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Original Article

Construction of transcriptional regulatory network in the intracranial aneurysms

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Abstract: Background: The pathophysiology of intracranial aneurysms (IAs) remains unclear. More comprehensive understanding of regulatory mechanism can help us to assess the risk of IAs and improve the diagnosis and treatment to IAs patients. Methods: The mRNA expression profiles of human IAs were downloaded from GEO database for integrated analysis. DEGs were screened and functional annotation of DEGs was conducted. And the IAs-specific transcriptional regulatory network was constructed. Results: Integrated analysis of six eligible IAs microarray datasets led to the discovery of 1093 DEGs (517 up-regulated and 576 down-regulated) in IAs compared with normal tissues. Functional analysis showed that the pathway of cell adhesion molecules was significantly enriched, which was highly in accordance with the previous studies. In the IAs-specific regulatory network, top ten TFs covering the most downstream DEGs were NFIC, NFATC2, FOXO3, ZNF354C, ZNF263, BRCA1, FOXL1, NR4A2, SP2 and EGR1, which may cooperated with each other and play roles in the pathogenesis of IAs. Moreover, the target genes (OIT3, CNTN5, PLA2G12B and TMPRSS4) could be genes of interest in IAs, and dysregulation of them may be closely associated with IAs pathology. Conclusion: Taken together, our findings provide clues to further understand the regulatory mechanisms of IAs pathology. These novel findings reveal potential new therapeutic targets for the disease.

Keywords: Intracranial aneurysms, integrated analysis, transcription factors

Introduction

Intracranial aneurysms (IAs) are pathological outward bulging of the cerebral arteries [1]. IA is common lesions with a prevalence of approximately 2% of the general population who has undetected IAs [2]. Most IAs are often asymptomatic and clinically silent until the devastating event of subarachnoid hemorrhage (SAH) because of rupture, leading to a higher mortality [3]. IA is considered as a complex disease that results from the interplay of environmental and genetic factors [4]. The pathogenesis of IAs formation is related to increased wall shear stress, endothelial dysfunction, atherosclerosis, and altered gene regulation [5].

Recent advancements shed light to the molecular mechanisms of IA formation and rupture. IA formation begins with endothelial dysfunction followed by pathological remodeling with degenerative changes of vascular walls [6]. Hypertension, smoking and alcohol consumption are major determinants of IA risk [7]. Moreover, chronic inflammation is closely associated with the pathogenesis of IAs, which is mediated by genetic variants in pro-inflammatory cytokines TNFA and IFNG [8]. Researches in molecular biology using rabbit models have revealed that differentially exprressed genes (DEGs) between the aneurysms and control tissues were closely related to some key pathways, including inflammation and antigen presentation [9]. A systematic review reported that the role of inflammatory and cell adhesion molecules, enzymes and hormones that effect cerebral vasculature, and other cerebral proteins related to brain and vascular damage in both the formation and progression to rupture of IAs [10].

Large-scale genome-wide association studies (GWAS) of Chinese patients showed that TSLC2A9 (rs7660895) and TOX (rs11777927)

Table 1. Characteristics of the individual studies for integrated analysis

GEO ID	Samples (control: case)	Platform	Country	Time	Author
GSE54083	10:13	GPL4133, Agilent-014850 Whole Human Genome Microarray 4x44K G4112F	Japan	2014	Nakaoka H
GSE46337	2:2	GPL6480, Agilent-014850 Whole Human Genome Microarray 4x44K G4112F	China	2013	Jing Y
GSE26969	3:3	GPL570, Affymetrix Human Genome U133 Plus 2.0 Array	China	2011	Li L
GSE15629	5:14	GPL6244, Affymetrix Human Gene 1.0 ST Array	Poland	2010	Pera J
GSE13353	0:19	GPL570, Affymetrix Human Genome U133 Plus 2.0 Array	Finland	2010	Kurki MI
GSE6551	5:5	GPL570, Affymetrix Human Genome U133 Plus 2.0 Array/GPL2507 Sentrix Human-6 Expression BeadChip	USA	2007	Weinsheimer S

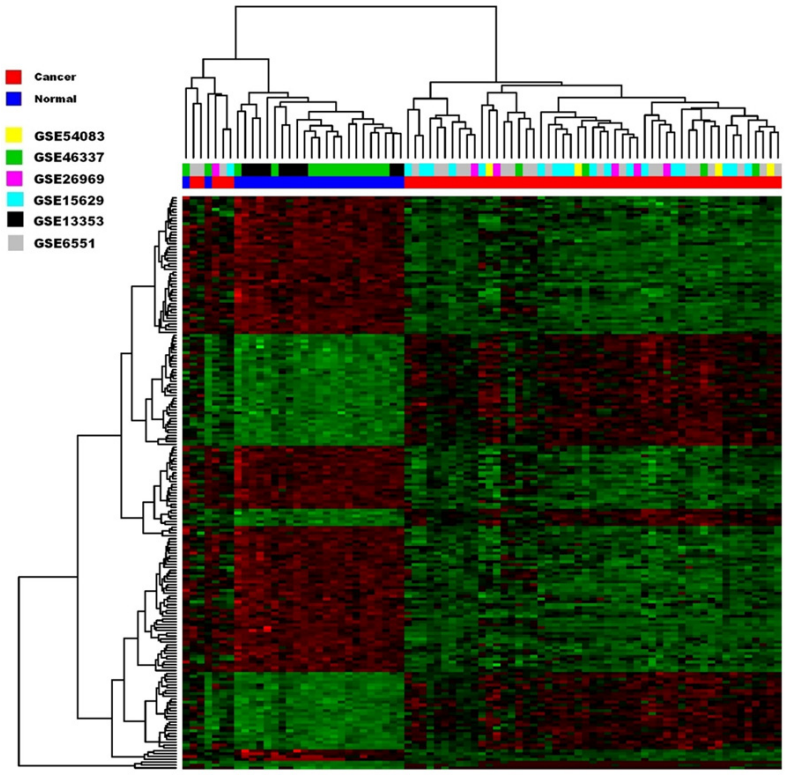

Figure 1. The hierarchical clustering analysis of IAs in different microarray studies.

gene polymorphisms may be associated with formation of IAs, and rs7660895 may be associated with IA rupture [11]. GWAS in Portugal revealed several genetic variants underlying the risk of IAs [12]. There is a tendency of an association between genotypes of eNOS3 gene with the mean size of the aneurysm, as well as clinical sequelae of the disease in patients with IAs [1]. FOS, CCL2, COL4A2 and CXCL5 might participate in the pathogenesis of IAs, and could serve as potential diagnosis targets [13]. Chen suggested that ACTA2, MYH11, MYLK, and MYL9 were most significantly related to vascular smooth muscle contraction and they can be beneficial for early diagnosis and treatment of IAs [14].

Up to now, there is no precise biochemical or phenotype marker for diagnosis of IAs, which still relies solely on imaging methods and the mechanisms of IA initiation, progression. Further clarification of molecular mechanisms of the formation and progression of IAs will shed light to the pathogenesis of IA development and provide insight into novel diagnostic and therapeutic strategies for IAs. In the present study, integrated analysis of IAs microarray data was conducted to identify more candidate biomarkers for improving the diagnostic rate of IAs, and the IAs-specific transcriptional regulatory network was constructed to further understand the regulatory mechanism.

Materials and methods

Microarray datasets

Microarray datasets of IA were downloaded from GEO (Gene Expression Omnibus) database (availble at http://www.ncbi.nlm.nih.gov/geo/) [15]. The following keywords were used:

Table 2. The most significantly enriched GO terms of DEGs

GO ID	GO term	No.of genes	FDR
Biological process			
GO:0007586	Digestion	6	5.63E-02
GO:0034765	Regulation of ion transmembrane transport	13	5.73E-01
GO:0044058	Regulation of digestive system process	2	4.16E-01
GO:0023041	Neuronal signal transduction	2	4.04E-01
GO:0034762	Regulation of transmembrane transport	13	5.05E-01
GO:0006996	Organelle organization	121	5.49E-01
GO:0031016	Pancreas development	2	5.09E-01
GO:0034220	ion transmembrane transport	16	4.69E-01
GO:0097114	N-methyl-D-aspartate receptor clustering	2	4.29E-01
GO:0097119	Postsynaptic density protein 95 clustering	2	3.86E-01
GO:0043031	Negative regulation of macrophage activation	1	5.49E-01
GO:0060453	Regulation of gastric acid secretion	1	5.03E-01
GO:0060455	Negative regulation of gastric acid secretion	1	4.64E-01
GO:0060457	Negative regulation of digestive system process	1	4.31E-01
Molecular function			
GO:0003723	RNA binding	53	6.91E-03
GO:0003676	Nucleic acid binding	125	1.49E-02
GO:0044822	Poly(A) RNA binding	42	2.16E-02
GO:0097159	Organic cyclic compound binding	194	2.29E-02
GO:1901363	Heterocyclic compound binding	188	3.34E-02
GO:0005215	Transporter activity	23	7.69E-02
GO:0015075	ion transmembrane transporter activity	17	6.93E-02
GO:0015370	Solute: sodium symporter activity	2	6.14E-02
GO:0015296	Anion: cation symporter activity	2	5.45E-02
GO:0022891	Substrate-specific transmembrane transporter activity	17	6.41E-02
GO:0022892	Substrate-specific transporter activity	19	5.87E-02
GO:0030228	Lipoprotein particle receptor activity	2	7.74E-02
GO:0005041	Low-density lipoprotein receptor activity	2	7.14E-02
GO:0031723	CXCR4 chemokine receptor binding	1	9.60E-02
Cellular component			
GO:0043229	Intracellular organelle	452	1.56E-03
GO:0044424	Intracellular part	654	1.84E-03
GO:0043231	Intracellular membrane-bounded organelle	393	1.65E-02
GO:0044422	Organelle part	367	2.51E-02
GO:0044446	Intracellular organelle part	343	2.72E-02
GO:0005737	Cytoplasm	238	3.23E-02
GO:0044428	Nuclear part	157	4.63E-02
GO:0005634	Nucleus	163	4.47E-02
GO:0034705	Potassium channel complex	6	5.01E-02
GO:0008076	Voltage-gated potassium channel complex	6	4.51E-02

"intracranial aneurysm" [MeSH Terms] OR Intracranial Aneurysm [All Fields]) AND ("gse" [Filter] AND "Homo sapiens" [Organism]. Study type was defined as "expression profiling by array".

Microarray data analysis

All the microarray datasets were processed by background correction and normalization. To identify differentially expressed genes

Table 3. Top 15 the most significantly enriched KEGG pathways of DEGs

KEGG ID	KEGG term	Count	FDR
hsa05200	Pathways in cancer	32	1.38E-06
hsa04514	Cell adhesion molecules (CAMs)	16	8.33E-05
hsa04360	Axon guidance	16	9.18E-05
hsa05222	Small cell lung cancer	13	9.60E-05
hsa05144	Malaria	4	1.19E-04
hsa04115	p53 signaling pathway	10	1.26E-03
hsa04512	ECM-receptor interaction	11	1.39E-03
hsa04110	Cell cycle	5	1.42E-03
hsa05212	Pancreatic cancer	5	1.42E-03
hsa05220	Chronic myeloid leukemia	5	1.42E-03
hsa04010	MAPK signaling pathway	21	1.45E-03
hsa04144	Endocytosis	17	2.13E-03
hsa05014	Amyotrophic lateral sclerosis (ALS)	4	2.21E-03
hsa04510	Focal adhesion	17	2.32E-03
hsa00350	Tyrosine metabolism	7	3.00E-03

(DEGs) between IAs and the normal tissues, we used the Limma package in R by two-tailed Student's t-test, and *P*-value was obtained. Further false discovery rate (FDR) was further calculated. The genes with FDR <0.01 were considered as DEGs.

Functional analysis

We hypothesized that DEGs between IAs and normal tissues may participate in processes which contributed to the biology of IA. To uncover such biological processes, all the DEGs were submitted to DAVID [16] to perform functional annotation clustering, including GO (Gene Ontology) categories [17] and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway annotation [18].

Transcription factors analysis

DEGs between IAs and normal tissues could be activated or repressed by transcription factors (TFs). The targets of such TFs may be significantly enriched in up-regulated or down-regulated DEGs. To discover the TFs, we downloaded all TFs in human genome and their genomic binding sites from TRANSFAC database. The position weight matrix (PWM) for gene promoter scanning was also downloaded [19]. Based on the DEGs obtained from integrated analysis, the DEGs which has the binding site of the TF in the promoter region were identified as targets. Moreover, the transcriptional regulatory network was constructed and visualized by Cytoscape software [20].

Results

Discover DEGs in IAs

Based on the inclusion criteria, six microarray datasets were included for integrated analysis, in which 56 cases of IAs and 25 cases of normal tissues were enrolled. The characteristics of the individual studies were displayed in **Table 1**. By integrated analysis, a total of 1093 DEGs with FDR <0.01 were identified between IAs and normal tissues, including 517 up-regulated DEGs and 576 down-regulated DEGs, respectively. Besides, the hierarchical clustering analysis indicated that the DEGs in IAs were distinguished from that in normal tissues (**Figure 1**).

Functional enrichment analysis of DEGs

GO enrichment analysis of DEGs was performed to understand their biological functions. The top GO terms consisted of digestion, regulation of ion transmembrane transport, regulation of digestive system process, neuronal signal transduction, regulation of transmembrane transport (**Table 2**).

To further evaluate the biological pathways the DEGs involved, the KEGG pathway enrichment analysis was conducted. Notably, Pathways in cancer was found to be most significantly enriched. Furthermore, Cell adhesion molecules and Axon guidance were also found to be significantly enriched (**Table 3**).

Construction of transcriptional regulatory network

Totally, 56 differentially expressed TFs were identified between IAs and normal tissues, including 16 up-regulated and 40 down-regulated. The transcriptional regulatory network consisted of 845 TF-target interactions between 50 TFs and 523 DEGs in the context of IAs (**Figure 2**). The top 10 TFs covering the most downstream DEGs were identified as crucial TFs involved in the development of IAs

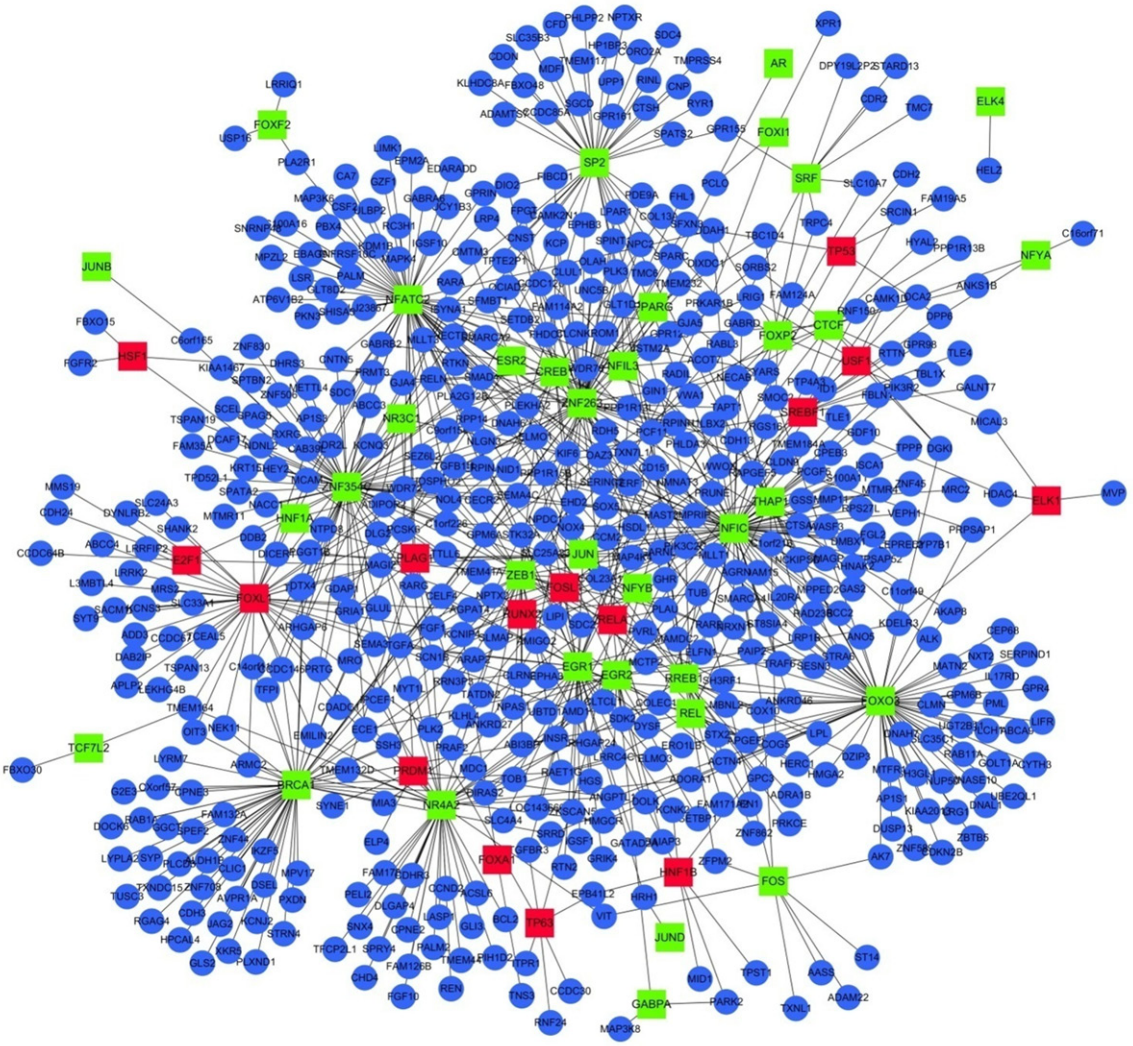

Figure 2. Transcriptional regulatory network of IAs. Red- and green-color nodes indicate up- and down-regulated TFs, respectively. Blue nodes indicate target genes regulated by the corresponding TFs.

and listed in **Table 4**, including NFIC, NFATC2, FOXO3, ZNF354C, ZNF263, BRCA1, FOXL1, NR4A2, SP2, EGR1.

Discussion

IAs remains a devastating clinical challenge and the pathophysiology of the IAs remains unclear. In this study, we compared six mRNA expression profiles in human IAs and normal arterial tissues. We have discovered that there are extensive changes in mRNA expression in IAs. Totally, 1093 DEGs were identified in IAs compared with normal tissues, of which 517 up-regulated and 576 down-regulated DEGs. Functional analysis demonstrated that these genes were significantly associated with pathways in cancer and cell adhesion molecules, which provided some clues on the potential functional connections between the altered mRNAs and pathogenesis of IAs. Consistent with this, the current literature also supported the role of cell adhesion molecules in cerebral vasculature in the formation and progression of IAs [10].

TFs play important roles in tumorigenesis. Combining with the TRANSFAC database, 56 differentially expressed TFs were identified between IAs and normal tissues, and an IAs-specific transcriptional regulatory network was constructed, which consisted of 845 TF-target interactions between 50 TFs and 523 DEGs in the context of IAs. The results suggested

Table 4. Top 10 TFs covering the most downstream DEGs involved in the development of IA

Transcription factor	LogFC	Up/down	Count	Genes
BRCA1	-4.71E-01	Down	58	OIT3,TGFA,GLS2,CCDC146,CXorf57,PLXND1,SSH3,MRO,PCSK6,SYP,TUSC3,KLHL4,DOCK6,AVPR1A,LYRM7,TMEM41A,IKZF5,CPNE3,HMGCR,G2E3,C14orf119,PXDN,GDAP1,ZNF44,KCNJ2,MPV17,GPM6A,TMEM132D,ALDH1B1,RAET1G,SRRD,FAM132A,DTX4,RAB1A,CELF4,SPEF2,GGCT,TFPI,DSEL,ARMC2,PLCD3,ARAP2,CLIC1,DIRAS2,ARHGAP6,RGAG4,JAG2,HPCAL4,XKR5,CDH3,TXNDC15,MIA3,ZNF708,STRN4,ECE1,NEK11,LYPLA2,INSR
EGR1	-1.92E+00	Down	36	IGSF1,RARG,STK32A,ELFN1,GATAD2A,SDC2,TGFBR3,JUN,RADIL,ELMO3,MAP4K4,KCNK2,SLC4A4,RTN2,HRH1,ERO1LB,RRN3P3,UBTD1,SEMA3F,RAPGEFL1,ZKSCAN5,PLEKHA2,CELF4,MAMDC2,SLC25A33,TGFA,NPTX2,CD151,MIA3,COG5,GRIK4,HGS,MDC1,TOB1,HSDL1,LOC143666
FOXL1	8.61E-01	Up	44	ARMC2,L3MBTL4,SLC24A3,DDB2,APLP2,NEK11,MAGI2,KCNQ3,TMEM164,CCDC146,PLEKHG4B,KCNIP4,SACM1L,PRTG,GLUL,ABCC4,CLRN3,MRS2,KCNS3,CDR2L,CAB39L,ADIPOR2,LRRFIP2,MCAM,LRRK2,DYNLRB2,CCDC67,ARHGAP6,OIT3,PHOSPHO2,DAB2IP,TCEAL5,AMD1,ADD3,CDADC1,EMILIN2,DTX4,TSPAN13,AGPAT4,SLC33A1,PCSK6,SHANK2,PLK2,SYT9
FOXO3	-3.54E-01	Down	67	DZIP3,DGKI,AP1S1,IL17RD,LIFR,RAPGEF5,MBNL2,ANO5,UGT2B11,GPR4,ZNF589,RAD23B,PLCH1,ABCA9,RAB11A,PVRL1,CDKN2B,UBE2QL1,HERC1,ANGPTL1,RAET1G,KIAA2013,COL23A1,ZBTB5,DUSP13,CEP68,RNASE10,NMNAT3,LRG1,SESN3,ALK,AMD1,YARS,STRA6,GOLT1A,MLLT1,NUP50,SMARCA4,HMGA2,NXT2,ADORA1,PRPSAP1,CLRN3,CYTH3,AK7,SLC35C1,SRRD,RAPGEFL1,TUB,COG5,DNAL1,MATN2,MTFR1,GPM6B,SERPIND1,DNAH7,PML,AKAP8,MAST3,SH3RF1,C11orf49,HMGCR,MPRIP,SH3GL1,CLMN,CDH13,ANKRD46
NFATC2	-1.73E+00	Down	68	MAPK4,PBX4,GLT8D2,ULBP2,NID1,ABCC3,SHISA5,SDC1,YARS,GUCY1B3,GPRIN1,CNTN5,EDARADD,CCM2,SNRNP48,SERPINA1,PCSK6,GABRA6,RARG,FHDC1,MAST3,PKN3,MAP3K6,MCAM,OAZ3,GIN1,MPZL2,FLJ23867,TUB,SFMBT1,EPM2A,CAB39L,IGSF10,LIPI,DTX4,DDAH1,CA7,ATP6V1B2,PALM,CDR2L,SMAD4,RDH,PLAG1,TNFRSF10C,EBAG9,MLLT3,LSR,LIMK1,NOL4,GJA5,KIF6,GZF1,SERINC2,KDM1B,CSF2,TGFB1I1,DNAH6,S100A16,PPP1R13L,WDR72,SEZ6L2,RPP14,WDR78,NMNAT3,RC3H1,LRP4,STK32A,PLA2R1
NFIC	-7.16E-01	Down	79	CCM2,CYP7B1,JUN,AHNAK2,RDH5,SCN1B,ISCA1,SEMA4C,LBX2,TRAF6,RPP14,SMOC2,WASF3,NCKIPSD,COLEC10,WDR78,PLAU,EHD2,S100A11,ZNF45,GABR,TMEM41A,ST8SIA4,C1orf226,PRUNE,GAS2,MTMR4,GDF10,TMEM184A,AGPAT4,VWA1,CPEB3,RGS16,TLE1,ANKRD46,KLHL4,SERINC2,SERPINH1,KCNIP4,ARAP2,ADAM15,MAMDC2,ATXN7L1,GSS,FGL2,PAIP2,TERF1,CDH13,IL20RA,RPS27L,CLTCL1,ELFN1,TUB,PTP4A3,ANO5,LEPREL2,VEPH1,RAD23B,RCC2,MMP11,MLLT3,PHLDA3,COX10,CTSA,SMAGP,PPP1R15B,C1orf216,NRXN1,DMBX1,PCGF5,PID1,RPSAP52,DDAH1,CLDN9,SLC25A33,NID1,RARB,OAZ3,MPPED2
NR4A2	-9.56E-01	Down	43	IPCEF1,CDHR3,VIT,BCL2,SNX4,ANGPTL1,GLUL,TMEM132D,GLI3,PRTG,COLEC10,TFCP2L1,AMIGO2,FAM126B,CLTCL1,DLG2,TMEM44,ACSL6,FAM178B,MDC1,ABI3BP,REN,PALM2,SLMAP,ADORA1,LASP1,LRRC4C,DLGAP4,CHD4,ARMC2,PRDM1,SPRY4,EMILIN2,CPNE2,TMEM164,RARG,SCN1B,AMD1,CDADC1,CCND2,FGF10,PELI2,PIH1D2
SP2	-4.94E-01	Down	40	SGCD,CD151,HECTD3,SDC4,RARA,MPRIP,ISYNA1,TMEM117,CDON,GPRIN1,RAPGEF5,CNST,PLEKHA2,CCDC85A,CORO2A,SLC35B3,SPATS2,HP1BP3,PCSK6,CTSH,TMEM232,UPP1,CAMK2N1,RADIL,GPR161,MDFI,TMPRSS4,KLHDC8A,CNP,NPTXR,RYR1,GPR155,ADAMTS7,CFD,PDE9A,PCF11,PHLPP2,GABRD,FBXO48,RINL
ZNF263	-1.34E-01	Down	60	MLLT1,PLK3,EHD2,CCDC126,CLUL1,PLA2G12B,NPC2,CLCNKB,NPDC1,NID1,NECAB1,OCIAD2,LBX2,GHR,TAPT1,HSDL1,RARB,LPAR1,SETDB2,SPARC,CDH13,SMARCA4,SPINT1,TGFA,NPTX2,SERPINH1,SOX5,EPHB3,GPM6A,DLG2,FAM114A2,PRMT3,SLMAP,C11orf49,CAMK2N1,PDE9A,KCP,CNST,FHL1,GDAP1,PHOSPHO2,OLAH,SFXN3,VSTM2A,PRPSAP1,GLT1D1,TMC6,SFMBT1,COL23A1,SMARCA2,PHLDA3,NLGN3,ADIPOR2,ROM1,AMIGO2,UNC5B,RTKN,TMEM232,TPTE2P1,DIXDC1
ZNF354C	-1.57E+00	Down	64	ZNF506,AP1S3,PLA2G12B,RTKN,TERF1,GPR123,RARG,ENTPD8,PLAG1,DDB2,FAM35A,HEY2,NLGN3,C14orf119,C9orf156,PPP1R15B,MRO,CECR2,DTX4,C1orf226,SCN1B,NACC1,TFPI,SPAG5,GLUL,SCEL,METTL4,SEMA3F,HECTD3,SPATA2,ISYNA1,CNTN5,ABCC3,MPRIP,ECE1,OAZ3,SDC1,TTLL6,KRT15,RELN,E2F1,MTMR11,C6orf165,PRTG,NOL4,ZNF830,DCAF17,GJA4,PVRL1,ARHGAP6,GABRB2,SPTBN2,KIAA1467,SESN3,TSPAN19,SMAD4,NDNL2,CCDC146,TPD52L1,RXRG,SEMA4C,SLC25A33,DHRS3,ELMO1

that the TFs may cooperate with each other and play roles in the pathogenesis of IAs. In the regulatory network, we identified some crucial TFs which covered the most downstream DEGs, including NFIC, NFATC2, FOXO3, ZNF354C, ZNF263, BRCA1, FOXL1, NR4A2, SP2 and EGR1. Some of these TFs have been reported to be highly correlated with various cancers.

The discovery of the BRCA1 gene has transformed the management of women who are at high risk of developing breast and ovarian cancer [21]. Fox proteins are at the junction of multiple signaling pathways and play critical roles in a variety of physiological and pathological processes including cancer [22]. FOXL1 has been implicated in the regulation of epithelial cell proliferation in gastrointestinal tracts [23]. And FOXL1 was supposed as a novel candidate tumor suppressor, which can inhibits tumor aggressiveness and predict outcome in human pancreatic cancer [22]. Sp2 was identified as a transcriptional repressor of carcinoembryonic antigen-related cell adhesion molecule 1 in tumorigenesis of prostate cancer [24]. It was reported that ZNF263 was implicated in tumor development [25]. ZNF354C was important in gastric cardiac adenocarcinomas [26]. NFATc2 was a transcription factor which played key regulatory role in bronchial adenocarcinoma [27]. In our present study, we found that all of the above TFs were differentially expressed, which may provide insight into the regulatory mechanism of IAs and researchers should pay attention to their functions in IAs.

To extract more information about the TFs involved in IAs from the integrated analysis and identify more candidate biomarkers for improving the diagnostic rate of IAs, we focus on the role of TF-targets, and several of targets may be involved in the development of IAs, including CNTN5, OIT3, PLA2G12B and TMPRSS4. CNTN5 belongs to the immunoglobulin superfamily, and it has been well documented that cell adhesion/recognition molecules of the immunoglobulin superfamily play a crucial role in the formation and maintenance of the nervous system [28]. Previous study revealed that OIT3 was possibly correlated with calcium binding and was regulated by a large number of transcription factors. OIT3 with loss of heterozygosity was closely related to the progression of pancreatic cancer [29]. Secreted phospholipases A2 (sPLA2s) represent attractive potential tumour biomarkers and therapeutic targets for various cancers. Among which PLA2G12B might be a good candidate as a novel biomarker for colon cancers [30]. It has been well documented that increased expression of the protease TMPRSS4 is associated with acquisition of epithelial to mesenchymal transition, invasion and metastasis in vivo, and high levels of TMPRSS4 have been found in several types of solid tumours in patients [31]. Herein, the dysregulation of OIT3, CNTN5, PLA2G12B and TMPRSS4 suggested that they should be genes of interest in IAs.

Altogether, 1093 genes were identified as being differentially expressed in IAs. Moreover, 56 differentially expressed TFs were identified, and the IAs-specific transcriptional regulatory network was constructed, which can help us understand the regulatory mechanisms of IAs pathology.

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Disclosure of conflict of interest

None.

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