Meningioma Protein-Protein Interaction Network

Hakimeh Zali PhD[1], Mostafa Rezaei Tavirani PhD•[2]

Abstract

Background: Meningioma is one of the most common central nervous system tumors derived from meningothelial (arachnoid cap) cells. This paper identified the network-based Protein-Protein Interactions (PPI) for meningioma compared to healthy controls.

Methods: Gene expression data, including 384 gene or protein names, were extracted from a number of previous investigations. Out of these 384 proteins, 176 were found to be exclusively expressed in meningiomas and 208 proteins were down-regulated. The networks of related differentially expressed genes were explored using cytoscape and the PPI analysis methods such as MCODE and ClueGO.

Results: Analysis introduced a number of hub proteins and 27 clusters (protein complex) with distinctive seed genes. Identified ClueGO Pathways based on subnetworks mined by MCODE was composed of positive regulation in RBC homeostasis, dysregulation of transport from ER to Golgi, disruption regulation of cell cycle and antigen processing and presentation of exogenous peptide antigen and neutralization of exogenous dsRNA. Combination of over-expression of TCEA1, UBE2E1, XRCC5, IFIT1, IFIT-3, MCM2, and MCM7 and under-expression of CDC25A, SEC31A, and CDK6 can serve as a diagnostic biomarker panel for meningiomas.

Conclusion: These proposed network-based biomarkers for the meningioma patterns may be helpful in diagnosis, prognosis and treatment processes, although biomarker validation is necessary.

Keywords: Biomarker, Hub, meningioma, protein complex, protein-protein interactions network

Introduction

Meningiomas are the most common non-glial neoplasm of the central nervous system (CNS) and account for one fifth of all intracranial tumors.[1,2] They are extra-axial tumors that originate from the arachnoid cap cells of the meninges. These tumors are more common in women and uncommon in patients before the age of 40; their incidence in younger patients might be due to neurofibromatosis type 2 (NF2). The World Health Organization (WHO) classification for CNS tumors[3,4] categorizes them as WHO I: meningioma (about 88–95%), WHO II: atypical meningioma (atypical, clear cell, chordoid- about 5–6%), WHO III: malignant meningioma (rhabdoid, anaplastic, papillary - about 1%), and finally WHO IV: meningioma with sarcomatous degeneration that is extremely rare.[4] Malignant tumors are rare and about 90% all meningiomas are benign.[5] The etiologies of meningiomas are not fully clear.[6] Familial cases are much lower in frequency than sporadic ones. In the past, cases with exposure to radiation suffered from brain injury[7], especially those who had frequent dental X-rays as the X-ray dose used to be higher than now.[8] Studies of cell phones have found no relationship between cell phone use and incidence of meningiomas.[5,9]

Although the Magnetic Resonance (MR) spectroscopy generally is not required for perfect diagnosis, it can be helpful for recognizing meningiomas from mimics i.e. increased alanine, glutamine / glutamate and choline indicate cellular tumor. Significant reduction in N-acetyl aspartate indicates non-neuronal origin. On MR perfusion, there is good correlation between volume transfer constant (k-trans) and histological grade.[10,11]

Mutations in NF-2 gene have been detected in 60% of meningiomas.[12] The NF2 gene, a tumor suppressor gene located at 22q12.2, is the main candidate for the genesis of meningiomas. Expression of other tumor suppressor genes, including THBS1, TIMP-3, p16 (INK4a), MGMT, p73, ER, GSTP1, RB1 and p14 (ARF), is inhibited in meningiomas.[13,14] Other possible genes/loci include AKT1, MN1,[15] PTEN,[16] SMO and an unknown gene at 1p13.[17,18] Sadetzki et al. showed that variations in Ki-RAS and ERCC2 are associated with an approximately 2-fold increased risk of meningioma.[19]

Apart from genetic aberrations, alterations in protein expression have been reported. Saydam et al. (2011) compared meningioma cells proteome to human primary arachnoidal cells to discover novel protein biomarkers for diagnostic and/or prognostic purposes. They found a significant increase in minichromosome maintenances (MCM) family (MCM2, MCM3, MCM4, MCM5, MCM6, and MCM7) in meningiomas.[20] The role of proteolytic enzymes, such as serine proteases and metalloproteinases, in tumor invasion and metastasis are previously indicated in several types of cancer.[21]

Although most meningiomas are slow growing benign tumors, huge meningiomas are believed to entail surgical risks. However, if a meningioma is diagnosed in early stages, it can be treated non-surgically. Since homogenous genotype of meningiomas is the characteristic of benign rather than malignant brain tumors, it makes it relatively easy to discover candidate biomarkers for meningiomas. On the other hand, study based systems lead to find useful diagnostic biomarkers of meningiomas as well as their drug targets. In addition, tumor therapy is based on information about molecular alterations; therefore, we searched different genes expression of meningioma in the literature to find genes and proteins with altered expression in meningiomas in comparison to the normal cells or tissues. So, quantitative and/or modification changes in 23 studies[20–43] based on genomic and proteomic studies

Authors' affiliations: [1]Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran. [2]Proteomics Research Center, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

•**Correspondence author and reprints:** Mostafa Rezaei Tavirani PhD, Proteomics Research Center, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran. E-mail: rezaei.tavirani@ibb.ut.ac.ir.

Accepted for publication: 29 January 2014

Table 1. The PPI subnetworks were clustered as highly connected regions in meningioma network by MCODE analysis.

Cluster	Score (Density*#Nodes)	Nodes	Edges	Seed	Degree
1	6.419	44	139	P40938	7
2	6	7	19	J3KPM5	2
3	5.2	21	52	P61024	6
4	4.444	10	23	—	—
5	4.308	27	59	B2RBZ4	2
6	4.159	89	184	P23193	73
7	4	4	6	—	—
8	4	8	15	CHEBI:17283	22
9	4	6	10	Q9Z0E3	2
10	3.957	48	93	O94979	54
11	3.333	4	5	—	—
12	3.333	4	5	Q5QNR8	2
13	3.333	4	5	Q6NW02	29
14	3.167	13	20	Q8TB30	4
15	3	3	3	—	—
16	3	7	10	—	—
17	3	3	4	A1L374	2
18	3	3	3	O76075	2
19	3	3	5	DIP-6092N	2
20	3	3	4	P30304	2
21	3	3	4	—	—
22	2.857	8	11	—	—
23	2.833	13	22	Q05397	7
24	2.833	13	18	—	—
25	2.5	5	6	P09914	2
26	2	2	3	Q15667	2
27	2	2	3	—	—
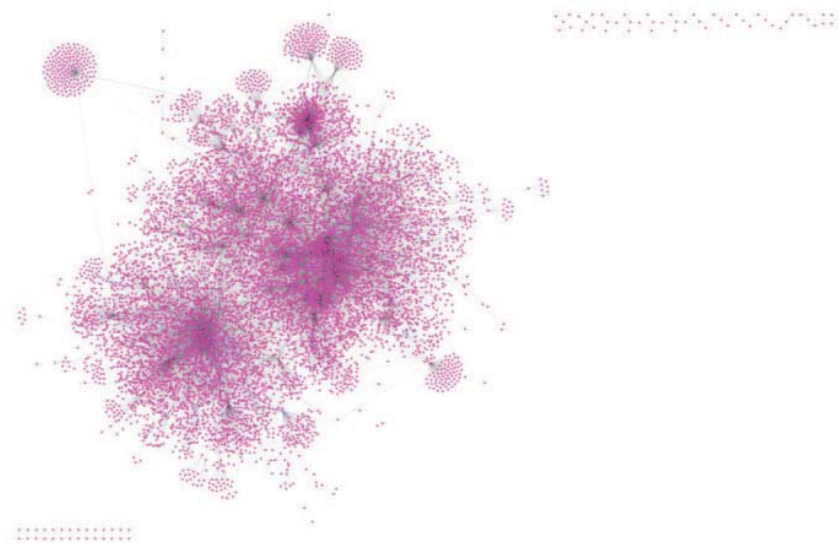
Figure 1. PPI Network of meningioma based on cytoscape 3 software. Green ellipses represent hubs in which the left side is related to down-regulated proteins in meningioma compared to the normal and right side green ellipses indicating up-regulated proteins. Blue ellipses represent neighbor nodes. All edges represent physical interactions.

in meningioma patients published since 1993 were investigated and analyzed.

Materials and Methods

Data Collection

During the last decade, there has been an exponential increase in the number of studies analyzing brain cancer tissue; so in this study, data were extracted from a number of these investigations. 84 papers were reviewed and the papers containing duplicated proteins and genes were eliminated. Finally, 384 non-redundant genes and/or proteins were extracted from 23 papers. All genes and proteins which had significantly different expressions (up-regulated, down-regulated) in meningioma tissues compared to normal tissues or cells (brain and arachnoid cap cells of the meninges) were selected. A minimal fold change of 1.4 (most papers considered ±2 folds but a few used ±1.4) was considered for comparison of genes and proteins between the two groups. Proteins and genes data from different studies were identified by various techniques; for instance, Saydam et al. isolated 281 proteins with

altered expression levels from meningioma cell line and human primary arachnoidal cells by Gel-nanoLC-MS/MS.[20] All genes and proteins were presented in supplementary Table 1.

UniProt accession numbers (http://www.uniprot.org), a publicly available web-based tool, was used to search for annotations that are significantly associated to the list of meningioma-related proteins in order to carry out a retrospective meta-analysis of the functional annotations.

Protein-Protein Interaction Analysis

Protein-Protein Interactions (PPIs) are the basic skeleton for self-organization and homeostasis of living organisms.[44] In this study, information on human PPI networks from selected genes was obtained from databases, including the MPIDB, MolCon, MBInfo, I2D-IMEx, BIND, UniProt, Interoporc, STRING, DIP, IntAct, and MINT. The PPI network was visualized using the Cytoscape 3 software.[45] We integrated the databases and networks and used Molecular COmplex DEtection (MCODE) to analyze the characteristics of the networks. The MCODE clusters a given network based on topology to find densely connected regions.[46] MCODE considers network as directed graphs and analysis is performed on directed network. Interactomes with a score greater than 2.0 and at least two nodes were selected as significant predictions. The second stage in MCOD algorithm recognizes seeds as a complex with the highest weighted vertex (forward and outward) the weight of which is above a given threshold.[46]

Gene ontology categories were analyzed to identify the function of each highly connected region that was generated by the MCODE. ClueGO v2.0.5, cytoscape plug-in tool, that visualizes the non-redundant biological terms for large clusters of genes in a functionally grouped network, was used to statistically evaluate groups of proteins with respect to the existing annotations of the Gene Ontology. The degree of functional enrichment for a given cluster was quantitatively assessed (*P*-value) using a hypergeometric distribution implemented in the ClueGO tool.[47]

Result

Three hundred eighty four (384) genes with different gene expression in meningioma were distinguished via literature survey. Among these regulated genes, 176 were up-regulated or newly expressed and 208 were down-regulated or repressed. All data are presented in table S1 (supplementary).

Results of PPI Analysis

The PPI networks of the significantly expressed genes (compared between the meningioma pattern and the control) contain 9860 nods and 11442 edges (Figure S1). Nods represent the proteins from our list and others that directly interact with them. Connections contain direct interaction partners and interconnections. It is necessary to mention that the edge represents physical or functional interaction between two proteins. In order to simplify the connection patterns, interactions for the nods with the greatest degrees (hubs) was selected. Cytoscape analysis revealed a great number of close interconnections that can be seen in Figure 1. The hub nods included Fibronectin 1 (FN1), Cyclin-dependent kinase 6 (CDK6), MmTRA1b, ubiquitin-conjugating enzyme E2E1 (UBE2E1), VCAM1, Poly[ADP-ribose] synthase 1, c-Myc, ISG60, CDK1, RNF96, XRCC5, FER1L1, MCM3, MCM7, Spectrin, FWP007, FLC3A, GEC1, Cyclin, DBC1,

ISG56, NEAS, MAP1LC3A, SNU114 homolog, BRR2 homolog, DNMT, ISG54, MCM2, CAD, DHC1, DXS423E, LRP130, BRG1-associated factor 170, LPC2D, MAP1ALC3 and MCM6. As depicted in figure 1, the left side is related to the down-regulated hubs and the right side corresponds to up-regulated hubs.

Further analysis of complex by MCODE revealed 27 subnetworks for the network (see Table 1). The PPI subnetworks correspond to the differently expressed genes made up of highly connected regions in meningioma pattern versus control samples. Four complexes were selected by comparing the complex with our list (see Figure 2). All subnetworks are represented in figure S2. The seed nodes of these complexes included Q5QNR8, Q9Z0E3, P30304, CHEBI:17283, Q15667, Q05397, P61024, J3KPM5, DIP-6092N, P09914, O76075, A1L374, B2RTS1, P23193, Q8TB30, O94979, P40938 and Q6NW02. The gene ontology analysis of four selected subnetworks were identified by MCODE and performed by ClueGO (results are depicted in Figure 3).

Discussion

Since proteins act as complex or collaborate in overlapping pathways, their deregulation results in disorders and diseases such as cancer. Cancer uses pre-existing pathways in different ways or combines some components of these pathways in a new fashion. Molecular mapping of brain cancer is a useful tool for evaluating the pathways.[48,49] In addition, gene clustering based on functions illustrates correlated expression patterns.[50,51] Because of the importance and value of networks in system biology, quantitative tools have been developed in recent years for analyzing the networks. Analyzing the network properties of gene-expression data might reveal the organizational pattern of gene expression in cancer, which might in turn help us to identify new potential drug targets; so, in this study, network is utilized to extract meningioma data. Here, identification of network-based gene expression biomarkers is analyzed by the appropriate software.

As represented in Figure 1, meningioma protein interaction network is made up of numerous nodes with the most degrees as hubs. FN1 is the hub with the most degrees but its altered expression is not very significant. It is expressed ubiquitously in various cell types such as squamous cell carcinomas, neuroblastomas, proliferating hematopoietic progenitor cells, and beta-cells of pancreatic islets of Langerhans.[52]

CDK6 is the next hub protein with its expression suppressed significantly in meningioma cells. Since this protein is over-expressed in some leukemias and malignancies including sarcoma, glioma, breast tumors, lymphoma and melanoma,[53] its expression pattern in meningioma might prove a good diagnosis biomarker in combination with other markers.

Another over-expressed hub is UBE2E1. It plays a role in the Ubl conjugation pathway and different pathways of Reactome databases such as cell cycle, cellular responses to stress, mitotic M-M/G1 phases, Cdc20:Phospho-APC/C mediated degradation of Cyclin A, immune System and APC-Cdc20 mediated degradation of Nek2A.[54] Tracing this protein in the peripheral blood cells could indicate disrupted homeostasis.
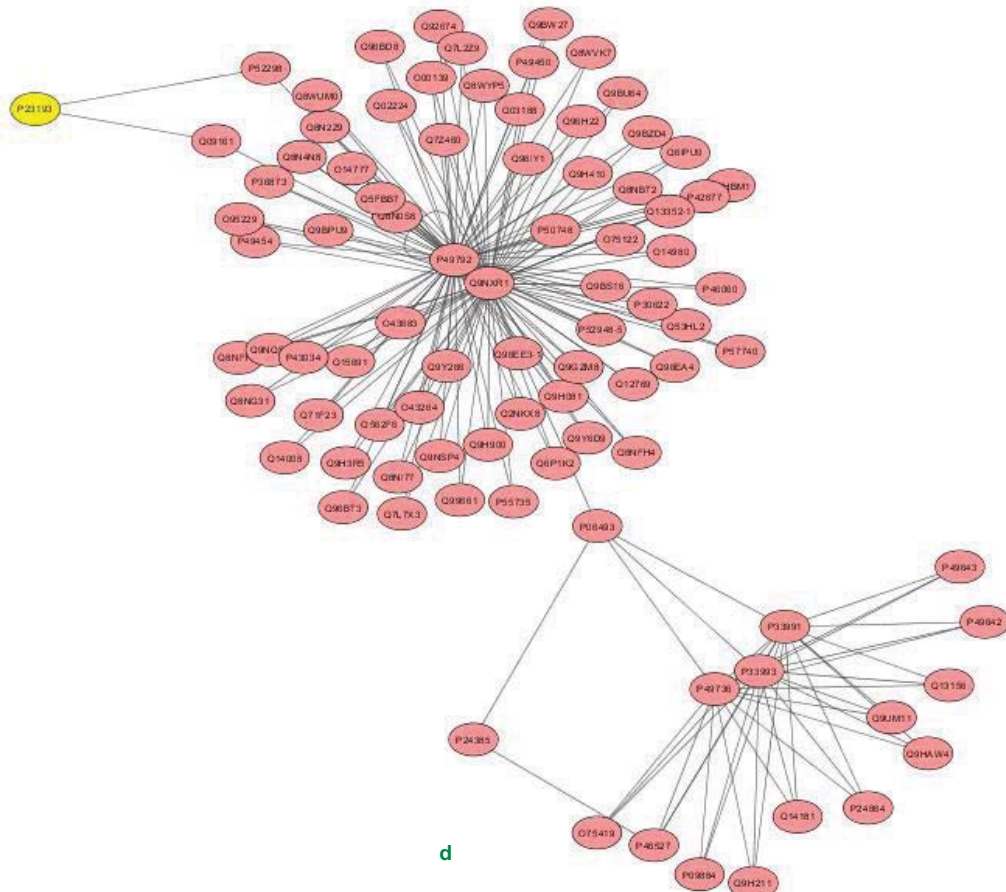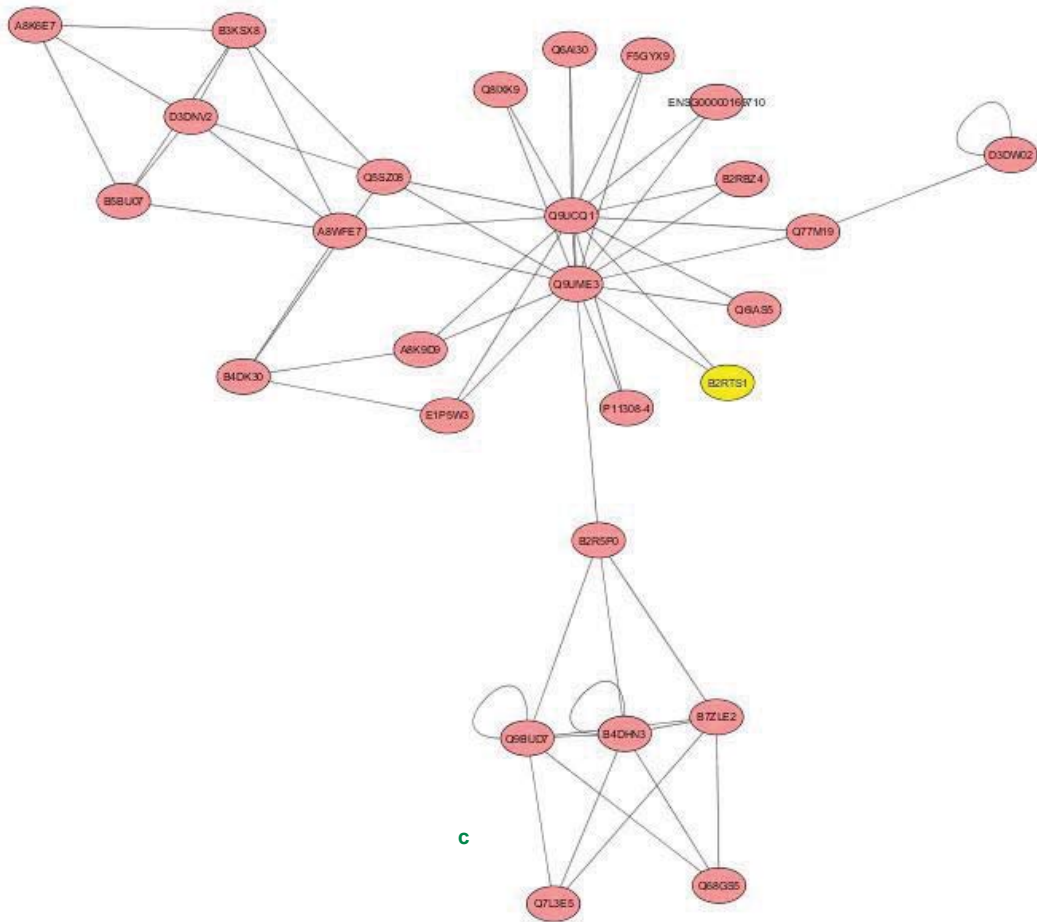
DNA repair protein XRCC5 is the next hub that is up-regulated in meningioma cells. It has a role in chromosome translocation. The XRCC5/6 dimerization apparently leads to stabilizing broken DNA ends and bringing them together. The complex of XRCC5/6 dimer and APEX1 constitutes a negative regulator of transcrip-
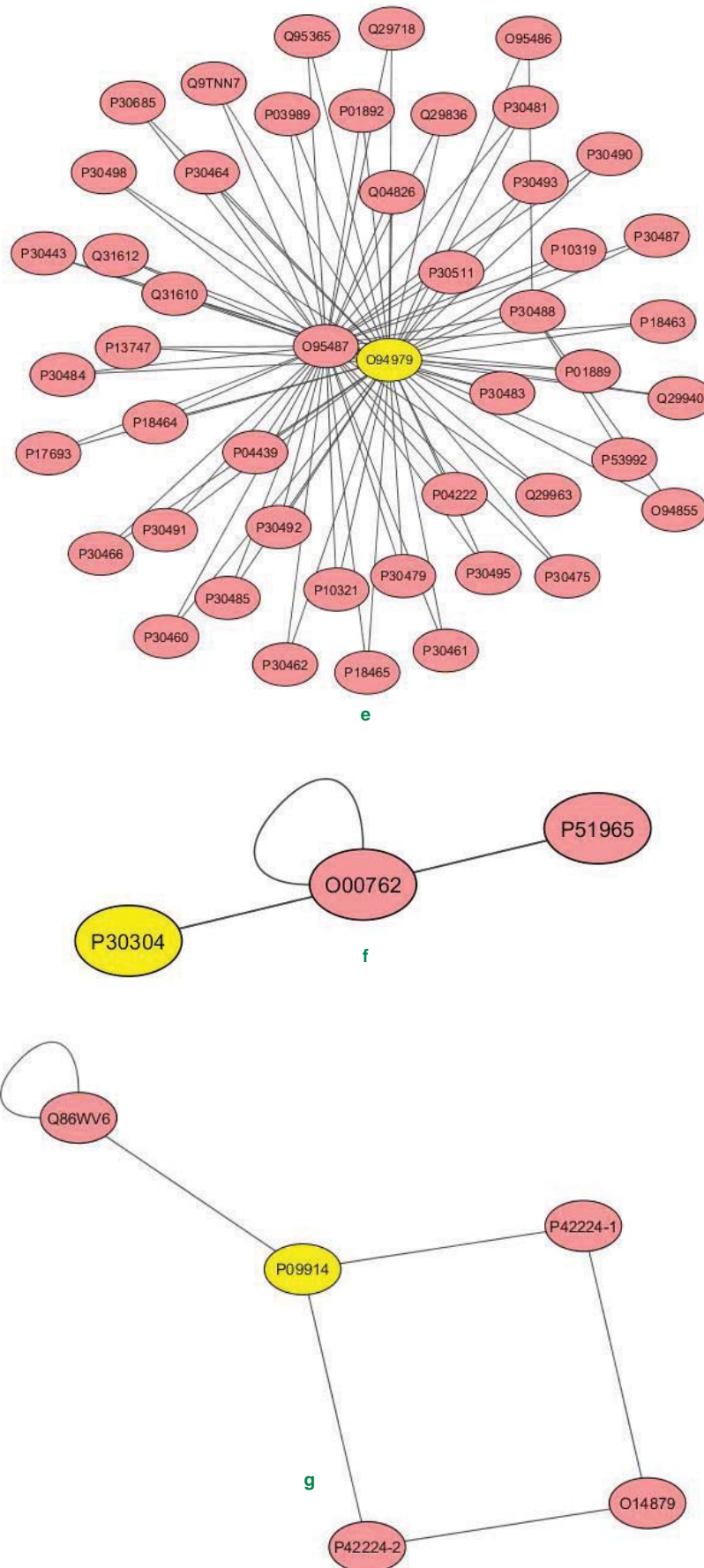
a

b

c

d
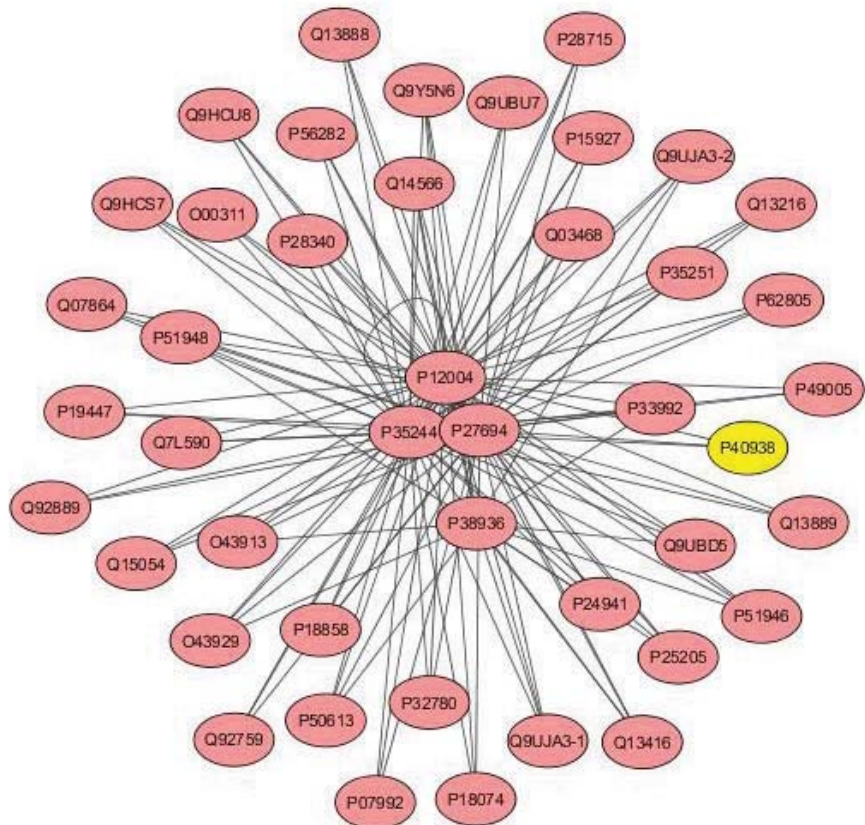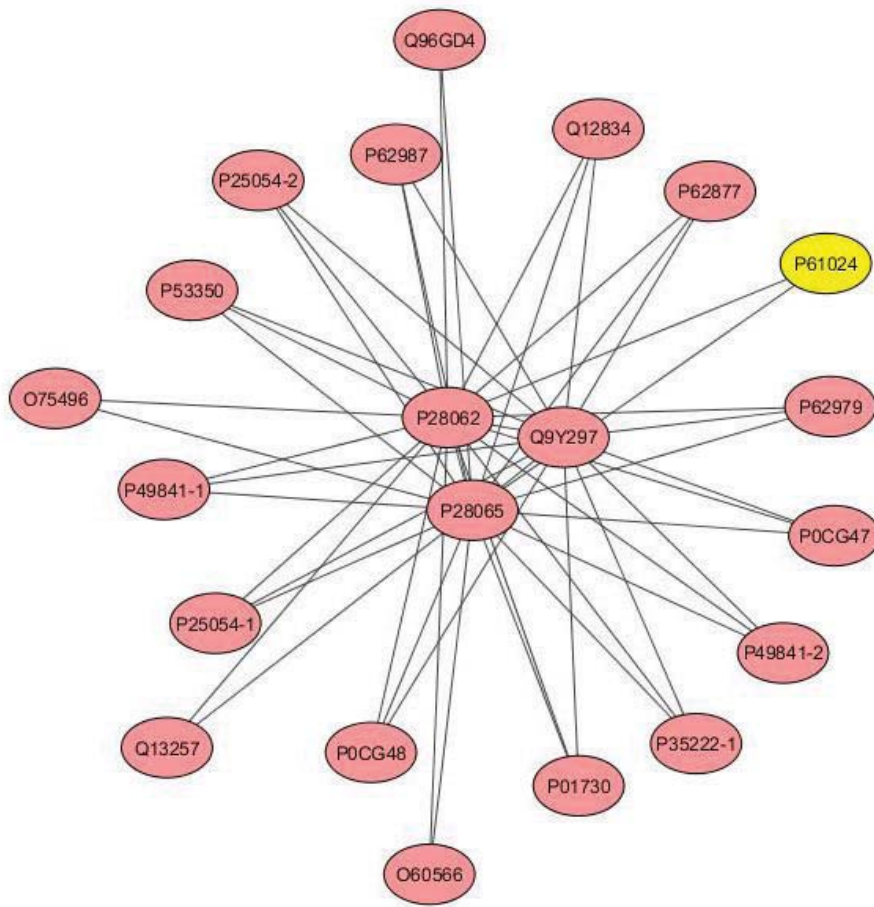
Figure 2. The PPI subnetworks based on the differently expressed genes made up of highly connected regions in meningioma pattern versus control sample. Clusters 6, 10, 20 and 25, whose seed genes are included in our list, are selected and represented as a, b, c and d, respectively. Yellow ellipses represent seed nodes. Pink ellipses represent neighbor nodes. All edges represent directed interactions that MCODE has not considered in result of complexes.

a

b

c
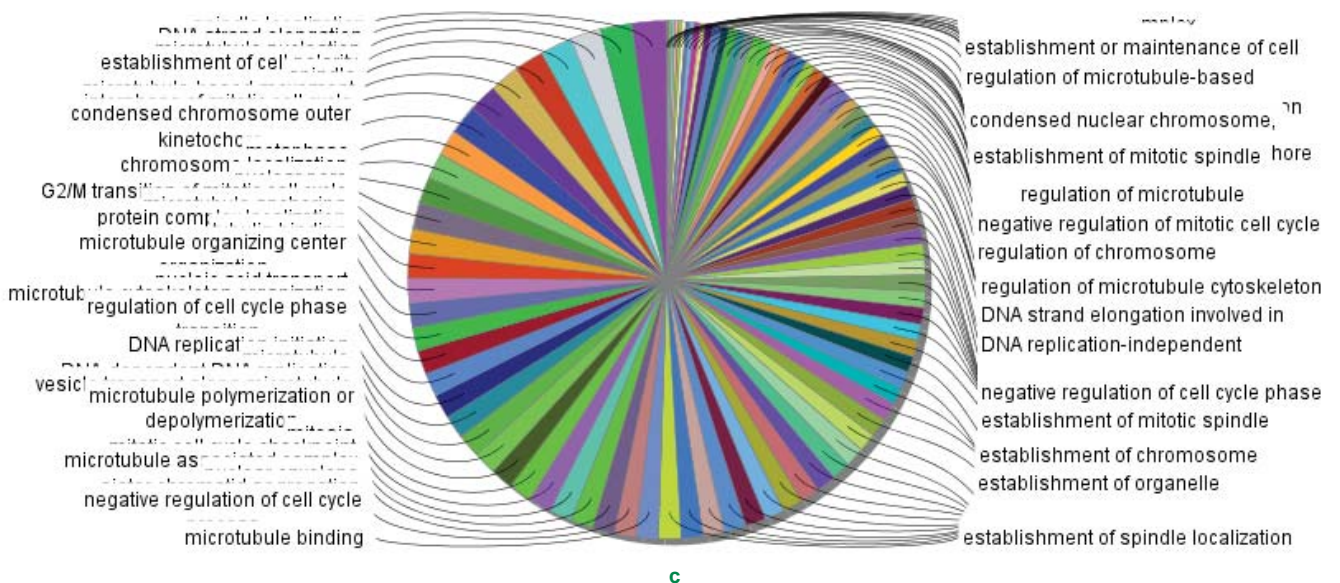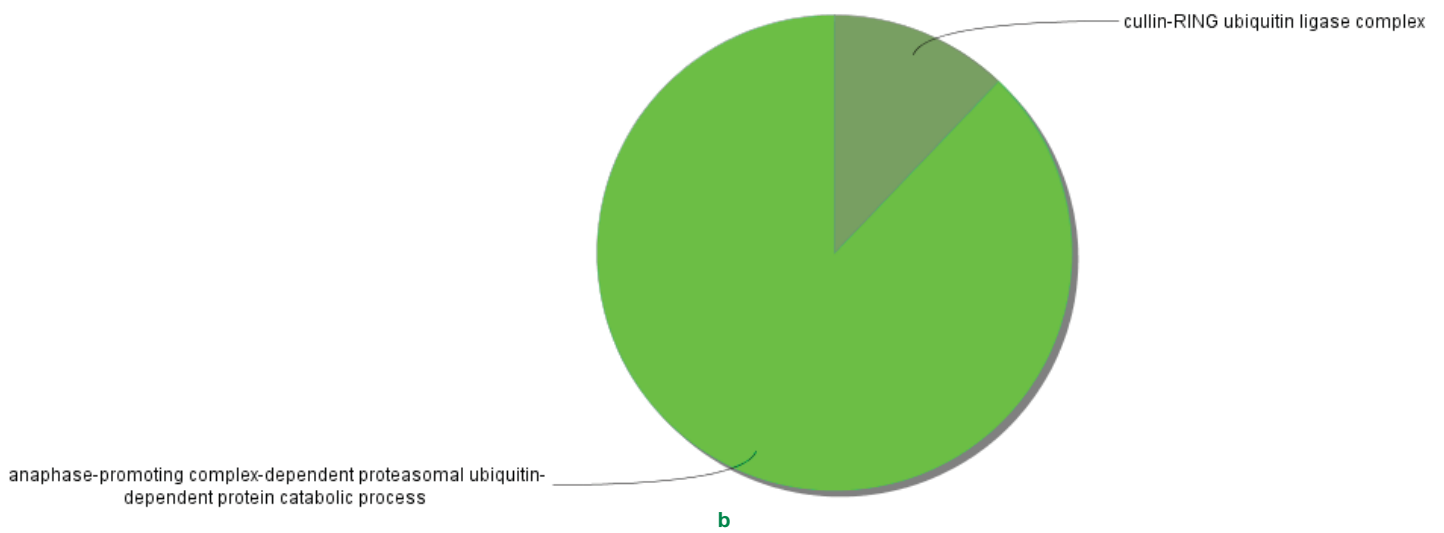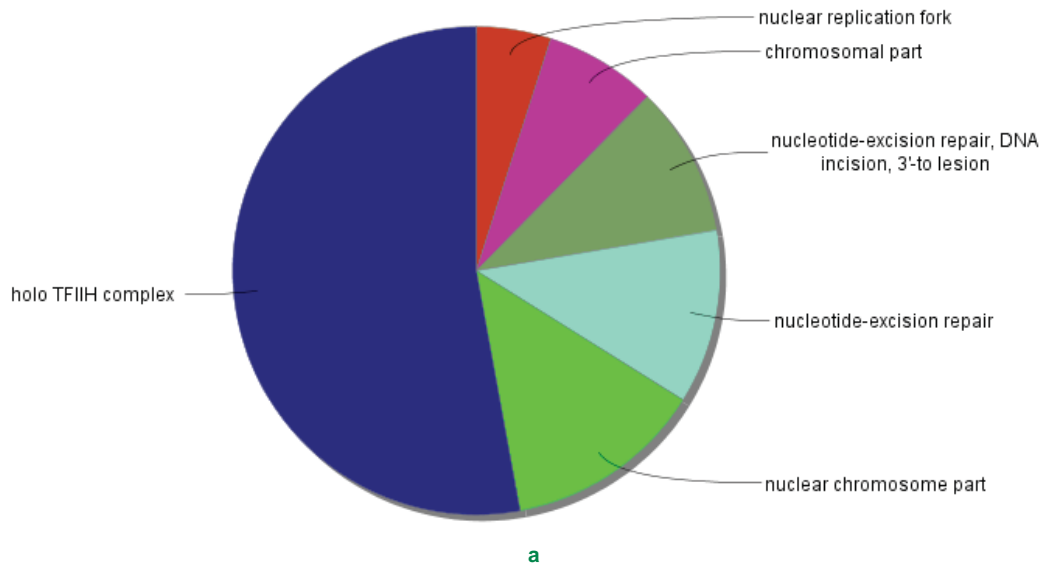
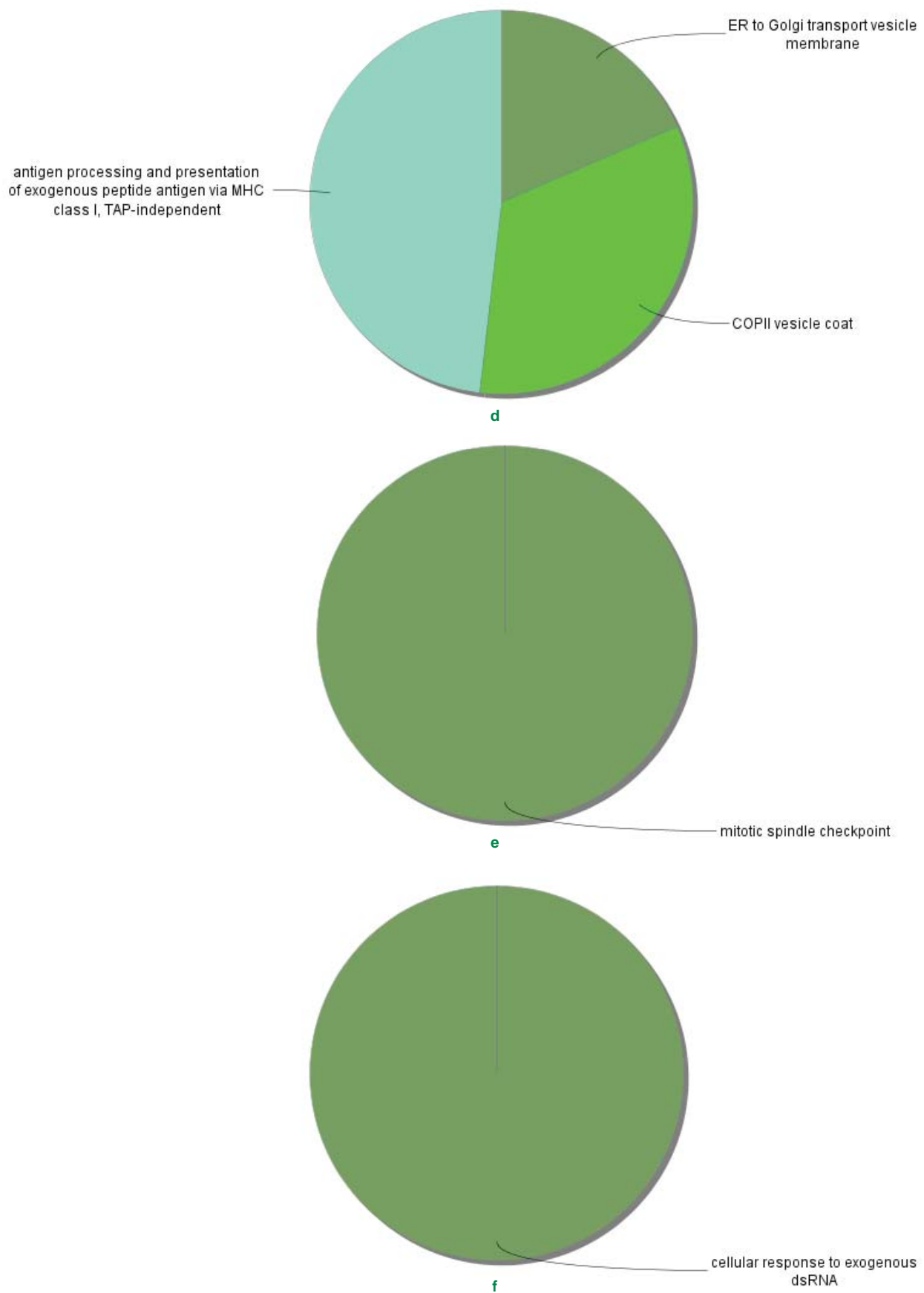**Figure 3.** The functional groups of gene ontology analysis from selected PPI subnetworks of the meningioma network performed by ClueGO. Clusters 6, 10, 20 and 25 are represented as a, b, c and d, respectively.

tion.[55] Its expression increases during promyelocyte differentia-tion[56]; so in meningioma, it may promote repair mechanisms in brain.

From the minichromosome maintenance complex family, MCM2 and MCM7 are two hubs over-expressed in meningioma. They are replicative helicases essential for DNA replication initiation and elongation in eukaryotic cells.[20,57]

Interferon-induced protein with tetratricopeptide repeats 3 (IFIT-3 or ISG60) is highly up-regulated in meningioma. IFN-induced antiviral protein acts as an inhibitor of cellular and viral processes, proliferation, signaling, cell migration and viral replication.[58] In patients with systemic lupus erythematosus, IFIT-3 is expressed significantly higher in peripheral blood mononuclear cells and monocytes (at protein level).[59]

Protein complexes were determined by powerful network analyzers. One of the original methods for subnetwork detection in biological data is the MCODE algorithm.[46] MCODE weights all nodes by local neighborhood density and identifies densely connected seed regions, which are subsequently modified by adding or removing nodes based on a connectivity criterion. MCODE has been widely applied for detection of complexes in protein interaction networks, and is available as a default plugin for the cytoscape network visualization and analytical tool.[60] Many of densely connected regions contribute to known molecular complexes and imply that large amounts of available knowledge are buried in large protein interaction networks. Further study of the complex through analyzing network with MCODE revealed 27 sub-networks described in Table 1. By comparing the complex with our list, we selected four complexes represented in figure 2 and analyzed them based on GO represented in Figure 3. Of seed genes participating in the pathogenesis pathways of meningioma, previously P61024, O76075, Q05397, O94979, Q8TB30, Q6NW02, P23193 were determined as brain tissue proteins,[61] but only four of these seed genes, P30304, P09914, P23193, and O94979 are included in our gene list. P09914[20] and P23193[20] were up-regulated while the expression of P30304[37] and O94979[20] play a down-regulatory role in meningioma.

The first subnetwork a (cluster 6) (Figure 2, 3) with the seed gene, P23193 (TCEA1), transcription elongation factor A1 is newly expressed in meningioma. It is also expressed in brain and some other tissues. It is necessary for efficient RNA polymerase II transcription elongation. It is composed of a transcription regulatory complex formation of UBR5, CDK9, RNAP II, and TFIIS/TCEA1 that can stimulate transcription of genes such as gamma fibrinogen/FGG.[62] The TCEA1-related family is involved in many essential cellular functions, especially positive regulation of the immune system process, nucleotide and nucleic acid metabolic process, regulation of transcription, nitrogen compound metabolic process, multicellular organismal development, metabolic process, biosynthetic process, regulation of gene expression, regulation of macromolecule biosynthetic process, hematopoiesis, myeloid cell differentiation, cell differentiation, erythrocyte differentiation, multicellular organismal process, developmental process, RNA biosynthetic process, erythrocyte homeostasis, and cellular nitrogen compound metabolic process. Up-regulated TCEA1 in meningioma has positive regulation in RBC homeostasis to eliminate erythrocytes in tissue because meningiomas are highly vascularized (before demonstrated increased expression of vascular endothelial growth factor (VEGF) in meningioma tumor).[63] Increased VEGF expression and increased expression

of vascular permeability factor are correlated with increased microvessel density and microcystic morphology of meningiomas.[64]

O94979 (SEC31A), which was down-regulated in meningioma, is expressed in a number of tissues such as blood, brain, epithelium, pancreas, placenta, PNS, spleen, testis and uterine endothelium.[61] This protein is a component of the coat protein complex II (COPII) which promotes the formation of transport vesicles of ER. The physical deformation of the ER membrane into vesicles and selection of cargo molecules are the main functions of coat.[65] The PPI analysis (Figures 2, 3) explained SEC31A in the subnetwork b (cluster 10) as the SEC31A related family is involved in many functional classes such as antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-independent, COPII vesicle coat, ER to Golgi transport vesicle membrane. Therefore, in meningioma, these processes are down-regulated.

The next subnetwork with P30304 (CDC25A) as seed gene operates as a dosage-dependent inducer of mitotic progression. It is a tyrosine protein phosphatase that directly dephosphorylates CDK1 and stimulates its kinase activity. It also dephosphorylates *in vitro* the complex of CDK2 and cyclin E.[66] Victor Martinez-Gleza et al. identified that P30304 is down-regulated in meningioma when compared to normal.[20] Decreasing CDC25A expression might exert an up-regulation effect on production of other cell cycle proteins to promote meningioma. The PPI analysis (Figures 2 and 3) showed that in the subnetwork c (cluster 20), the P30304 related family (P51965 and O00762) is involved only in mitotic spindle checkpoint. Therefore, in meningioma, the regulation of mitotic spindle checkpoint protein in the cell cycle is down-regulated.

The last seed gene is P09914 (IFIT1 or ISG56), interferon-induced protein with tetratricopeptide repeats 1 that was up-regulated in meningioma pathogenesis. The PPI analysis (Figures 2 and 3) revealed as the subnetwork d (cluster 25), the IFIT1 related family is only involved in cellular response to exogenous dsRNA, thereby acting as a sensor of viral single-stranded RNAs and preventing expression of viral messenger RNAs. It exhibits antiviral activity against several viruses including human papilloma and hepatitis C viruses.[67,68] As depicted in figure 1, IFIT1 is also one of the hub proteins; so, this protein might be a putative biomarker. Over the past few decades, many studies have identified pathways within meningioma, examples of which include the RAF-1-MEK-1-MAPK/ERK pathway,[69–71] and the P13K-Akt/protein kinase BP7056 pathway, loss of alkaline phosphatase activity,[70–72] and expression of minichromosome maintenance-2 protein.[20] Recently, Okay Saydam et al. investigated novel potential tumor markers for meningiomas and found that meningioma pathogenesis is associated with various biological functions such as DNA replication, recombination, cell cycle, and apoptosis.[20] Our major findings of pathways-based network, that illustrated difference between the meningioma pattern and normal one, are composed of positive regulation in RBC homeostasis to eliminate erythrocytes in brain, dysregulation of transport from ER to Golgi and antigen processing and presentation of exogenous peptide antigen via MHC class I, disrupted regulation of mitotic spindle checkpoint protein and finally neutralization of exogenous dsRNA. Combination of over-expression of TCEA1, UBE2E1, XRCC5, IFIT1, IFIT-3, MCM2 and MCM7 and under-expression of CDC25A, SEC31A and CDK6 can serve as a diagnostic biomarker panel for meningiomas. To unravel the possible role(s) of these proteins

in meningioma tumorigenesis, further investigations are needed.

References

1. Elster AD, Challa VR, Gilbert TH, Richardson DN, Contento JC. Meningiomas: MR and histopathologic features. *Radiology*. 1989; **170:** 857 – 862.
2. Filippi CG, Edgar MA, Ulug AM, Prowda JC, Heier LA, Zimmerman RD. Appearance of meningiomas on diffusion-weighted images: correlating diffusion constants with histopathologic findings. *AJNR Am J Neuroradiol*. 2001; **22:** 65 – 72.
3. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, et al. The 2007 WHO classification of tumor of the central nervous system. *Acta Neuropathol*. 2007; **114:** 97 – 109.
4. Jaaskelainen J, Haltia M, Servo A. Atypical and anaplastic meningiomas: radiology, surgery, radiotherapy, and outcome. *Surg Neurol*. 1986; **25:** 233 – 242.
5. Barnholtz-Sloan JS, Kruchko C. Meningiomas: causes and risk factors. *Neurosurg Focus*. 2007; **23:** E2.
6. Marosi C, Hassler M, Roessler K, Reni M, Sant M, Mazza E, et al. Meningioma. *Crit Rev Oncol Hematol*. 2008; **67:** 153 – 171.
7. Longstreth WT Jr, Dennis LK, McGuire VM, Drangsholt MT, Koepsell TD. Epidemiology of intracranial Meningioma. *Cancer*. 1993; **72:** 639 – 648.
8. Claus EB, Calvocoressi L, Bondy ML, Schildkraut JM, Wiemels JL, Wrensch M. Dental x-rays and risk of meningioma. *Cancer*. 2012; **118:** 4530 – 4537.
9. Rabin BM, Meyer JR, Berlin JW, Marymount MH, Palka PS, Russell EJ. Radiation-induced changes in the central nervous system and head and neck. *Radiographics*. 1996; **16:** 1055 – 1072.
10. Lee W, Chang KH, Choe G, Chi JG, Chung CK, Kim IH, et al. MR imaging features of clear-cell meningioma with diffuse leptomeningeal seeding. *AJNR Am J Neuroradiol*. 2000; **21:** 130 – 132.
11. Sanverdi SE, Ozgen B, Oguz KK, Mut M, Dolgun A, Soylemezoglu F, et al. Is diffusion-weighted imaging useful in grading and differentiating histopathological subtypes of meningiomas? *Eur J Radiol*. 2012; **81:** 2389 – 2395.
12. Kleihues P, Cavenee WK .*World Health Organization Classification of Tumours. Pathology and genetics of tumours of the nervous system*. 2th ed. Lyon: IARC Press; 2000.
13. Bello MJ, Aminoso C, Lopez-Marin I, Arjona D, Gonzalez- Gomez P, Alonso ME, et al. DNA methylation of multiple promoter-associated CpG islands in meningiomas: relationship with the allelic status at 1p and 22q. *Acta Neuropathol (Berl)*. 2004; **108:** 413 – 421.
14. Liu Y, Pang JC, Dong S, Mao B, Poon WS, Ng HK. Aberrant CpG island hypermethylation profile is associated with atypical and anaplastic meningiomas. *Hum Pathol*. 2005; **36:** 416 – 425.
15. Lekanne Deprez RH, Riegman PH, Groen NA, Warringa UL, van Biezen NA, Molijn AC, et al. Cloning and characterization of MN1, a gene from chromosome 22q11, which is disrupted by a balanced translocation in a meningioma. *Oncogene*. 1995; **10:** 1521 – 1528.
16. Staal FJ, van der Luijt RB, Baert MR, van Drunen J, van Bakel H, Peters E, et al. A novel germline mutation of PTEN associated with brain tumor of multiple lineages. *Br J Cancer*. 2002; **86:** 1586 – 1591.
17. Zattara-Cannoni H, Roll P, Figarella-Branger D, Lena G, Dufour H, Grisoli F, et al. Cytogenetic study of six cases of radiation-induced meningiomas. *Cancer Genet Cytogenet*. 2001; **126:** 81 – 84.
18. Brastianos PK, Horowitz PM, Santagata S, Jones RT, McKenna A, Getz G, et al. Genomic sequencing of meningiomas identifies oncogenic SMO and AKT1 mutations. *Nat Genet*. 2013; **45:** 285 – 289.
19. Sadetzki S, Flint-Richter P, Starinsky S, Novikov I, Lerman Y, Goldman B, et al. Genotyping of patients with sporadic and radiation- associated meningiomas. *Cancer Epidemiol Biomarkers Prev*. 2005; **14:** 969 – 976.
20. Saydam O, Senol O, Schaaij-Visser T, Pham TV, Piersma SR, Stemmer-Rachamimov AO, et al. Comparative protein profiling reveals minichromosome maintenance (MCM) proteins as novel potential tumor markers for meningiomas. *J Proteome Res*. 2010; **9:** 485 – 494.
21. Tummalapalli P, Gondi CS, Dinh DH, Gujrati M, Rao JS. RNA interference-mediated targeting of urokinase plasminogen activator receptor and matrix metalloproteinase-9 gene expression in the IOMM-Lee malignant meningioma cell line inhibits tumor growth, tumor cell invasion and angiogenesis. *Int J Oncol*. 2007; **31:** 5 – 17.
22. Martinez-Glez V, Franco-Hernandez C, Alvarez L, De Campos JM, Isla A, Vaquero J, et al. Meningiomas and schwannomas: mo-lecular subgroup classification found by expression arrays. *Int J Oncol*. 2009; **34:** 493 – 504.
23. Okamoto H, Li J, Vortmeyer AO, Jaffe H, Lee YS, Gläsker S, et al. Comparative proteomic profiles of meningioma subtypes. *Cancer Res*. 2006; **66:** 10199 – 10204.
24. Baines AJ, Lu HC, Bennett PM. The Protein 4.1 family: Hub proteins in animals for organizing membrane proteins. *Biochim Biophys Acta*. 2014; **1838:** 605 – 619.
25. Laurendeau I, Ferrer M, Garrido D, D'Haene N, Ciavarelli P, Basso A, et al. Gene expression profiling of ErbB receptors and ligands in human meningiomas. *Cancer Invest*. 2009; **27:** 691 – 698.
26. Das A, Tan WL, Smith DR. p53 point mutation is rare in meningiomas from Singaporean patients. *Asian J Surg*. 2005; **28:** 7 – 10.
27. Siddique K, Yanamandra N, Gujrati M, Dinh D, Rao JS, Olivero W. Expression of matrix metalloproteinases, their inhibitors, and urokinase plasminogen activator in human meningiomas. *Int J Oncol*. 2003; **22:** 289 – 294.
28. Mawrin C, Sasse T, Kirches E, Kropf S, Schneider T, Grimm C, et al. Different activation of mitogen-activated protein kinase and Akt signaling is associated with aggressive phenotype of human meningiomas. *Clin Cancer Res*. 2005; **11:** 4074 – 4082.
29. Watson MA, Gutmann DH, Peterson K, Chicoine MR, Kleinschmidt-DeMasters BK, Brown HG, et al. Molecular characterization of human meningiomas by gene expression profiling using high-density oligonucleotide microarrays. *Am J Pathol*. 2002; **161:** 665 – 672.
30. Wrobel G, Roerig P, Kokocinski F, Neben K, Hahn M, Reifenberger G, et al. Microarray-based gene expression profiling of benign, atypical and anaplastic meningiomas identifies novel genes associated with meningioma progression. *Int J Cancer*. 2005; **114:** 249 – 256.
31. Hankins GR, Sasaki T, Lieu AS, Saulle D, Karimi K, Li JZ, et al. Identification of the deleted in liver cancer 1 gene, DLC1, as a candidate meningioma tumor suppressor. *Neurosurgery*. 2008; **63:** 771 – 780.
32. Arnold SA, Brekken RA. SPARC: a matricellular regulator of tumorigenesis. *J Cell Commun Signal*. 2009; **3:** 255 – 273.
33. Murphy M, Pykett MJ, Harnish P, Zang KD, George DL. Identification and characterization of genes differentially expressed in meningiomas. *Cell Growth Differ*. 1993; **4:** 715 – 722.
34. Aruga J, Nozaki Y, Hatayama M, Odaka YS, Yokota A. Expression of ZIC family genes in meningiomas and other brain tumors. *BMC Cancer*. 2010; **10:** 79.
35. Laurendeau I, Ferrer M, Garrido D, D'Haene N, Ciavarelli P, Basso A, et al. Gene expression profiling of the hedgehog signaling pathway in human meningiomas. *Mol Med*. 2010; **16:** 262 – 270.
36. Terzia A, Saglama A, Barak A, Soylemezoglu F. The significance of immunohistochemical expression of Ki-67, p53, p21, and p16 in meningiomas tissue arrays. *Pathol Res Pract*. 2008; **204:** 305 – 314.
37. MartInez-Gleza V, Alvareza L, Franco-Hernandeza C, Torres-Martina M, de Camposb JM, Islac A, et al. Genomic deletions at 1p and 14q are associated with an abnormal cDNA microarray gene expression pattern in meningiomas but not in schwannomas. *Cancer Genet Cytogenet*. 2010; **196:** 1 – 6.
38. von Randowa AJ, Schindlerc S, Tews DS. Expression of extracellular matrix-degrading proteins in classic, atypical, and anaplastic meningiomas. *Pathol Res Pract*. 2006; **202:** 365 – 372.
39. Di Vinci A, Brigati C, Casciano I, Banelli B, Borzi L, Forlani A, et al. HOXA7, 9, and 10 are methylation targets associated with aggressive behavior in meningiomas. *Transl Res*. 2012; **160:** 355 – 362.
40. Piaskowski S, Rieske P, Szybka M, Wozniak K, Bednarek A, Płuciennik E, et al. GADD45A and EPB41 as tumor suppressor genes in meningioma pathogenesis. *Cancer Genet Cytogenet*. 2005; **162:** 63 – 67.
41. Muller P, Henn W, Niedermayer I, Ketter R, Feiden W, Steudel WI, et al. Deletion of chromosome 1p and loss of expression of alkaline phosphatase indicate progression of meningiomas. *Clin Cancer Res*. 1999; **5:** 3569 – 3577.
42. Gutmann DH, Donahoe J, Perry A, Lemke N, Gorse K, Kittiniyom K, et al. Loss of DAL-1, a protein 4.1-related tumor suppressor, is an important early event in the pathogenesis of meningiomas. *Hum Mol Genet*. 2000; **9:** 1495 – 1500.
43. Robb VA, Li W, Gascard P, Perry A, Mohandas N, Gutmann DH. Identification of a third Protein 4.1 tumor suppressor, Protein 4.1R, in meningioma pathogenesis. *Neurobiol Dis*. 2003; **13:** 191 – 202.
44. Real-Chicharro A, Ruiz-Mostazo I, Navas-Delgado I, Kerzazi A, Chniber O, Sánchez-Jiménez F, et al. Protopia: a protein-protein interaction tool. *BMC Bioinformatics*. 2009; **10:** S17.
45. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et

al. Cytoscape: a software environment for integrated models of bio-molecular interaction networks. *Genome Res*. 2003; **13:** 2498 – 2504.

46. 45. Bader JD, Hogue CW. An automated method for finding molecular complexes in large protein interaction networks. *BMC Bioinformatics*. 2003; **4:** 2.

47. Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, et al. ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology annotation networks. *Bioinformatics*. 2009; **25:** 1091 – 1093.

48. Whitfield ML, Sherlock G, Saldanha AJ, Murray JI, Ball CA, Alexander KE, et al. Identification of genes periodically expressed in the human cell cycle and their expression in tumors. *Mol Biol Cell*. 2002; **13:** 1977 – 2000.

49. Ficenec D, Osborne M, Pradines J, Richards D, Felciano R, Cho RJ, et al. Computational knowledge integration in biopharmaceutical research. *Brief Bioinform*. 2003; **4:** 260 – 278.

50. Bergmann S, Ihmels J, Barkai N. Similarities and differences in genome-wide expression data of six organisms. *PLoS Biol*. 2004; **2:** E9.

51. Stuart JM, Segal E, Koller D, Kim SK. A genecoexpression network for global discovery of conserved genetic modules. *Science*. 2003; **302:** 249 – 255.

52. Meyerson M, Harlow E. Identification of G1 kinase activity for cdk6, a novel cyclin D partner. *Mol Cell Biol*. 1994; **14:** 2077 – 2086.

53. Fiaschi-Taesch NM, Salim F, Kleinberger J, Troxell R, Cozar-Castellano I, Selk K, et al. Induction of human beta-cell proliferation and engraftment using a single G1/S regulatory molecule, cdk6. *Diabetes*. 2010; **59:** 1926 – 1936.

54. David Y, Ziv T, Admon A, Navon A. The E2 ubiquitin-conjugating enzymes direct polyubiquitination to preferred lysines. *J Biol Chem*. 2010; **285:** 8595 – 8604.

55. Tuteja N, Tuteja R, Ochem A, Taneja P, Huang NW, Simoncsits A, et al. Human DNA helicase II: a novel DNA unwinding enzyme identified as the Ku autoantigen. *EMBO J*. 1994; **13:** 4991 – 5001.

56. Oderwald H, Hughes MJ, Jost JP. Non-histone protein 1 (NHP1) is a member of the Ku protein family which is upregulated in differentiating mouse myoblasts and human promyelocytes. *FEBS Lett*. 1996; **382:** 313 – 318.

57. Todorov IT, Pepperkok R, Philipova RN, Kearsey SE, Ansorge W, Werner D. A human nuclear protein with sequence homology to a family of early S phase proteins is required for entry into S phase and for cell division. *J Cell Sci*. 1994; **107:** 253 – 265.

58. Xiao S, Li D, Zhu HQ, Song MG, Pan XR, Jia PM, et al. RIG-G as a key mediator of the antiproliferative activity of interferon-related pathways through enhancing p21 and p27 proteins. *Proc Natl Acad Sci USA*. 2006; **103:** 16448 – 16453.

59. Huang X, Shen N, Bao C, Gu Y, Wu L, Chen S. Interferon-induced protein IFIT4 is associated with systemic lupus erythematosus and promotes differentiation of monocytes into dendritic cell-like cells.

Arthritis Res Ther. 2008; **10:** R91.

60. Killcoyne S, Carter GW, Smith J, Boyle J. Cytoscape: a community-based framework for network modeling. *Methods Mol Biol*. 2009; **563:** 219 – 239.

61. da Huang W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc*. 2009; **4:** 44 – 57.

62. Cojocaru M, Bouchard A, Cloutier P, Cooper JJ, Varzavand K, Price DH, et al. Transcription factor IIS cooperates with the E3 ligase UBR5 to ubiquitinate the CDK9 subunit of the positive transcription elongation factor B. *J Biol Chem*. 2011; **286:** 5012 – 5022.

63. Yoshioka H, Hama S, Taniguchi E, Sugiyama K, Arita K, Kurisu K. Peritumoral brain edema associated with meningioma: influence of vascular endothelial growth factor expression and vascular blood supply. *Cancer*. 1999; **85:** 936 – 944.

64. Christov C, Lechapt-Zalcman E, Adle-Biassette H, Nachev S, Gherardi RK. Vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) and its receptor flt-1 in microcystic meningiomas. *Acta Neuropathol (Berl)*. 1999; **98:** 414 – 420.

65. Tang BL, Zhang T, Low DYH, Wong ET, Horstmann H, Hong W. Mammalian homologues of yeast sec31p. An ubiquitously expressed form is localized to endoplasmic reticulum (ER) exit sites and is essential for ER-Golgi transport. *J Biol Chem*. 2000; **275:** 13597 – 13604.

66. Falck J, Mailand N, Syljuaasen RG, Bartek J, Lukas J. the ATM-Chk2-Cdc25A checkpoint pathway guards against radioresistant DNA synthesis. *Nature*. 2001; **410:** 842 – 847.

67. Terenzi F, Saikia P, Sen GC. Interferon-inducible protein, P56, inhibits HPV DNA replication by binding to the viral protein E1. *EMBO J*. 2008; **27:** 3311 – 3321.

68. Li Y, Li C, Xue P, Zhong B, Mao AP, Ran Y, et al. ISG56 is a negative-feedback regulator of virus-triggered signaling and cellular antiviral response. *Proc Natl Acad Sci USA*. 2009; **106:** 7945 – 7950.

69. Wang JL, Nister M, Hermansson M, Westermark B, Ponten J. Expression of PDGF beta-receptors in human meningioma cells. *Int J Cancer*. 1990; **46:** 772 – 778.

70. Johnson MD, Woodard A, Kim P, Frexes-Steed M. Evidence for mitogen-associated protein kinase activation and transduction of mitogenic signals by platelet-derived growth factor in human meningioma cells. *J Neurosurg*. 2001; **94:** 293 – 300.

71. Johnson M, Toms S. Mitogenic signal transduction pathways in meningiomas: novel targets for meningioma chemotherapy? *J Neuropathol Exp Neurol*. 2005; **64:** 1029 – 1036.

72. Johnson MD, Okedli E, Woodard A, Toms SA, Allen GS. Evidence for phosphatidylinositol 3-kinase-Akt-p7S6K pathway activation and transduction of mitogenic signals by platelet-derived growth factor in meningioma cells. *J Neurosurg*. 2002; **97:** 668 – 675.