Gene expression profiling analysis of MENX-associated rat pituitary adenomas contributes to understand molecular mechanisms of human pituitary adenomas

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Abstract. The present study aimed to screen potential genes associated with pituitary adenomas to obtain further understanding with regard to the pathogenesis of pituitary adenomas. The microarray GSE23207 dataset, containing 16 pituitary adenoma samples from multiple endocrine neoplasia syndrome-associated rats and 5 normal pituitary tissue samples, was downloaded from Gene Expression Omnibus. The Linear Models for Microarray Data package was used to identify the differentially-expressed genes (DEGs) with the cut-off criteria of a $|\log_2$fold change (FC)$|>1$ and adjusted P-values of <0.05. The potential functions of the DEGs were predicted by functional and pathway enrichment analysis with the Database for Annotation, Visualization and Integrated Discovery. Furthermore, the interaction associations of the up- and downregulated DEGs obtained from the Search Tool for the Retrieval of Interacting Genes database were respectively revealed by the protein-protein interaction networks visualized with Cytoscape. A total of 391 upregulated and 238 downregulated DEGs in were screened in the pituitary adenoma samples. The upregulated DEGs with a higher degree in the protein-protein interaction network (e.g., *CCNA2*, *CCNB1* and *CDC20*) were significantly involved in cell cycle and cell division. Notably, *PTTG1* was enriched in every functional term. These DEGs interacted with each other. The downregulated DEGs (e.g., *GABRA1*, *GABRA4* and *GABRB1*) also interacted with each other, and were relevant to neuroactive ligand-receptor interaction; the DEG *POU1F1*, interacting with *POMC*, was correlated with the development of the pituitary gland, adenohypophysis and endocrine system. Certain DEGs, including *CCNB1*, *CCNA2*, *CDC20*, *GABRA1*, *GABRA4*, *GABRB1*, *POU1F1* and *POMC*, and particularly *PTTG1*, were shown to be closely involved in the pathogenesis of pituitary adenomas.

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Introduction

Pituitary adenomas, accounting for ~15% of all diagnosed intracranial tumors, are benign monoclonal adenomas that originate from cells of the anterior pituitary gland (1). Surgical resection, with or without adjuvant radiotherapy, is always the first line of treatment for the majority of pituitary adenomas, with the exception of prolactinomas (2). However, these treatments cannot usually control invasive pituitary adenomas due to the limited understanding of the underlying molecular mechanisms. Thereby, further research into the tumorigenesis will contribute to identifying novel therapeutic targets, which will be conductive to the development of novel therapeutic approaches for pituitary adenomas.

In past years, considerable progress has been made in identifying the key players in pituitary adenomas. A previous study has shown that the phosphoinositide 3-kinase/AKT signaling pathway is activated and enhanced in pituitary adenomas, which may be due to the mutation and amplification of an oncogene, *PIK3CA* (3). Mutation in another oncogene, *GNAS*, which encodes the guanine nucleotide-activating α subunit has also been suggested to be involved in pituitary hyperplasia (4). Meanwhile, a tumor suppressor aryl hydrocarbon receptor-interacting protein has been demonstrated to function in modulating cellular signaling and cAMP signaling pathways via regulation of the localization of the aryl hydrocarbon receptor (5). Also, the absence of expression of another two tumor suppressors, growth arrest and DNA-damage-inducible β (GADD45β) and γ (GADD45γ), has been observed in human pituitary adenomas (6,7). Aberrant methylation of a number of genes, such as *DAPK* (8) and *FGFR2* (9) has been confirmed to have a momentous role in pituitary tumorigenesis. Additionally, certain cell cycle regulators, such as p16,

p21, p27, cyclin D1 and cyclin E, have also been demonstrated to function in pituitary tumorigenesis (8,10). Recently, certain microRNAs (miRNA/miR) have been found to be crucial in pituitary adenomas. For instance, the expression levels of miR-431 and miR-770-5p have been found to be slightly higher in non-functioning pituitary adenomas compared to their levels in the normal pituitary gland (11). Recently, another study has shown that miRNA-dependent impairment of the HMGA/E2F1 pathway functions as pro-oncogene signaling in pituitary adenomas. Several miRNAs targeting *HMGA2* (miR-326, miR-570 and miR-432) or *E2F1* (miR-326 and miR-603) could inhibit the growth of pituitary cell lines (HP75 and GH3) (12).

Lee *et al* demonstrated that gonadotroph adenomas in MENX-affected rats closely resemble their human counterparts (13). The study further found that *CYP11A1* and *NUSAP1*, two commonly dysregulated differentially-expressed genes (DEGs) in the gonadotroph adenomas of rats and humans, are upregulated in 77 and 95% of human gonadotroph adenomas, respectively. Using the microarray data deposited by Lee *et al*, the present study aimed to further identify genes that were differentially expressed between pituitary adenomas samples and normal controls. Following Gene Ontology (GO) functional and pathway enrichment analysis of the screened DEGs, Protein-Protein Interaction (PPI) networks were constructed for the up- and downregulated DEGs, respectively, in order to learn more about the interaction of proteins encoded by DEGs, which may aid in our understanding of the molecular mechanisms of pituitary adenomas. The results are expected to assist in elucidating the etiology of pituitary adenomas, and provide novel insights for the clinical diagnosis of this disease.

Materials and methods

Affymetrix microarray data. The gene expression profile data of GSE23207 (13) were acquired from Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/), which was based on the platform of the GPL6247 [RaGene-1_0-st] Affymetrix Rat Gene 1.0 ST Array. This dataset contains 16 samples of pituitary homozygous mutants (p27Kip1/Cdknb1) from MENX-associated rats, aged 7-8 months, with large tumors 1-2 mm in size, and 5 samples of normal pituitary tissues purchased from BioChain Inc. (Hayward, CA, USA).

Data preprocessing and screening of DEGs. CEL files and probe annotation files were downloaded, and the gene expression data of all the samples were preprocessed via the Robust Multichip Averaging background correction (14), quantile normalization and probe summarization methods using the Oligo package (15). The Linear Models for Microarray Data package (16) of R was used for the identification of genes that were significantly differentially expressed in pituitary adenomas samples. The raw P-value was adjusted by the Benjamin and Hochberg method (17), and only the genes meeting the cut-off criteria of a $|\log_2$fold change (FC)$|$ of >1 and an adjusted P-value of <0.05 were selected as DEGs.

GO and pathway enrichment analysis. The Database for Annotation, Visualization and Integrated Discovery (DAVID) gene functional classification tool now provides a comprehensive set of novel and powerful tools for researchers to understand the biological meaning behind abundant genes (18). Pathway enrichment analysis was conducted to identify the significant metabolic pathways for the DEGs (19). P<0.05 and a count number of >2 were used as the cut-off criteria for GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses by DAVID.

PPI network construction. The Search Tool for the Retrieval of Interacting Genes database was used to analyze the PPIs for DEGs by calculating the combined scores (20), and a score >0.4 was chosen as the cut-off. Next, PPI networks for up- and downregulated DEGs were visualized using Cytoscape (http://cytoscape.org/) (21). The highly connected nodes (hub proteins) were detected by calculating the degree of each node protein based on the scale-free property of interaction networks (22).

Results

Identification of DEGs. Based on the cut-off criteria, a total of 629 DEGs were screened from the pituitary adenomas samples, including 391 upregulated and 238 downregulated DEGs.

Enrichment analysis of up- and downregulated DEGs. According to GO functional annotation, the upregulated DEGs were mainly enriched in GO terms associated with the cell cycle and cell division. For example, DEGs such as *CCNA2*, *NUSAP1*, *CCNB1*, *CENPF*, *CDC20* and *SPC25* were significantly involved in the cell cycle ($P=1.08\times10^{-12}$); *NUSAP1*, *CENPF*, *SPC25*, *CDC20* and *CCNB1* were involved in the M phase ($P=3.09\times10^{-11}$); and DEGs such as *CCNB1*, *SPC25*, *TOP2A*, *CDC20* and *CCNA2* were correlated with cell division ($P=1.25\times10^{-7}$). Notably, *PTTG1* was found to be enriched in every GO term (Table I). The downregulated DEGs, such as *KCND3*, *GABRA1*, *GABRA4* and *GABRB1*, were markedly associated with ion transport ($P=6.05\times10^{-7}$); DEGs such as *KCNJ5*, *KCND3*, *KCNJ6* and *KCNT2* were relevant to metal ion transport ($P=3.87\times10^{-6}$) and potassium ion transport ($P=7.01\times10^{-5}$); DEGs such as *DRD2*, *POU1F1* and *GHRHR* were distinctly associated with the positive regulation of multicellular organism growth ($P=2.47\times10^{-4}$), pituitary gland development ($P=5.70\times10^{-4}$), adenohypophysis development ($P=8.52\times10^{-4}$), diencephalon development ($P=1.24\times10^{-3}$) and endocrine system development ($P=6.09\times10^{-3}$); and DEGs such as *NOTCH2*, *ERBB4* and *POU1F1* were involved in cell fate commitment ($P=3.22\times10^{-2}$) and the regulation of cell proliferation ($P=4.55\times10^{-2}$) (Table II).

According to the KEGG pathway enrichment analysis, the upregulated DEGs were mainly enriched in 10 pathways. For example, *CDC20*, *CCNB1*, *CCNB2*, *BUB1*, *CDKN1A* and *MCM3* were enriched in the pathway of the cell cycle ($P=5.01\times10^{-7}$); *CDC20*, *CCNB1*, *CCNB2*, *BUB1* and *PLK1* were distinctly enriched in the pathway of oocyte meiosis ($P=1.058\times10^{-3}$); *CCNB1*, *CCNB2*, *CCNA2*, *BUB1* and *PLK1* were significantly enriched in the pathway of progesterone-mediated oocyte maturation ($P=1.150\times10^{-3}$); and *CDKN1A*, *CCNB1*, *CCNB2* and *CASP8* were markedly enriched in the p53 signaling pathway ($P=3.4841\times10^{-2}$) (Table III). Meanwhile, the downregulated

Table I. Top two enriched GO biological process term clusters with the highest enrichment score for the upregulated differentially-expressed genes.

Enrichment score	Term	Description	Count	P-value	Genes
7.432	GO:0007049	Cell cycle	31	1.08×10^{-12}	*SPC25, CCNA2, CENPF, NUSAP1, CDC20, NDC80, CCNB1, PTTG1, CCNB2, BUB1B…*
	GO:0022403	Cell cycle phase	23	1.10×10^{-12}	*NUSAP1, CENPF, NDC80, CDC20, CCNB1, PTTG1, SPC25, CDKN1A, PLK1, BUB1B…*
	GO:0022402	Cell cycle process	27	3.26×10^{-12}	*PTTG1, SPC25, CDKN2C, CENPF, NUSAP1, CDC20, NDC80, CCNB1, CDKN1C, BUB1B…*
	GO:0000279	M phase	19	3.09×10^{-11}	*NUSAP1, CENPF, NDC80, CDC20, PTTG1, CCNB1, SPC25, PLK1, BUB1B, SKA3…*
	GO:0051301	Cell division	14	1.25×10^{-7}	*NUSAP1, CDC20, PTTG1, CCNB1, SPC25, CCNB2, PLK1, BUB1B, TOP2A, CCNA2…*
	GO:0000087	M phase of mitotic cell cycle	12	2.16×10^{-7}	*CCNB1, SPC25, PLK1, NUF2, NUSAP1, BUB1B, SKA3, CENPF, CDC20, PTTG1…*
	GO:0000278	Mitotic cell cycle	16	5.40×10^{-7}	*NUSAP1, CENPF, NDC80, CDC20, PTTG1, CCNB1, SPC25, CDKN1A, CDKN2C, BUB1B…*
	GO:0000280	Nuclear division	10	1.09×10^{-5}	*CCNB1, SPC25, KIF11, PLK1, NUF2, NUSAP1, BUB1B, SKA3, CDC20, PTTG1*
	GO:0007067	Mitosis	10	1.09×10^{-5}	*CCNB1, SPC25, KIF11, PLK1, NUF2, NUSAP1, BUB1B, SKA3, CDC20, PTTG1*
	GO:0048285	Organelle fission	10	1.74×10^{-5}	*CCNB1, SPC25, KIF11, PLK1, NUF2, NUSAP1, BUB1B, SKA3, CDC20, PTTG1*
	GO:0051439	Regulation of ubiquitin-protein ligase activity during mitotic cell cycle	4	4.86×10^{-2}	*CCNB1, PLK1, BUB1B, CDC20*
5.335	GO:0000279	M phase	19	3.09×10^{-11}	*NUSAP1, CENPF, NDC80, CDC20, PTTG1, CCNB1, SPC25, PLK1, BUB1B, SKA3…*
	GO:0051327	M phase of meiotic cell cycle	7	2.32×10^{-4}	*ADCY3, KIF2C, MKI67, PLK1, SGOL2, PTTG1, RAD51*
	GO:0007126	Meiosis	7	2.32×10^{-4}	*ADCY3, KIF2C, MKI67, PLK1, SGOL2, PTTG1, RAD51*
	GO:0051321	Meiotic cell cycle	7	2.76×10^{-4}	*ADCY3, KIF2C, MKI67, PLK1, SGOL2, PTTG1, RAD51*

GO, gene ontology.

Table II. Top two enriched GO biological process term clusters with the highest enrichment score for the downregulated differentially-expressed genes.

Enrichment score	Term	Description	Count	P-value	Genes
4.727	GO:0006811	Ion transport	16	6.05×10^{-7}	*KCND3, GABRA1, GABRA4, GABRB2, GABRB1, CACNG6, ATP2B4, KCNT2, KCNH7, CACNA1A……*
	GO:0030001	Metal ion transport	12	3.87×10^{-6}	*KCNJ5, KCND3, KCNJ6, ATP2B4, CACNG6, KCNH7, KCNH8, SCNN1G, KCNJ3, CACNA1A……*
	GO:0006812	Cation transport	12	2.54×10^{-5}	*KCNJ5, KCND3, KCNJ6, ATP2B4, CACNG6, KCNH7, KCNH8, SCNN1G, KCNJ3, CACNA1A……*
	GO:0006813	Potassium ion transport	7	7.01×10^{-5}	*KCNJ5, KCND3, KCNJ6, KCNT2, KCNH7, KCNH8, KCNJ3*
	GO:0015672	Monovalent inorganic cation transport	8	5.52×10^{-4}	*KCNJ5, KCND3, KCNJ6, KCNT2, KCNH7, KCNH8, SCNN1G, KCNJ3*
2.321	GO:0040018	Positive regulation of multicellular organism growth	4	2.47×10^{-4}	*GH1, DRD2, POU1F1, GHRHR*
	GO:0021983	Pituitary gland development	4	5.70×10^{-4}	*DRD2, POU1F1, GHRHR, TBX19*
	GO:0021984	Adenohypophysis development	3	8.52×10^{-4}	*DRD2, POU1F1, GHRHR*
	GO:0021536	Diencephalon development	4	1.24×10^{-3}	*DRD2, POU1F1, GHRHR, TBX19*
	GO:0043567	Regulation of insulin-like growth factor receptor signaling pathway	3	1.82×10^{-3}	*GH1, POU1F1, GHRHR*
	GO:0040014	Regulation of multicellular organism growth	4	4.82×10^{-3}	*GH1, DRD2, POU1F1, GHRHR*
	GO:0035270	Endocrine system development	4	6.09×10^{-3}	*DRD2, POU1F1, GHRHR, TBX19*
	GO:0030900	Forebrain development	5	9.58×10^{-3}	*ERBB4, DRD2, POU1F1, GHRHR, TBX19*
	GO:0045927	Positive regulation of growth	4	9.75×10^{-3}	*GH1, DRD2, POU1F1, GHRHR*
	GO:0048732	Gland development	5	1.75×10^{-2}	*ERBB4, DRD2, POU1F1, GHRHR, TBX19*
	GO:0045165	Cell fate commitment	4	3.22×10^{-2}	*NOTCH2, ERBB4, POU1F1, TBX19*
	GO:0051240	Positive regulation of multicellular organismal process	5	3.49×10^{-2}	*GH1, ERBB4, DRD2, POU1F1, GHRHR*
	GO:0042127	Regulation of cell proliferation	8	4.55×10^{-2}	*NOTCH2, ERBB4, DRD2, NR3C2, CDK6, POU1F1, GHRHR, TBX19*

GO, gene ontology.

Table III. Results of pathway enrichment analysis of the up- and downregulated differentially-expressed genes.

Category	Term	Description	Count	P-value	Genes
Upregulated	rno04110	Cell cycle	14	5.01×10^{-7}	*CDC20, MCM3, CDKN1C, CCNB1, CDKN1A, CCNB2, CDKN2C, BUB1, BUB1B, CCNA2……*
	rno04114	Oocyte meiosis	9	1.06×10^{-3}	*CCNB1, ADCY3, AR, CCNB2, MAPK12, PLK1, BUB1, CDC20, PTTG1*
	rno00601	Glycosphingolipid biosynthesis	5	1.10×10^{-3}	*B4GALT1, B3GALT2, B3GALT5, FUT4, B4GALT4*
	rno04914	Progesterone-mediated oocyte maturation	8	1.15×10^{-3}	*CCNB1, ADCY3, CCNB2, KRAS, MAPK12, PLK1, BUB1, CCNA2*
	rno04062	Chemokine signaling pathway	9	1.46×10^{-2}	*ADCY3, KRAS, LYN, ARRB1, PREX1, GRK5, PRKCD, CCL6, SHC4*
	rno00330	Arginine and proline metabolism	5	1.70×10^{-2}	*ARG1, GOT1, NOS1, ASS1, GAMT*
	rno05219	Bladder cancer	4	2.94×10^{-2}	*CDKN1A, KRAS, PGF, VEGFA*
	rno04115	p53 signaling pathway	5	3.48×10^{-2}	*CCNB1, CDKN1A, CCNB2, CASP8, IGFBP3*
	rno04610	Complement and coagulation cascades	5	4.19×10^{-2}	*C1QA, A2M, C1S, C1QC, F2R*
	rno00510	N-glycan biosynthesis	4	4.89×10^{-2}	*B4GALT1, MAN2A1, ALG5, MAN1A1*
Downregulated	rno04080	Neuroactive ligand-receptor interaction	9	4.35×10^{-4}	*GH1, GABRA1, GABRA4, GABRB2, DRD2, GABRB1, TSHB, LHB, GHRHR*
	rno00410	β-alanine metabolism	3	1.05×10^{-2}	*ALDH2, ALDH1A7, DPYD*
	rno00340	Histidine metabolism	3	1.25×10^{-2}	*MAOB, ALDH2, ALDH1A7*
	rno00982	Drug metabolism	4	1.44×10^{-2}	*GSTA4, MAOB, MGST1, GSTM7*
	rno00380	Tryptophan metabolism	3	3.75×10^{-2}	*MAOB, ALDH2, ALDH1A7*
	rno04020	Calcium signaling pathway	5	3.99×10^{-2}	*ATP2B4, ERBB4, PLCG2, PLCB1, CACNA1A*
	rno00480	Glutathione metabolism	3	4.93×10^{-2}	*GSTA4, MGST1, GSTM7*

Table IV. Upregulated DEGs with connection degrees of >30 and the downregulated DEGs with connection degrees of at least 3 in the protein-protein interaction networks.

Category	Degree
Upregulated DEGs	
CDK1	51
CCNB1	47
CCNA2	46
BUB1	44
ECT2	43
TPX2	42
NDC80	42
PRC1	42
NUSAP1	41
TOP2A	41
CCNB2	41
PBK	41
RACGAP1	41
TTK	41
PIK1	40
SPC25	40
BUB1B	40
CENPF	40
CDKN3	39
NUF2	38
CDC20	38
KIF11	38
DLGAP5	38
SGOL2	37
DTL	36
KIF20A	36
CDCA2	36
KIF20B	35
ESCO2	35
RAD51	34
ARHGAP11A	34
CKAP2	33
HMMR	33
KIF2C	32
DEPDC1	32
Downregulated DEGs	
POMC	6
GSTA4	5
GSTA1	5
POU1F1	4
ERBB4	4
GABRA1	3
MGST1	3
GABRA4	3
GSTM7	3
ALDH2	3
GH1	3
NR4A2	3
MAOB	3
KCND3	3
NOTCH2	3
GABRB1	3

DEGs, differentially-expressed genes.

DEGs were mainly enriched in 7 pathways. *GH1*, *GABRA1*, *GABRA4* and *GABRB1* were enriched in the pathway of neuroactive ligand-receptor interaction ($P=4.35x10^{-4}$); *MAOB*, *ALDH2* and *ALDH1A7* were mainly enriched in the pathways of histidine metabolism ($P=1.2476x10^{-2}$) and tryptophan metabolism ($P=3.7487x10^{-2}$); *ATP2B4*, *ERBB4* and *PLCG2* were enriched in the calcium signaling pathway ($P=3.9919x10^{-2}$); and *GSTA4*, *MGST1*, *GSTM7* were significantly enriched in the pathways of drug metabolism ($P=1.4397x10^{-2}$) and glutathione metabolism ($P=4.9294x10^{-2}$) (Table III).

Analysis of PPI network. The PPI networks constructed with the up- and downregulated DEGs consisted of 1,044 and 69 PPI pairs, respectively. In the former, *PTTG1*, along with *CCNB1*, *CCNA2*, *SPC25*, *CENPF*, *NUSAP1*, *CDC20*, *TOP2A* and *BUB1*, were observed to interact with each other (Fig. 1). Within the PPI network built with downregulated DEGs, *GABRA1*, *GABRA4*, *GABRB1* and *GABRB1* were observed to interact with each other; *GSTA3*, *GSTA4*, *GSTM7* and *MGST1* were also found to interact with each other, and *POU1F1* was observed to interact with *POMC* (Fig. 2). The connection degrees of the top 15% highly-connected upregulated DEGs were each >30, and those of *CDK1*, *CCNB1*, *CCNA2* and *BUB1* were 51, 47, 46 and 44, respectively (Table IV). The top 20% highly-connected downregulated DEGs all had connection degrees of at least 3, and the degrees of *POMC*, *GSTA4*, *POU1F1*, *ERBB4*, *KCND3* and *NOTCH2* were 6, 5, 4, 4, 3 and 3, respectively (Table IV).

Discussion

In the present study, 391 DEGs were identified to be significantly upregulated and 238 were significantly downregulated in the pituitary adenomas samples. According to the constructed PPI network with the upregulated DEGs, *PTTG1* interacted with other DEGs with higher connection degrees, such as *CCNB1*, *CCNA2*, *SPC25*, *CENPF*, *NUSAP1*, *CDC20*, *TOP2A* and *BUB1*.

PTTG1, a tumorigenic gene *in vivo* (23), is abundantly expressed in pituitary tumors (24). As a securin protein, *PTTG1* is correlated with the mitotic checkpoint that prevents abnormal chromosome segregation (25), and peaks at the G_2/M phase (26). The overexpression of *PTTG1* results in cell transformation and induces aneuploidy (27), and this exists in >90% of pituitary tumors (28). *PTTG1*, together with *CCNB1*, *CCNA2*, *BUB1*, *SPC25*, *CENPF*, *NUSAP1*, *TOP2A* and *CDC20*, were all found to be enriched in GO terms associated with the cell cycle or cell division, which are indispensable for tumor growth. It

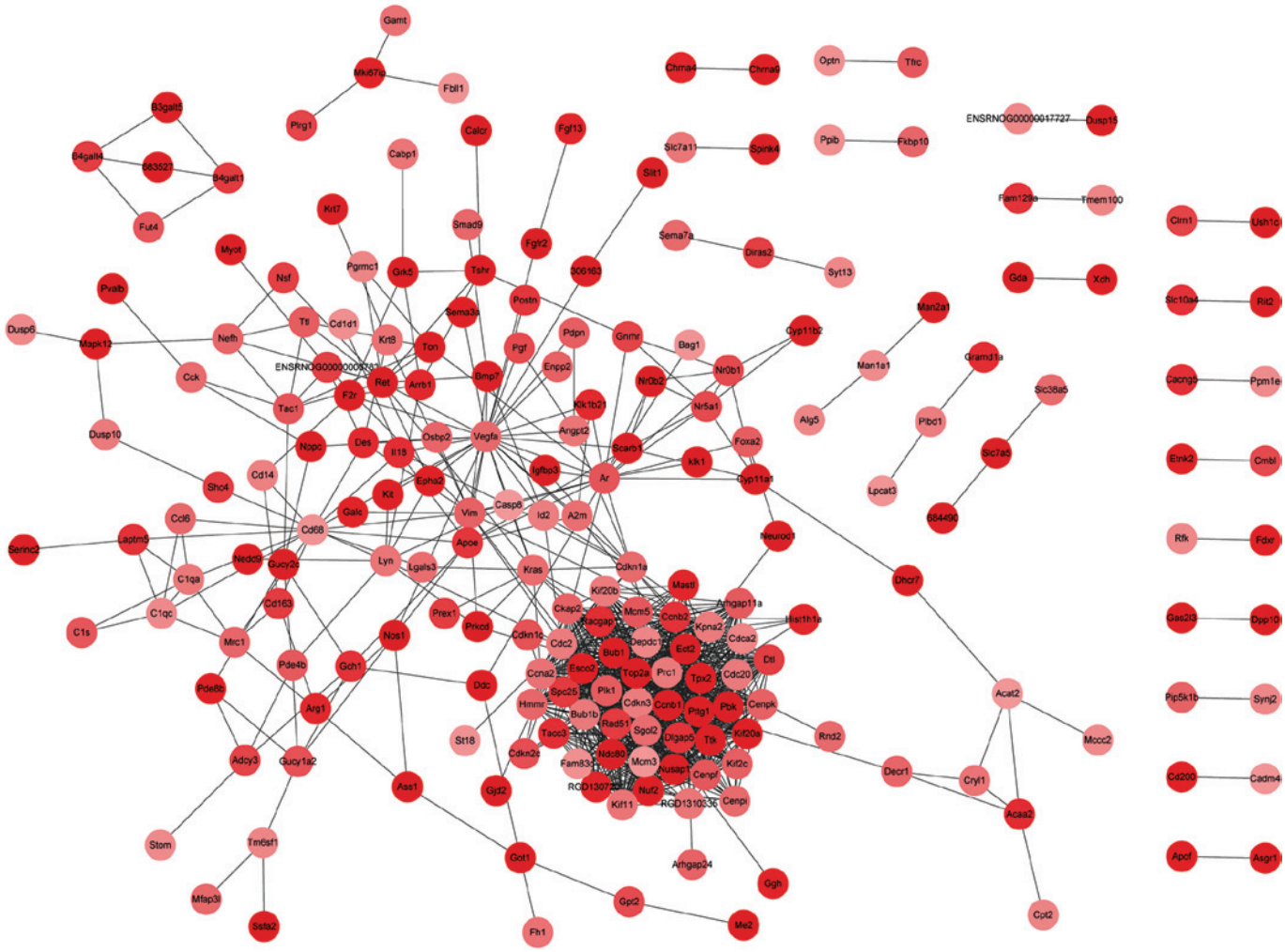

Figure 1. Protein-protein interaction network constructed with the upregulated differentially-expressed genes. Different shades of nodes colors represent the degree of up- or downregulation.

has been reported that *CDC20* is involved in the degradation of *PTTG1*-encoding products (29). Meanwhile, previous studies have also reported the abnormal expression of *CCNB1* (30), *CCNA2* (31), and *BUB1* (32) in pituitary adenomas. Furthermore, *CCNB1* was enriched in the p53 signaling pathway. *PTTG1*-encoding protein can cooperate with p53 to take part in cell apoptosis and DNA damage/repair (33,34). Altered p53 expression has been reported in pituitary carcinomas (35). Also, *PTTG1* can activate β-fibroblast growth factor, cyclin D3 and c-myc to promote cell proliferation (36,37). Therefore, *PTTG1* may play a crucial role in the occurrence of pituitary adenomas via interaction with *CCNB1*, *CCNA2*, *CENPF*, *NUSAP1*, *CDC20*, *TOP2A*, *BUB1* and p53.

Within the PPI network constructed with downregulated DEGs, *GABRA1*, *GABRA4* and *GABRB1* had higher degrees of connection to other genes. These genes were enriched in the pathway of neuroactive ligand-receptor interaction. *GABRA1*, *GABRA4* and *GABRB1* encode γ-aminobutyric acid (GABA) receptors. GABA is the major inhibitory neurotransmitter in the mammalian brain and may act as a paracrine or autocrine regulating factor in the human pituitary gland and human pituitary growth hormone adenomas (38). It has been reported that GABA has a specific effect on the electrical activity of a tumoral line of rat pituitary cells, and that it inhibits prolactin secretion directly at the pituitary level (39). Additionally, *POU1F1* was also observed to have a higher connection degree in the PPI network. This gene encoding a member of the POU family of transcription factors (40), was correlated with the development of the pituitary gland, adenohypophysis and endocrine system. In humans, *POU1F1* mutation has been shown to be associated with combined pituitary hormone deficiency (41). *POU1F1* is also implicated in cell growth and prevents cell apoptosis (42). In the present study, it was observed to interact with *POMC*, which encodes a polypeptide hormone precursor. The encoded polypeptide hormone precursor is synthesized mainly in corticotrophin cells of the anterior pituitary (43). A previous study has shown that in silent pituitary adenomas, *POMC* mRNA has a diffuse low level or is absent (44). Thus, *GABRA1*, *GABRA4*, *GABRB1*, *POU1F1* and *POMC* may also have critical roles in pituitary adenoma occurrence via close interaction.

In conclusion, upregulated DEGs, such as those associated with the cell cycle or cell division (e.g., *CCNB1*, *CCNA2*, *BUB1*, *CENPF*, *NUSAP1*, *CDC20*, *TOP2A* and particularly *PTTG1*) and downregulated DEGs, such as those relevant to neuroactive ligand-receptor interaction (e.g., *GABRA1*, *GABRA4* and

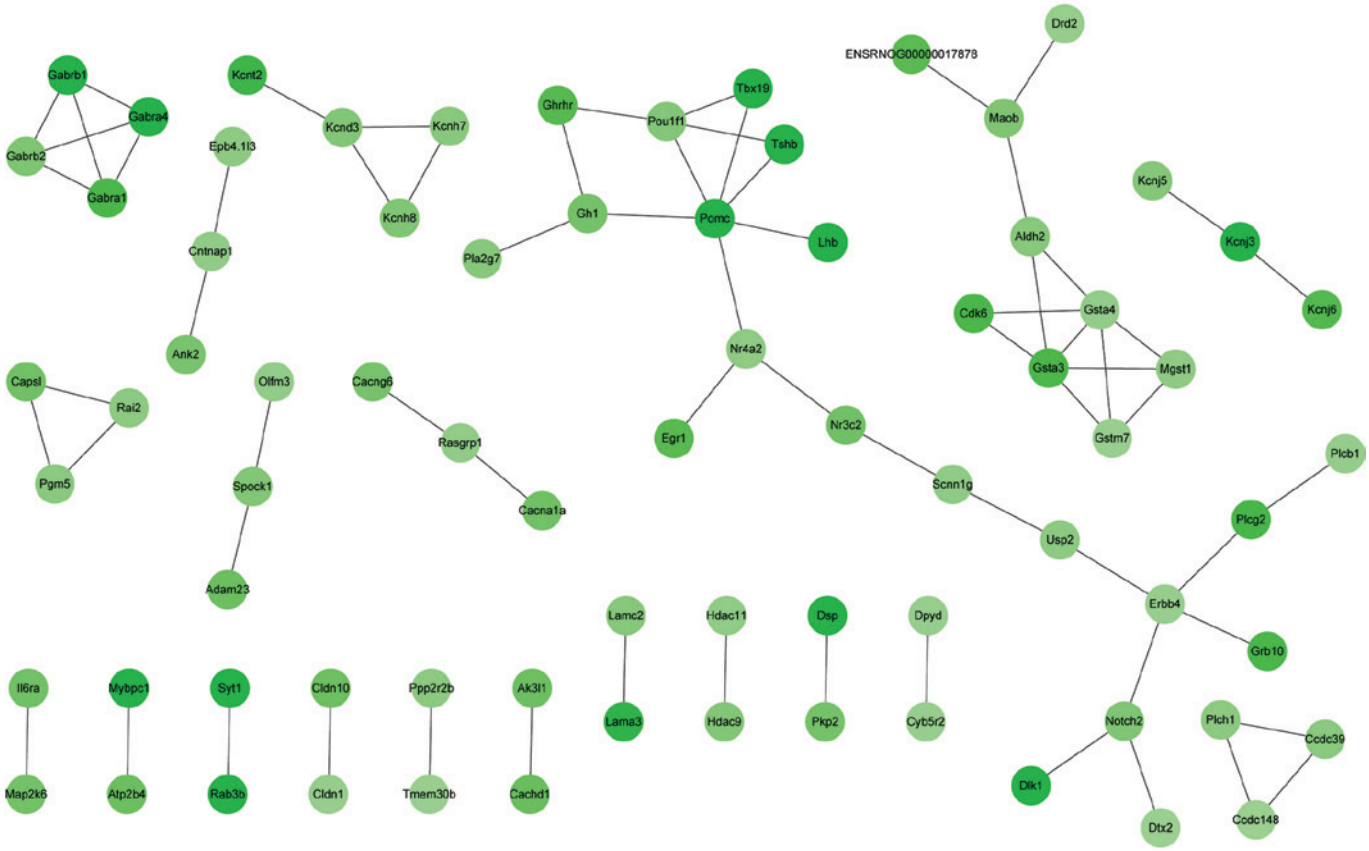

Figure 2. Protein-protein interaction network constructed with the downregulated differentially-expressed genes. Different shades of nodes colors represent the degree of up- or downregulation.

GABRB1), as well as those correlated with the development of the pituitary gland, adenohypophysis and endocrine system (e.g., *POU1F1*) may have essential roles in the pathogenesis of pituitary adenomas. The present study provides novel information for the clinical diagnosis of this disease.

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