Int J Clin Exp Med 2020;13(12):9331-9342
www.ijcem.com /ISSN:1940-5901/IJCEM0114961

Original Article

Variations in adamantinomatous craniopharyngioma: a gene set enrichment analysis

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Received May 25, 2020; Accepted September 17, 2020; Epub December 15, 2020; Published December 30, 2020

Abstract: Adamantinomatous craniopharyngioma (ACP) is an epithelial tumor that occurs in the sellar region; it may be derived from embryonic residue of the Rathke fissure epithelium. Surgery is difficult to perform and resection success rates are low because the tumor can invade and compress important surrounding structures. Therefore, it is necessary to find a safer and more effective treatment for ACP. Our research involved access to microarray data (GSE68015), and we identified differentially expressed genes (DEGs) between ACP and healthy samples using the limma package. We performed a gene set enrichment analysis (GSEA) based on Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG), constructed and analyzed a protein-protein interaction (PPI) network, and identified the significant modules. We identified 75 DEGs, comprising 64 upregulated genes and 11 downregulated genes in ACP samples compared with their expressions in healthy brain samples. The GSEA of these DEGs provided a comprehensive overview of some major pathophysiological mechanisms involved in ACP, including the roles played by the intermediate filaments, transport vesicles, and membranes. We also built a PPI network and identified five key genes: CDH1, KRT19, KRT16, KRT5, and KRT14. When comparing the ACP and healthy brain samples, 75 DEGs and five hub genes were identified that may be involved in the occurrence and progression of ACP, in particular the genes involved with the intermediate filaments.

Keywords: Adamantinomatous craniopharyngioma, gene set enrichment analysis, intermediate filament, gene expression omnibus

Introduction

Adamantinomatous craniopharyngioma (ACP) is an epithelial tumor that occurs in the sellar region [1, 2]. ACP can affect patients' vision and visual field and is caused by the tumor's compression of the optic nerve [3, 4]. Some patients may also suffer from increased intracranial pressure, hydrocephalus, and consciousness disorders [5]. However, this tumor's propensity to invade or compress the surrounding important structures makes surgery very difficult, so its resection success rate is low [6]. Furthermore, ACP can develop into squamous cell carcinoma following radiotherapy [7]. Therefore, it is necessary to find a safer and more effective treatment for ACP.

With developments in molecular biology and technologies for targeted gene therapy, an increasing number of studies of the molecular pathology of ACP have recently been conducted [8-11]. Studies have confirmed that the overactivation of the WNT/β-Catenin signaling pathway is the molecular basis of ACP [12-15]. However, due to the lack of an effective diagnosis and treatment for ACP in its early stages, its early diagnosis and treatment remain difficult

[16, 17]. Therefore, in order to develop an effective diagnosis and treatment for ACP, it is vital that we ascertain the molecular mechanisms underlying its occurrence, proliferation, and recurrence.

Bioinformatics can help us to identify differentially expressed genes (DEGs) and functional pathways related to the occurrence and development of tumors, such as ACP [18-20].

In this study, we analyzed data downloaded from the Gene Expression Omnibus (GEO) database to screen DEGs between samples of brain tissue from healthy individuals and samples from patients with ACP, in order to explore the potentially important molecular biological mechanisms.

Materials and methods

Access to microarray data

A gene expression profile, GSE68015, was downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/geo/) [21]. The GSE68015 array data comprised mRNA expression profiles of 15 samples from patients with ACP and 16 samples from healthy individuals. These gene expression profiles were sequenced using Affymetrix HG-U133plus2 chips (Platform GPL570). All the ACP samples were collected from craniopharyngioma surgery tissues, and the control samples were obtained from the brain tissues of healthy individuals without ACP.

Identification of DEGs

The linear models for microarray data (limma) package is a full-featured package that contains both a RAW data input, a pre-processing (normalization) function for cDNA chips, and limma for the analysis of DEGs, especially for multifactor-design experiments. The limma package was used to identify the DEGs between the ACP and healthy brain tissue samples. The cut-off criteria were set as an adjusted *p*-value < 0.001 and a $|\log FC| \geq 7$.

GO and KEGG gene set enrichment analysis

Gene Ontology (GO) includes three aspects of biology: cellular components, molecular functions, and biological processes [22]. *The Kyoto Encyclopedia of Genes and Genomes* (KEGG) (https://www.kegg.jp/) aims to ascertain advanced functions and biological systems [23]. Gene set enrichment analysis (GSEA) can be used to analyze all sequenced genes in two sample groups. Its input is a gene expression matrix in which the genes are divided into two groups; all the genes are first sequenced and then used to indicate changing trends in the expression of the genes between the two groups. GSEA analyzes whether all the genes of an *a priori* defined set are enriched at the top or bottom of this sequence list [24]. GO and KEGG pathway enrichment analysis was performed for the identified DEGs using GSEA. GSEA was also conducted for all the sequenced genes from the ACP tumor tissues and the healthy brain tissues, using GSEA software, after importing the gene annotation files, reference function sets, and all gene data from both the ACP tumor tissues and the healthy brain tissues. This software performs analyses and sequences genes according to an algorithm, to produce a gene sequence list. It then analyzes the position of all the genes in the sequence list and scores them to obtain an enrichment score (ES). Once the ES has been standardized, we can obtain a comprehensive understanding of the biological functions of the genes through the enrichment of functional sets. The cut-off criterion is $P < 0.05$.

Construction and analysis of the protein-protein interaction (PPI) network and significant modules

The common DEGs were imported into the Search Tool for the Retrieval of Interacting Genes (STRING; http://string-db.org) (version 10.5), an online tool used to predict and trace-out PPI networks [25]. Cytoscape (version 3.6.1), a free visualization software application, was used to visualize the PPI networks [26]. Based on topology principles, the molecular complex detection (MCODE) (version 1.5.1) plug-in of Cytoscape can be used to discover tightly coupled regions [27]. MCODE was used to screen the modules of the PPI network with a degree cut-off of 6 and a node score cut-off of 0.2. Once the degrees were set (degrees 10), the hub genes were excavated.

Statistical analysis

SPSS software (version 25.0; IBM, Chicago, IL, USA) was used to perform the statistical analy-

Table 1. Functional enrichment analysis of the DEGs in ACP, using GSEA

Gene set name	SIZE	ES	NES	*P*-value
Upregulated				
GO_REGULATION_OF_TRANSCRIPTION_ELONGATION_FROM_RNA_POLYMERASE_II_PROMOTER	22	0.68	2.13	0.000
GO_INTERMEDIATE_FILAMENT_BASED_PROCESS	32	0.69	1.91	0.000
GO_REGULATION_OF_ACTIVIN_RECEPTOR_SIGNALING_PATHWAY	23	0.66	1.82	0.000
GO_LATERAL_PLASMA_MEMBRANE	44	0.63	1.81	0.000
GO_REGULATION_OF_DNA_BINDING	82	0.59	1.80	0.000
GO_REGULATION_OF_MITOCHONDRIAL_OUTER_MEMBRANE_PERMEABILIZATION_INVOLVED_IN_APOPTOTIC_SIGNALING_PATHWAY	37	0.66	1.79	0.000
Downregulated				
GO_GLUTAMATE_RECEPTOR_BINDING	34	-0.75	-1.70	0.000
GO_TRANSPORT_VESICLE_MEMBRANE	128	-0.61	-1.68	0.000
GO_REGULATION_OF_RESPONSE_TO_FOOD	16	-0.74	-1.66	0.000
GO_EXOCYTIC_VESICLE_MEMBRANE	49	-0.85	-1.63	0.000
GO_VESICLE_DOCKING_INVOLVED_IN_EXOCYTOSIS	32	-0.56	-1.61	0.017
GO_ADULT_BEHAVIOR	110	-0.69	-1.60	0.000

DEGs: differentially expressed genes; ACP: adamantinomatous craniopharyngioma; ES: enrichment score; NES: normalized enrichment score.

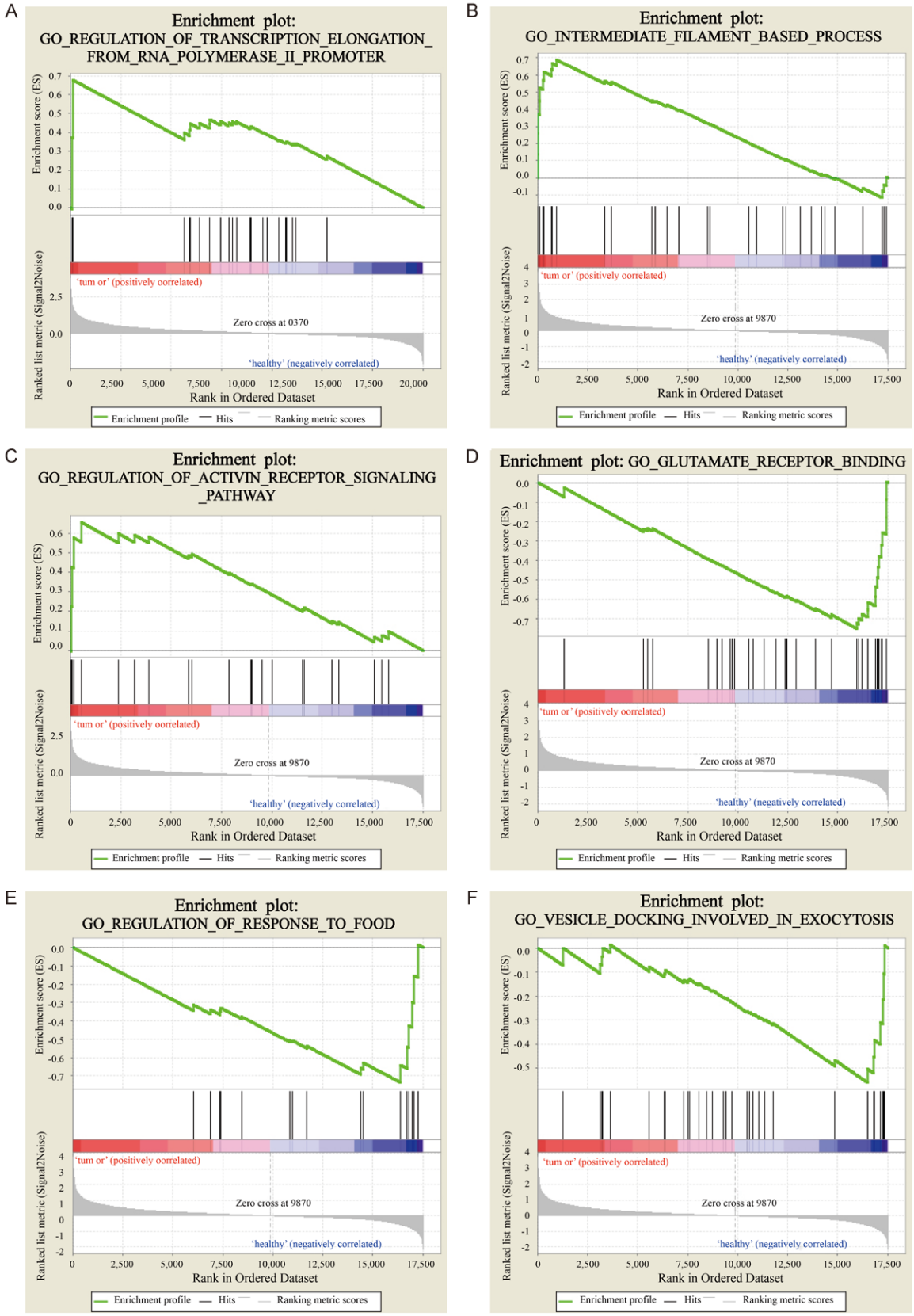

Figure 1. Six significant enrichment plots from the functional enrichment analyses of the DEGs in ACP, using GSEA. A. GO_REGULATION_OF_TRANSCRIPTION_ELONGATION_FROM_RNA_POLYMERASE_II_PROMOTER. B. Enrichment

plot: GO_INTERMEDIATE_FILAMENT_BASED_PROCESS. C. GO_REGULATION_OF_ACTIVIN_RECEPTOR_SIGNALING_PATHWAY. D. GO_GLUTAMATE_RECEPTOR_BINDING. E. GO_REGULATION_OF_RESPONSE_TO_FOOD. F. GO_VESICLE_DOCKING_INVOLVED_IN_EXOCYTOSIS.

Table 2. Pathway enrichment analysis of the DEGs in ACP, using GSEA

Gene set name	SIZE	ES	NES	*P*-value
Upregulated				
KEGG_PATHWAYS_IN_CANCER	287	0.56	1.67	0.000
KEGG_BLADDER_CANCER	37	0.69	1.67	0.002
KEGG_CHRONIC_MYELOID_LEUKEMIA	63	0.59	1.66	0.006
KEGG_SMALL_CELL_LUNG_CANCER	77	0.64	1.63	0.000
KEGG_PATHOGENIC_ESCHERICHIA_COLI_INFECTION	42	0.55	1.60	0.025
KEGG_MELANOMA	64	0.53	1.59	0.006
Downregulated				
KEGG_PROXIMAL_TUBULE_BICARBONATE_RECLAMATION	23	-0.55	-1.44	0.036
KEGG_CARDIAC_MUSCLE_CONTRACTION	62	-0.46	-1.37	0.103
KEGG_ALANINE_ASPARTATE_AND_GLUTAMATE_METABOLISM	30	-0.49	-1.36	0.096
KEGG_NEUROACTIVE_LIGAND_RECEPTOR_INTERACTION	203	-0.47	-1.30	0.080

DEGs: differentially expressed genes; ACP: adamantinomatous craniopharyngioma; ES: enrichment score; NES: normalized enrichment score.

ses. The measurement data were presented as the means ± standard error. Comparisons among the two groups were made using Student's t-tests. A *P*-value < 0.05 was considered statistically significant.

Results

Identification of DEGs in ACP

The analysis of the GSE68015 dataset identified 75 DEGs (adjusted P < 0.001, logFC ≥ 7 or ≤ -7), comprising 64 upregulated genes and 11 downregulated genes in the ACP samples, compared with their expressions in healthy brain tissues.

GO and KEGG pathway enrichment analysis of DEGs in ACP using GSEA

GSEA was used to analyze GO and KEGG to explore the functions and pathways of the DEGs. The GO enrichment analysis showed that 3211/4136 gene sets were upregulated in ACP, 950 gene sets were significantly enriched at a nominal *P*-value < 0.05, and 334 gene sets were significantly enriched at a nominal *P*-value < 0.01. In addition, 925/4136 gene sets were downregulated in ACP, 125 gene sets were significantly enriched at a nominal *P*-value < 5%, and 30 gene sets were significantly enriched at a nominal *P*-value < 0.01. The most significant enrichments for both up- and down-regulated gene sets, in order of significance of the normalized enrichment score (NES), are listed in **Table 1**. Six significant enrichment plots are shown in **Figure 1**, such as "GO_REGULATION_OF_TRANSCRIPTION_ELONGATION_FROM_RNA_POLYMERASE_II_PROMOTER", "GO_INTERMEDIATE_FILAMENT_BASED_PROCESS", "GO_REGULATION_OF_ACTIVIN_RECEPTOR_SIGNALING_PATHWAY", "GO_GLUTAMATE_RECEPTOR_BINDING", "GO_TRANSPORT_VESICLE_MEMBRANE", "GO_REGULATION_OF_RESPONSE_TO_FOOD". Our GO enrichment analysis found that the gene sets upregulated in ACP were mainly associated with intermediate filaments and DNA binding, and the downregulated gene sets were frequently correlated with transport-vesicle membranes. The KEGG enrichment analysis showed that 130/168 gene sets were upregulated in ACP compared with their expressions in the healthy brain samples, 34 gene sets were significantly enriched at a nominal *P*-value < 5%, and nine gene sets were significantly enriched at a nominal *P*-value < 1%. There were 38/168 gene sets downregulated in ACP, and one gene set was significantly enriched at a nominal *P*-value < 5%. The top-ten gene sets correlated with ACP, according to their NES, are shown in **Table 2**. Six significant enrichment plots are shown in **Figure 2**, such as "KEGG_PATHWAYS_IN_CANCER", "KEGG_

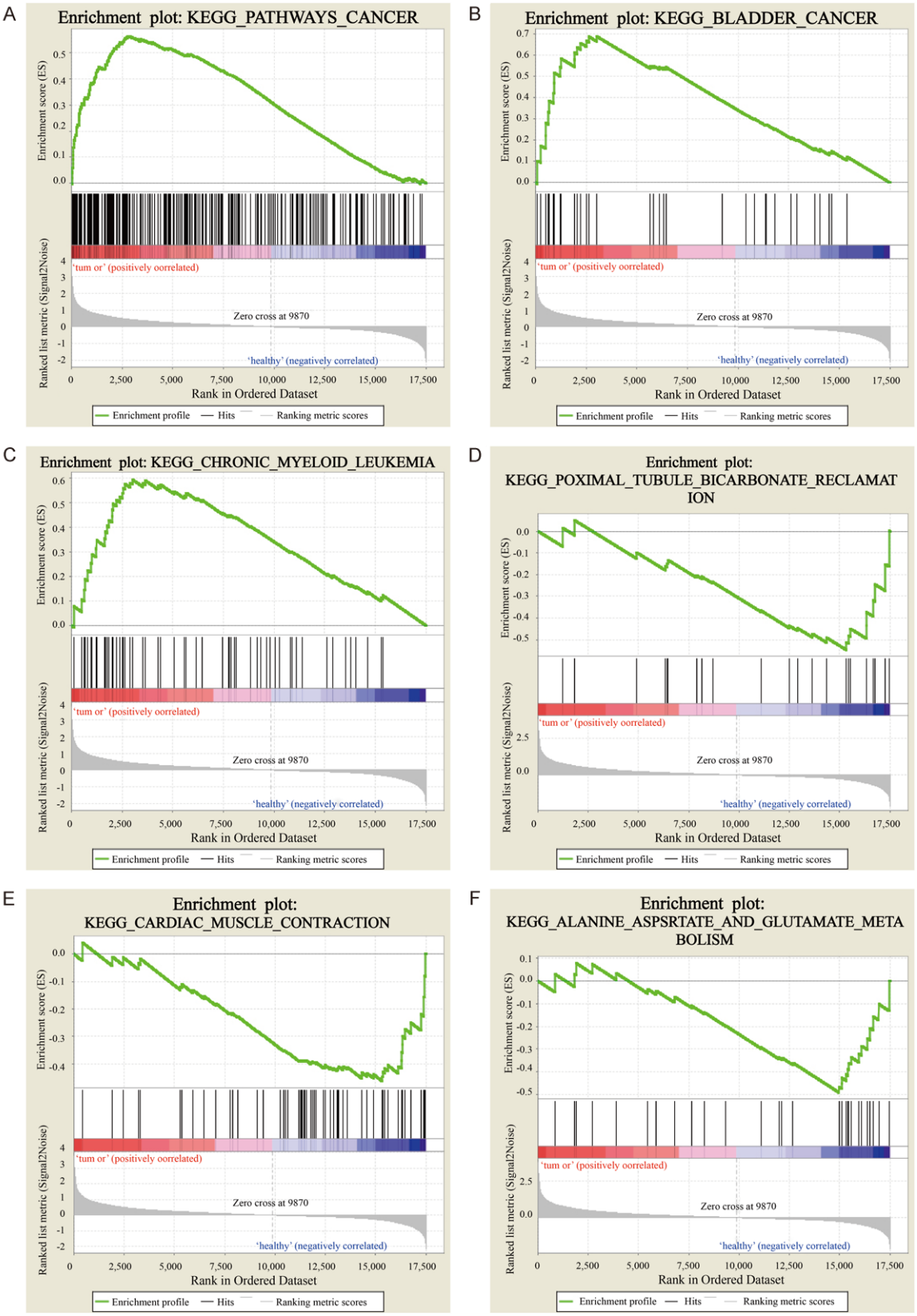

Figure 2. Six significant enrichment plots from the pathway enrichment analyses of the DEGs in ACP, using GSEA. A. KEGG_PATHWAYS_IN_CANCER. B. KEGG_BLADDER_CANCER. C. KEGG_CHRONIC_MYELOID_LEUKEMIA. D. KEGG_

PROXIMAL_TUBULE_BICARBONATE_RECLAMATION. E. KEGG_CARDIAC_MUSCLE_CONTRACTION. F. KEGG_ALANINE_ASPARTATE_AND_GLUTAMATE_METABOLISM.

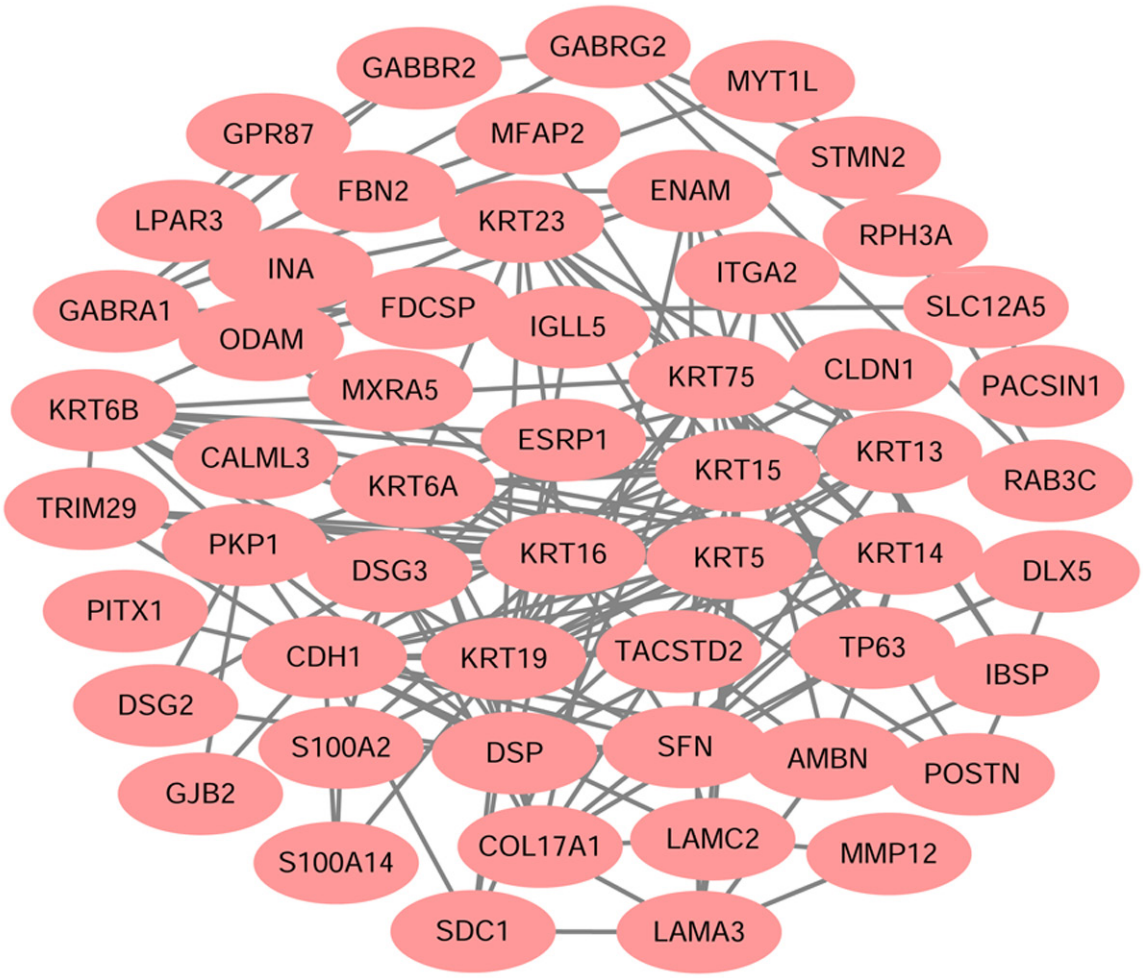

Figure 3. The protein-protein interaction (PPI) network of the differentially expressed genes (DEGs).

BLADDER_CANCER", "KEGG_CHRONIC_MYELOID_LEUKEMIA", "KEGG_PROXIMAL_TUBULE_BICARBONATE_RECLAMATION", "KEGG_ALANINE_ASPARTATE_AND_GLUTAMATE_METABOLISM". According to the KEGG pathway enrichment analysis, the gene sets upregulated in ACP were mostly associated with pathways linked with cancer, and the downregulated gene sets participated in the alanine, aspartate, and glutamate metabolism and neuroactive ligand receptor interactions.

PPI network construction and module analysis

The PPI network of the DEGs comprised 55 nodes and 162 edges, with a PPI enrichment *P*-value < 1.0e-16 (**Figure 3**). The cytoHubba plug-in for Cytoscape was used to screen the hub genes in the PPI network. A significant module, including 10 nodes and 45 edges, was generated from the PPI network of DEGs by MCODE (**Figure 4**). Five genes were identified as hub genes: CDH1, KRT19, KRT16, KRT5, and KRT14 (**Figure 5**). These five hub genes were screened out in the heatmap (**Figure 6**).

Discussion

There are different growth patterns of ACP, and within the sellar region, where this tumor occurs, there are many important structures, including the pituitary stalk, the optic nerve, the optic chiasm, and the hypothalamus [28-30]. Therefore, it remains a major challenge for neu-

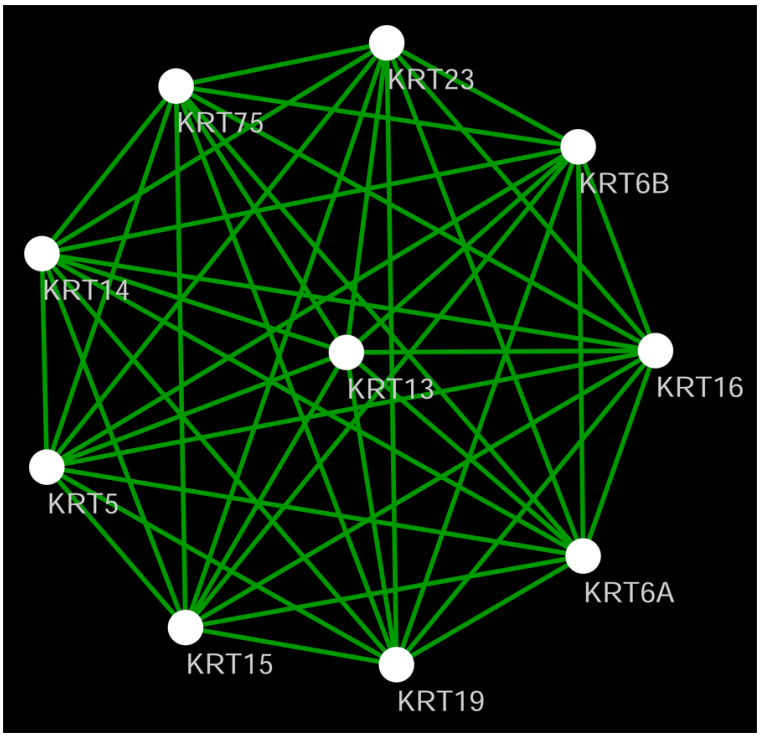

Figure 4. The significant module, including 10 nodes and 45 edges.

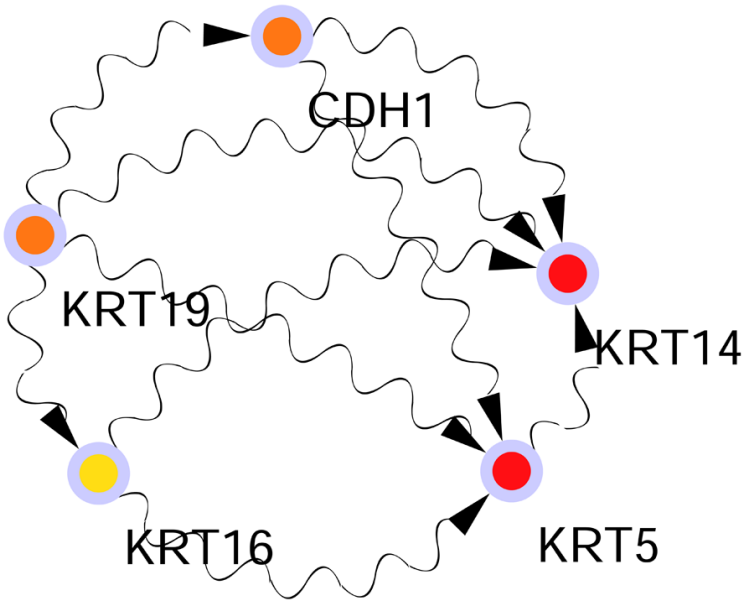

Figure 5. Five genes were selected as hub genes: CDH1, KRT19, KRT16, KRT5, and KRT14.

rologists to effectively treat ACP without damaging the surrounding tissues [31].

For this study, a total of 75 DEGs were identified, comprising 64 upregulated genes and 11 downregulated genes in the ACP samples compared with their expressions in the healthy brain tissues. The GSEA of DEGs provided a comprehensive overview of some of the major pathophysiological mechanisms involved in ACP, including intermediate filaments, transport vesicles, and membranes.

Intermediate filaments (IF) are one of the main types of protein filament that form the cytoskeleton [32, 33]. Many diseases are attributable to changes in cytoskeleton proteins, especially changes in the IF protein [34-36]. Gene deletions and the irregular expression of IF proteins can give rise to some types of tumors. Mu-Min Shao [37] concluded that cytokeratin (CK) immunohistochemistry plays an important role in the development of breast carcinoma. High CK7, CK8, CK18, and CK19 expression rates have also been observed in breast carcinoma. Furthermore, CK8 and CK20 play a very significant role in colorectal cancer, with high diagnostic and prognostic relevance [38]. In addition, Minsun Jung found that the CK20-positive state was associated with poorer prognoses and shorter survival times in patients with urinary bladder transitional cell carcinoma [39, 40]. The present study found that the expression of IF was noticeably upregulated in the patients with ACP compared with its expression in the healthy control group. Therefore, we infer that the over-expression of IF proteins might lead to ACP, which indicates IF could be a potential target for the treatment and prevention of ACP.

In this work we also built a PPI network and identified a total of five key genes: CDH1, KRT19, KRT16, KRT5, and KRT14. It was also

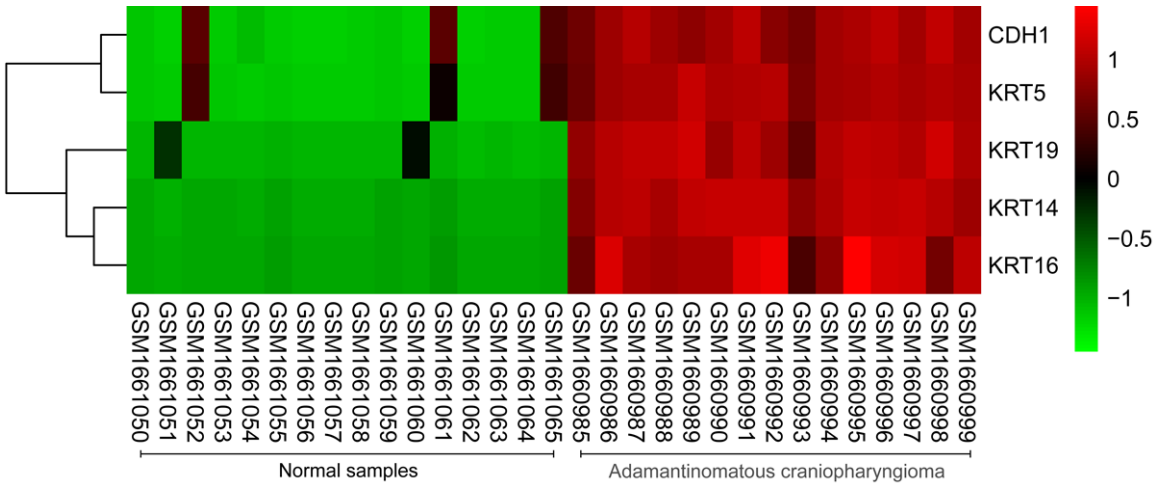

Figure 6. Hierarchical clustering showing that the hub genes could differentiate the adamantinomatous craniopharyngioma (ACP) samples from the healthy samples. The upregulation of the genes is indicated by red; the downregulation of the genes is indicated by green.

discovered that the expression of CDH1 was enhanced in patients with ACP. CDH1 is the gene that codes for E-cadherin, which plays a role in suppressing tumor progression [41]. E-cadherin is a vital adhesion molecule. Tumor metastasis begins with a reduction in cell adhesion, which relies on adhesion molecules [42-44]. Jun Yang found that the expression of CDH1 is significantly upregulated in patients with ACP [20]. Other researchers have observed CDH1 mutations in patients with hereditary diffuse gastric cancer, including CDH1 gene deletions, E-cadherin deletions, and decreased E-cadherin expressions [45]. Hereditary diffuse gastric cancer is an autosomal dominant inherited disease associated with CDH1 gene mutations, and after lobular breast cancer is the second most frequent type of neoplasia [46]. CDH1 mutations, including gene deletions and decreases in expression, are also present in other frequently occurring tumors, such as ovarian tumors and colorectal tumors [47, 48].

However, our study has some limitations. First, the results are based only on a bioinformatics analysis, so laboratory studies will be necessary to verify the functions of the key genes, using numerous tissue samples from patients with ACP.

In conclusion, using a bioinformatics analysis, we identified 75 DEGs and five hub genes when we compared the ACP and the healthy brain samples. These genes may be involved in the occurrence and development of ACP, especially the genes involved with the intermediate filaments.

Acknowledgements

We are thankful to Qing-qing Wang (Tianjin Medical University) for her statistical assistance and suggestions. This work was funded by Capital Characteristic Clinical Application Research (grant number: Z161100000516-236).

Disclosure of conflict of interest

None.

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References

[1] Wang Y, Deng J, Guo G, Tong A, Peng X, Chen H, Xu J, Liu Y, You C and Zhou L. Clinical and prognostic role of annexin A2 in adamantinomatous craniopharyngioma. J Neurooncol 2017; 131: 21-29.

[2] Scagliotti V, Avagliano L, Gualtieri A, Graziola F, Doi P, Chalker J, Righini A, Korbonits M, Bulfamante G, Jacques TS, Massa V and Gaston-Massuet C. Histopathology and molecular characterisation of intrauterine-diagnosed

congenital craniopharyngioma. Pituitary 2016; 19: 50-56.
[3] Metwali H, Gerganov V and Fahlbusch R. Optic nerve mobilization to enhance the exposure of the pituitary stalk during craniopharyngioma resection: early experience. J Neurosurg 2016; 125: 683-688.
[4] Gonzalez-Meljem JM, Haston S, Carreno G, Apps JR, Pozzi S, Stache C, Kaushal G, Virasami A, Panousopoulos L, Mousavy-Gharavy SN, Guerrero A, Rashid M, Jani N, Goding CR, Jacques TS, Adams DJ, Gil J, Andoniadou CL and Martinez-Barbera JP. Stem cell senescence drives age-attenuated induction of pituitary tumours in mouse models of paediatric craniopharyngioma. Nat Commun 2017; 8: 1819.
[5] Müller HL. Craniopharyngioma. Endocr Rev 2014; 35: 513-543.
[6] Müller HL, Merchant TE, Puget S and Martinez-Barbera JP. New outlook on the diagnosis, treatment and follow-up of childhood-onset craniopharyngioma. Nat Rev Endocrinol 2017; 13: 299-312.
[7] Fountas A, Chai ST, Ayuk J, Gittoes N, Chavda S and Karavitaki N. A rare challenging case of co-existent craniopharyngioma, acromegaly and squamous cell lung cancer. Endocrinol Diabetes Metab Case Rep 2018; 2018: 18-0018.
[8] Brastianos PK and Santagata S. Endocrine tumors: BRAF V600E mutations in papillary craniopharyngioma. Eur J Endocrinol 2016; 174: R139-144.
[9] Martinez-Gutierrez JC, D'Andrea MR, Cahill DP, Santagata S, Barker FG 2nd and Brastianos PK. Diagnosis and management of craniopharyngiomas in the era of genomics and targeted therapy. Neurosurg Focus 2016; 41: E2.
[10] Robinson LC, Santagata S and Hankinson TC. Potential evolution of neurosurgical treatment paradigms for craniopharyngioma based on genomic and transcriptomic characteristics. Neurosurg Focus 2016; 41: E3.
[11] Arginteanu MS, Hague K, Zimmerman R, Kupersmith MJ, Shaiu JH, Schaeffer J and Post KD. Craniopharyngioma arising de novo in middle age. Case report. J Neurosurg 1997; 86: 1046-1048.
[12] Gaston-Massuet C, Andoniadou CL, Signore M, Jayakody SA, Charolidi N, Kyeyune R, Vernay B, Jacques TS, Taketo MM, Le Tissier P, Dattani MT and Martinez-Barbera JP. Increased wingless (Wnt) signaling in pituitary progenitor/stem cells gives rise to pituitary tumors in mice and humans. Proc Natl Acad Sci U S A 2011; 108: 11482-11487.
[13] Andoniadou CL, Gaston-Massuet C, Reddy R, Schneider RP, Blasco MA, Le Tissier P, Jacques TS, Pevny LH, Dattani MT and Martinez-Barbera JP. Identification of novel pathways involved in the pathogenesis of human adamantinomatous craniopharyngioma. Acta Neuropathol 2012; 124: 259-271.
[14] Hussain I, Eloy JA, Carmel PW and Liu JK. Molecular oncogenesis of craniopharyngioma: current and future strategies for the development of targeted therapies. J Neurosurg 2013; 119: 106-112.
[15] Hara T, Akutsu H, Takano S, Kino H, Ishikawa E, Tanaka S, Miyamoto H, Sakamoto N, Hattori K, Sakata-Yanagimoto M, Chiba S, Hiyama T, Masumoto T and Matsumura A. Clinical and biological significance of adamantinomatous craniopharyngioma with CTNNB1 mutation. J Neurosurg 2018; 131: 217-226.
[16] Clark AJ, Cage TA, Aranda D, Parsa AT, Auguste KI and Gupta N. Treatment-related morbidity and the management of pediatric craniopharyngioma: a systematic review. J Neurosurg Pediatr 2012; 10: 293-301.
[17] Klimo P Jr, Venable GT, Boop FA and Merchant TE. Recurrent craniopharyngioma after conformal radiation in children and the burden of treatment. J Neurosurg Pediatr 2015; 15: 499-505.
[18] Tyanova S, Temu T, Sinitcyn P, Carlson A, Hein MY, Geiger T, Mann M and Cox J. The perseus computational platform for comprehensive analysis of (prote) omics data. Nat Methods 2016; 13: 731-740.
[19] Nobile MS, Cazzaniga P, Tangherloni A and Besozzi D. Graphics processing units in bioinformatics, computational biology and systems biology. Brief Bioinform 2017; 18: 870-885.
[20] Yang J, Hou Z, Wang C, Wang H and Zhang H. Gene expression profiles reveal key genes for early diagnosis and treatment of adamantinomatous craniopharyngioma. Cancer Gene Ther 2018; 25: 227-239.
[21] Wang Z, Monteiro CD, Jagodnik KM, Fernandez NF, Gundersen GW, Rouillard AD, Jenkins SL, Feldmann AS, Hu KS, McDermott MG, Duan Q, Clark NR, Jones MR, Kou Y, Goff T, Woodland H, Amaral FMR, Szeto GL, Fuchs O, Schüssler-Fiorenza Rose SM, Sharma S, Schwartz U, Bausela XB, Szymkiewicz M, Maroulis V, Salykin A, Barra CM, Kruth CD, Bongio NJ, Mathur V, Todoric RD, Rubin UE, Malatras A, Fulp CT, Galindo JA, Motiejunaite R, Jüschke C, Dishuck PC, Lahl K, Jafari M, Aibar S, Zaravinos A, Steenhuizen LH, Allison LR, Gamallo P, de Andres Segura F, Dae Devlin T, Pérez-García V and Ma'ayan A. Extraction and analysis of signatures from the gene expression omnibus by the crowd. Nat Commun 2016; 7: 12846.
[22] Cheng L, Lin H, Hu Y, Wang J and Yang Z. Gene function prediction based on the gene ontolo-

gy hierarchical structure. PLoS One 2014; 9: e107187.
[23] Tanabe M and Kanehisa M. Using the KEGG database resource. Curr Protoc Bioinformatics 2012; Chapter 1: Unit1.12.
[24] Holden M, Deng S, Wojnowski L and Kulle B. GSEA-SNP: applying gene set enrichment analysis to SNP data from genome-wide association studies. Bioinformatics 2008; 24: 2784-2785.
[25] Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, Simonovic M, Roth A, Santos A, Tsafou KP, Kuhn M, Bork P, Jensen LJ and von Mering C. STRING v10: protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res 2015; 43: D447-452.
[26] Su G, Morris JH, Demchak B and Bader GD. Biological network exploration with Cytoscape 3. Curr Protoc Bioinformatics 2014; 47: 8.13.1-24.
[27] Bader GD and Hogue CW. An automated method for finding molecular complexes in large protein interaction networks. BMC Bioinformatics 2003; 4: 2.
[28] Okada T, Fujitsu K, Ichikawa T, Mukaihara S, Miyahara K, Kaku S, Uryuu Y, Niino H, Yagishita S and Shiina T. Coexistence of adamantinomatous and squamous-papillary type craniopharyngioma: case report and discussion of etiology and pathology. Neuropathology 2012; 32: 171-173.
[29] Petito CK, DeGirolami U and Earle KM. Craniopharyngiomas: a clinical and pathological review. Cancer 1976; 37: 1944-1952.
[30] Renn WH and Rhoton AL Jr. Microsurgical anatomy of the sellar region. J Neurosurg 1975; 43: 288-298.
[31] Bi WL, Greenwald NF, Ramkissoon SH, Abedalthagafi M, Coy SM, Ligon KL, Mei Y, MacConaill L, Ducar M, Min L, Santagata S, Kaiser UB, Beroukhim R, Laws ER Jr and Dunn IF. Clinical identification of oncogenic drivers and copy-number alterations in pituitary tumors. Endocrinology 2017; 158: 2284-2291.
[32] Duncan EJ, Larivière R, Bradshaw TY, Longo F, Sgarioto N, Hayes MJ, Romano LEL, Nethisinghe S, Giunti P, Bruntraeger MB, Durham HD, Brais B, Maltecca F, Gentil BJ and Chapple JP. Altered organization of the intermediate filament cytoskeleton and relocalization of proteostasis modulators in cells lacking the ataxia protein sacsin. Hum Mol Genet 2017; 26: 3130-3143.
[33] Tang DD and Gerlach BD. The roles and regulation of the actin cytoskeleton, intermediate filaments and microtubules in smooth muscle cell migration. Respir Res 2017; 18: 54.
[34] Israeli E, Dryanovski DI, Schumacker PT, Chandel NS, Singer JD, Julien JP, Goldman RD and Opal P. Intermediate filament aggregates cause mitochondrial dysmotility and increase energy demands in giant axonal neuropathy. Hum Mol Genet 2016; 25: 2143-2157.
[35] Omary MB. Intermediate filament proteins of digestive organs: physiology and pathophysiology. Am J Physiol Gastrointest Liver Physiol 2017; 312: G628-G634.
[36] Hawley ZCE, Campos-Melo D and Strong MJ. MiR-105 and miR-9 regulate the mRNA stability of neuronal intermediate filaments. Implications for the pathogenesis of amyotrophic lateral sclerosis (ALS). Brain Res 2019; 1706: 93-100.
[37] Shao MM, Chan SK, Yu AM, Lam CC, Tsang JY, Lui PC, Law BK, Tan PH and Tse GM. Keratin expression in breast cancers. Virchows Arch 2012; 461: 313-322.
[38] Knösel T, Emde V, Schlüns K, Schlag PM, Dietel M and Petersen I. Cytokeratin profiles identify diagnostic signatures in colorectal cancer using multiplex analysis of tissue microarrays. Cell Oncol 2006; 28: 167-175.
[39] Jung M, Kim B and Moon KC. Immunohistochemistry of cytokeratin (CK) 5/6, CD44 and CK20 as prognostic biomarkers of non-muscle-invasive papillary upper tract urothelial carcinoma. Histopathology 2019; 74: 483-493.
[40] Li SS, Xu LZ, Zhou W, Yao S, Wang CL, Xia JL, Wang HF, Kamran M, Xue XY, Dong L, Wang J, Ding XD, Bella L, Bugeon L, Xu J, Zheng FM, Dallman MJ, Lam EWF and Liu Q. p62/SQSTM1 interacts with vimentin to enhance breast cancer metastasis. Carcinogenesis 2017; 38: 1092-1103.
[41] Liu H, Ma Y, He HW, Zhao WL and Shao RG. SPHK1 (sphingosine kinase 1) induces epithelial-mesenchymal transition by promoting the autophagy-linked lysosomal degradation of CDH1/E-cadherin in hepatoma cells. Autophagy 2017; 13: 900-913.
[42] Gan WJ, Wang JR, Zhu XL, He XS, Guo PD, Zhang S, Li XM, Li JM and Wu H. RARγ-induced E-cadherin downregulation promotes hepatocellular carcinoma invasion and metastasis. J Exp Clin Cancer Res 2016; 35: 164.
[43] Hugo HJ, Gunasinghe N, Hollier BG, Tanaka T, Blick T, Toh A, Hill P, Gilles C, Waltham M and Thompson EW. Epithelial requirement for in vitro proliferation and xenograft growth and metastasis of MDA-MB-468 human breast cancer cells: oncogenic rather than tumor-suppressive role of E-cadherin. Breast Cancer Res 2017; 19: 86.
[44] Reardon SN, King ML, MacLean JA 2nd, Mann JL, DeMayo FJ, Lydon JP and Hayashi K. CDH1 is essential for endometrial differentiation,

gland development, and adult function in the mouse uterus. Biol Reprod 2012; 86: 141, 1-10.
[45] Hansford S, Kaurah P, Li-Chang H, Woo M, Senz J, Pinheiro H, Schrader KA, Schaeffer DF, Shumansky K, Zogopoulos G, Santos TA, Claro I, Carvalho J, Nielsen C, Padilla S, Lum A, Talhouk A, Baker-Lange K, Richardson S, Lewis I, Lindor NM, Pennell E, MacMillan A, Fernandez B, Keller G, Lynch H, Shah SP, Guilford P, Gallinger S, Corso G, Roviello F, Caldas C, Oliveira C, Pharoah PD and Huntsman DG. Hereditary diffuse gastric cancer syndrome: CDH1 mutations and beyond. JAMA Oncol 2015; 1: 23-32.
[46] Corso G, Intra M, Trentin C, Veronesi P and Galimberti V. CDH1 germline mutations and hereditary lobular breast cancer. Fam Cancer 2016; 15: 215-219.
[47] Graffeo R, Livraghi L, Pagani O, Goldhirsch A, Partridge AH and Garber JE. Time to incorporate germline multigene panel testing into breast and ovarian cancer patient care. Breast Cancer Res Treat 2016; 160: 393-410.
[48] Rivu SF, Apu MNH, Shabnaz S, Nahid NA, Islam MR, Al-Mamun MMA, Nahar Z, Rabbi SNI, Ahmed MU, Islam MS and Hasnat A. Association of TP53 codon 72 and CDH1 genetic polymorphisms with colorectal cancer risk in Bangladeshi population. Cancer Epidemiol 2017; 49: 46-52.