Caligiuri MA, Chan KK, Wu LC, Marcucci G. Bortezomib induces DNA hypomethylation and silenced gene transcription by interfering with Sp1/NF-kappaB-dependent DNA methyltransferase activity in acute myeloid leukemia. Blood 2008; 111: 2364-2373.

36) Xiang J, Zang W, Che J, Chen K, Hang J. Regulation network analysis in the esophageal squamous cell carcinoma. Eur Rev Med Pharmacol Sci 2012; 16: 2051-2056.

37) Koshiji M, To KK, Hammer S, Kumamoto K, Harris AL, Modrich P, Huang LE. HIF-1alpha induces genetic instability by transcriptionally downregulating mutsalpha expression. Mol Cell 2005; 17: 793-803.

38) Kardassis D, Papakosta P, Pardali K, Moustakas A. c-Jun transactivates the promoter of the human p21(WAF1/Cip1) gene by acting as a superactivator of the ubiquitous transcription factor Sp1. J Biol Chem 1999; 274: 29572-29581.

39) Alfranca A, Gutierrez MD, Vara A, Aragones J, Vidal F, Landazuri MO. c-Jun and hypoxia-inducible factor 1 functionally cooperate in hypoxia-induced gene transcription. Mol Cell Biol 2002; 22: 12-22.

40) Boon RA, Dimmeler S. Micrornas and aneurysm formation. Trends Cardiovasc Med 2011; 21: 172-177.

41) Chen L, Zhang W, Yan W, Han L, Zhang K, Shi Z, Zhang J, Wang Y, Li Y, Yu S, Pu P, Jiang C, Jiang T, Kang C. The putative tumor suppressor miR-524-5p directly targets Jagged-1 and Hes-1 in glioma. Carcinogenesis 2012; 33: 2276-2282.

42) Vetter G, Saumet A, Moes M, Vallar L, Le Bechec A, Laurini C, Sabbah M, Arar K, Theillet C, Lecellier CH, Friederich E. miR-661 expression in SNAI1-induced epithelial to mesenchymal transition contributes to breast cancer cell invasion by targeting Nectin-1 and StarD10 messengers. Oncogene 2010; 29: 4436-4448.

43) Xu K, Liang X, Cui D, Wu Y, Shi W, Liu J. miR-1915 inhibits Bcl-2 to modulate multidrug resistance by increasing drug-sensitivity in human colorectal carcinoma cells. Mol Carcinog 2011; 52: 70-78.