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RESEARCH

Identification of Differentially Coexpressed Genes in Gonadotrope Tumors and Normal Pituitary Using Bioinformatics Methods

Tao Cai · Jie Xiao · Zhi-fei Wang · Qiang Liu · Hao Wu · Yuan-zheng Qiu

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Abstract To investigate the underlying molecular mechanisms of pituitary tumor by using the microarray expression profiles of pituitary tumor and normal tissue samples. The gene expression profile of GSE26966 was downloaded from Gene Expression Omnibus, including nine normal samples and 14 pituitary tumor samples. The differentially coexpressed genes (DEGs) were identified by Affy package in R Software. The functional and pathway enrichment analysis of the screened DEGs were performed by DAVID. Then, differential coexpression networks were contructed and further analyzed. Functional and pathway enrichment analysis of the 1220 identified DEGs revealed that phosphatidylinositol signaling system, p53 signaling pathway and inositol phosphate metabolism were disturbed in pituitary tumors. The degree of DLK1, CDKN2A and ITGA4 in the constructed differential coexpression network was 46, 45 and 44, respectively. In addition, MPP2 and ASAP2 were the obvious hub genes in the constructed differential coexpression network. Through exploring genes in the differential coexpression networks, the results suggested that DLK1, CDKN2A, ITGA4, MPP2 and ASAP2 may potentially be used as biomarkers for pituitary tumor.

T. Cai · Y.-z. Qiu (✉)
Department of Otolaryngology, The First Xiangya Hospital of Central South University, Changsha, Hunan Province, China
e-mail: songyechun@hotmail.com

Y.-z. Qiu
e-mail: yanghui12@hotmail.com

J. Xiao
Department of Emergency, The Third Xiangya Hospital of Central South University, Changsha, Hunan Province, China

Z.-f. Wang · Q. Liu · H. Wu
Department of Neurosurgery, The Third Xiangya Hospital of Central South University, Changsha, Hunan Province, China

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Introduction

Pituitary tumors are the most common (accounting for up to 25 %) intracranial neoplasm. Clinically detected pituitary adenomas develop in one per 10,000 persons, and are found in 20 % of the population now [1]. Clinical presentations include erectile dysfunction, headache, dizziness, visual field defects resulting from compression of the optic chiasm, and hormonal deficits resulting from normal pituitary gland compression [2, 3]. Surgical resection of the tumor can be highly effective but often fails to cure those with invasive or recurrent tumors. In addition, there are no animal models that optimally model gonadotrope tumorigenesis.

Factors underlying the pathogenesis and progression of these tumors remain poorly understood despite extensive investigation. Researchers reported that mutations in several classic oncogenes and tumor-suppressor genes, such as HIF 1α (hypoxia inducible factor 1, alpha subunit) and p53, are rarely associated with pituitary tumors [4, 5].

These difficulties underscore the need for improved predictive biomarkers of disease progression and the development of mechanism-targeted medical therapies. The advances in genomic microarray technology provide an opportunity to identify and characterize the pathways and genes responsible for the pathogenesis and progression of human pituitary tumors [6]. Previous studies usually analyze the differentially expressed genes (DEGs), such as the down-regulated GADD45β (Growth Arrest and DNA-Damage-Inducible Gene β) and the up-regulated RPRM (reprimo, TP53 dependent G2 arrest mediator candidate) [7, 8], but rarely analyze the differentially coexpressed genes (DCGs). Thus, we used the differential coexpression analysis (DCEA)

to explore the global transcriptional mechanisms underlying phenotypic changes. Compared with traditional differential expression analysis (DEA), the new DCEA tools, which are link-based DCEA algorithms with broader spectrum, overcome the current weakness of popular DCEA methods [9].

Here we used the advanced computational methods to predict the differentially coexpressed gene transcripts in gonadotrope tumors and normal pituitary genomic profiles. Firstly, the DEGs were identified by Affy package in R Software. The functional and pathway enrichment analysis of the screened DEGs were performed by DAVID. Then, differential coexpression networks were contructed and further analyzed. We anticipate that our work could contribute to the understanding for the mechanisms of pituitary tumor.

Methods

Affymetrix Microarray Data

Microarray expression profile of GSE26966 was downloaded from GEO (Gene Expression Omnibus, http://www.ncbi.nlm.nih.gov/geo/) database, which consist of nine samples from normal tissues and 14 samples from tissues of patients with pituitary tumor. Platform information was GPL570 (HG-U133_Plus_2) Affymetrix Human Genome U133 Plus 2.0 Array. The original files (.raw) and annotation files of platform were also downloaded.

Data Preprocessing and Identification of DEGs

Data were preprocessed to remove all probes considered absent or having multiple targets by an Affy package available in R Software. If a gene was mapped into more than one probes, we recorded their mean value. Only transcripts that mapped to the genome singly (once) were downloaded, resulting in 19,803 sequences. DEGs were identified by student's t-test between normal samples and pituitary tumor samples. We calculated the p-values and adjusted the raw p-values into false discovery rate (FDR) using the Benjamini-Hochberg (BH) method [10] to circumvent the multi-test problem which may induce too much false positive results. The genes only with FDR<0.01 and fold change >2 were selected as DCGs.

Pathway Enrichment Analysis for DCGs

Enrichment analysis of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway for the interesting genes were done by the DAVID (the Database for Annotation, Visualization and Integrated Discovery) software [11]. The DAVID identifies canonical pathways associated with a given list of genes by calculating the hypergeometric test p-value for probability that association between this set of genes and a canonical pathway. We chose p-value<0.01 as the cut-off criterion.

Construction of Differential Coexpression Network

From the perspective of systems biology, functionally related genes frequently coexpressed. We used differential coexpression enrichment (DCe) method in differentially coexpressed genes and links (DCGL) package to build the links between DCGs (differentially coexpressed links, DCLs [12]). DCGL package is a robust statistical method, originally proposed for selecting DCGs from microarray data based on the 'Limit Fold Change' (LFC) model. We obtained all the DCGs at two conditions (normal pituitary and gonadotrope tumors) with FDR less than 0.05 . In addition, the DCLs were also identified, which included two categories of links of DCGs. One was that the pair of DCGs only coexpressed in control or gonadotrope tumors samples (note as CO group). Another was that coexpressed in both control and gonadotrope tumors samples while switched at two contrastive conditions (note as CB group). For the CO group, the obtained DCLs were further analysed with the criterion of $-0.4<r<0.4$. What's more, clustering analysis of samples was performed according to the identified DCGs in CB group.

Results

Identification of DCGs

We found 1220 differentially expressed genes, at the criteria of FDR<0.01, fold change >2. Among those genes, 501 were up-regulated and 719 were down-regulated. Those identified differentially expressed genes may participate in the development of gonadotrope tumors.

GO Enrichment

In order to gain functional annotation of large lists of differential gene expression in gonadotrope tumors, we performed online biological classification on the base of the annotation resources provided by DAVID. Total 28 GO functions were enriched at the threshold of FDR<0.05. GO enrichment analysis revealed that the DEGs were strongly correlated with regulation of cell proliferation (p =2.00 E - 05), and response to organic substance (p =4.03 E - 05). In addition, cell adhesion, biological adhesion, and response to wounding were also shown evidence of association with the DEGs (p <0.01).

Differential Coexpression Network of DCGs

For DEGs, we used the DCe method to build up two gene coexpression networks at the two contrastive conditions (normal pituitary and gonadotrope tumors). Under the criterion of FDR<0.01, we obtained 1839 DCLs in CO group. After the r ($-0.4<r<0.4$) based filtering, a number of 1401 DCLs remained, involved 597 DCGs in CO group (Fig. 1). For 1401 pairs of DCGs, 1389 pairs were identified as coexpression in normal pituitary but not in gonadotrope tumors, while 12 pairs were only coexpressed in gonadotrope tumors but not in normal pituitary. We found that the degrees of some genes in the network were relatively high (for instance, the degree of DLK1[Delta-like 1 homolog], CDKN2A[Cyclin-dependent kinase inhibitor 2A] and ITGA4 [integrin, alpha 4] was 46, 45 and 44, respectively), which indicated that those genes were correlated with the development of the gonadotrope tumors. Furthermore, pathway enrichment analysis revealed that the DCGs were strongly associated with phosphatidylinositol signaling system ($p=0.0168$), p53 signaling pathway ($p=0.0374$) and inositol phosphate metabolism ($p=0.0466$).

In addition, we also obtained 20 pairs of DCLs in CB group involving 28 DCGs. Among these DCGs, MPP2 (Palmitoylated membrane protein 2) and ASAP2 (ArfGAP with SH3 domain, ankyrin repeat and PH domain 2) were the obvious hub genes in the constructed differential coexpression network (Fig. 2), suggesting their potential roles in the pathogenesis of pituitary tumors.

Hierarchical Clustering Analysis of DCGs in CB Group

The sample clustering analysis was conducted based on the expressions of the obtained 28 DCGs in the differential coexpression network. Twelve DCGs (WBP2NL, SEMA6D, ETNK2, FAM43B, CDH8, GAL3ST3, TOMM22, C3orf39, SUGT1L1, MPP2, CLDN9, and FRMPD1) were down-regulated while the other 16 DCGs (SUSD4, C10orf11, FAM174B, F3, POR, GPRC5B, TLE3, TFEB, SLC45A4, ETHE1, BCAT1, KDELR3, COL18A1, PAMR1, DEPDC6, and ASAP2) were up-regulated in the samples of gonadotrope tumors (Fig. 3). The clustering could distinguish the normal sample from those failures well, and the correlation coefficient between normal pituitary and gonadotrope tumors group was greater than 0.94, except the GSM663758 in gonadotrope tumors group. The correlation coefficient between GSM663758 and other samples in gonadotrope tumors group was 0.91.

Discussion

In this work, we have analyzed gene expression data using advanced computational methods with the aim of uncovering genes that the expression were interfered in gonadotrope tumors. A total of 1220 DEGs were identified between gonadotrope tumor samples and normal pituitary samples and 28 GO functions were enriched. We predicated several molecular biomarkers, which are useful to improve diagnosis

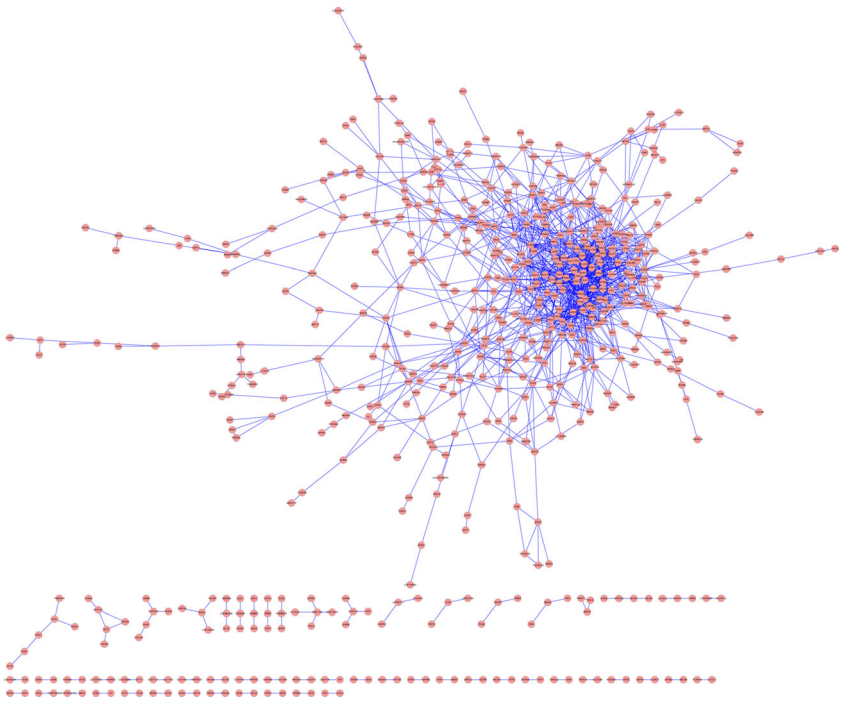

Fig. 1 The differential coexpression network of CO group. The nodes represent differentially co-expressed genes

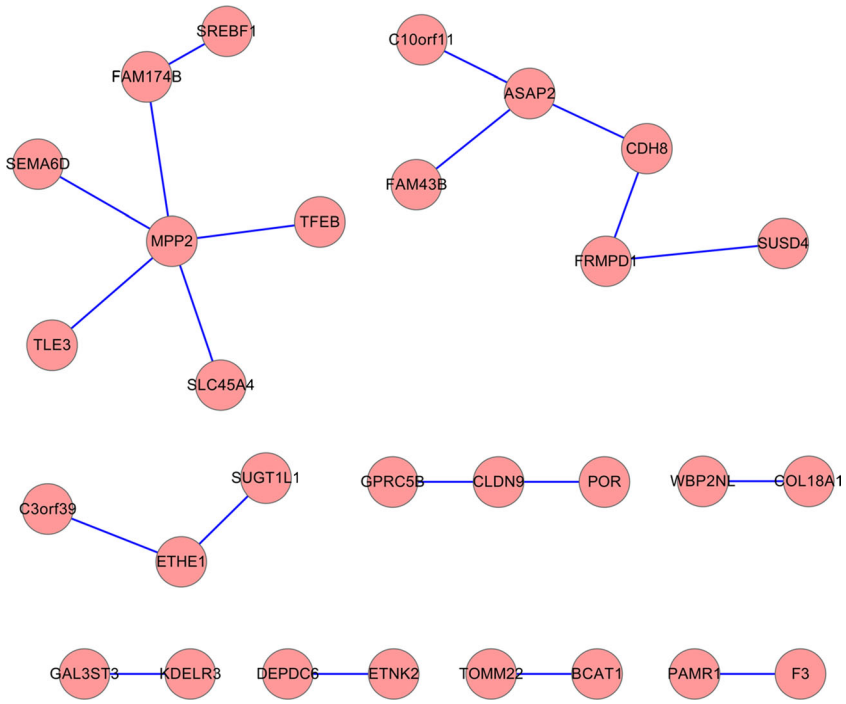

Fig. 2 The differential coexpression network of CB group. The nodes represent differentially co-expressed genes and lines represent differentially co-expressed links

and therapeutic efficacy. By conducting the GO functional analysis, we found that the three GO functions which were enriched by most DEGs are cell adhesion (GO:0007155), biological adhesion(GO:0022610) and respond to wounding (GO:0009611). With the help of KEGG, we found these genes are most enriched into the phosphatidylinositol signaling system, p53 signaling pathway and inositol phosphate metabolism.

In the constructed differential coexpression network DLK1 is a protein coding gene with a degree of 46, and is usually thought

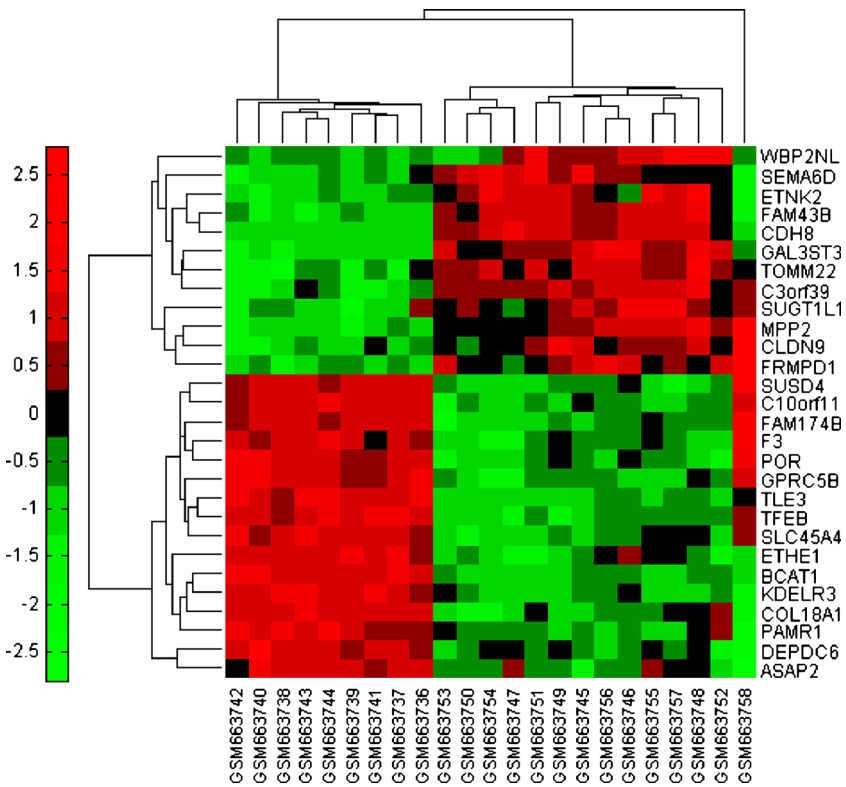

Fig. 3 Heat map of gene expression for samples of normal pituitary and gonadotrope tumors. Nine columns at lift are expression data obtained from normal pancreas specimens, and the remaining eight columns correspond to expression data obtained from gonadotrope tumors. Each row represents the expression of a single gene in all the samples

to be a tumor suppressor. In previous work, Altenberger et al. have reported that it is correlated with the development of gonadotrope tumors [13, 14]. What's more, CDKN2A with a degree of 45 is a cyclin-dependent kinase inhibitor which can encode at most three proteins. It is found to be served as a target gene of p53 in the p53 signaling pathway. There are also researches suggested that it is related to pituitary adenomas [15–17]. Meanwhile, the product of ITGA4 (integrin, alpha 4) with a degree of 44 belongs to the integrin alpha chain family of proteins. Current studies have report thated the promoter methylation of ITGA4 is sensitivity and specificity for the detection of neoplasm [18] and its decreased expression is significantly associated with relative loss of gene copy in prostate cancer [19]. Therefore, ITGA4 may also play an important role in gonadotrope tumors.

Furthermore,, we found that the MPP2 and ASAP2 were coexpressed with not only one gene. Those two genes were located in the hub axis, indicating that they may play important roles in the development of gonadotrope tumors. MPP2 is a member of a family of membrane-associated proteins termed MAGUKs (membrane-associated guanylate kinase homologs). MAGUKs, as tumor suppressor protein, interact with the cytoskeleton and regulate cell proliferation, signaling pathways, and intracellular junctions. MPP2 contains a conserved sequence, called the SH3 (src homology 3) motif, found in several other proteins that associate with the cytoskeleton and are suspected to play important roles in signal transduction. MPP 2 frequently disrupted in tumors and this may lead to the development of tumors [20]. ASAP2 gene encodes a multidomain protein Arf6GAPs, namely GIT1 and AMAP2/DDEF2. This protein contains an N-terminal alpha-helical region with a coiled-coil motif, followed by a pleckstrin homology (PH) domain, an Arf-GAP domain, an ankyrin homology region, a proline-rich region, and a C-terminal Src homology 3 (SH3) domain [21]. The protein localizes in the Golgi apparatus and at the plasma membrane, where it colocalizes with protein tyrosine kinase 2-beta (PYK2). The encoded protein forms a stable complex with PYK2 in vivo. ASAP2 functions as a substrate and downstream target for PYK2 and SRC, a pathway that may be involved in the regulation of vesicular transport. Multiple transcript variants encoding different isoforms have been found for this gene. Currently, researchers reported that multi-SH3 domain-containing proteins are expressed in the cancer cells. The interaction of those proteins may lead to the formation of dynamic protein complexes that function in pancreatic cancer cell signaling [22]. As DDEF 2 contains a SH3 domain, it may be involved in the activation of cancer cell signaling.

In a word, through exploring genes in the differential coexpression networks, the results suggested that DLK1, CDKN2A, ITGA4, MPP2 and ASAP2 may potentially be used as biomarkers for pituitary tumor. However, the results of our study need to be confirmed by further experiments.

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Conflict of Interest The authors do not have any conflicts of interest to declare.

References

1. Michaelis KA, Knox AJ, Xu M, Kiseljak-Vassiliades K, Edwards MG, Geraci M, Kleinschmidt-DeMasters BK, Lillehei KO, Wierman ME (2011) Identification of growth arrest and DNA-damage-inducible gene beta (GADD45beta) as a novel tumor suppressor in pituitary gonadotrope tumors. Endocrinology 152(10):3603–3613. doi:10.1210/en.2011-0109
2. Alexander JM, Biller BM, Bikkal H, Zervas NT, Arnold A, Klibanski A (1990) Clinically nonfunctioning pituitary tumors are monoclonal in origin. J Clin Invest 86(1):336–340. doi:10.1172/JCI114705
3. Pivonello R, Matrone C, Filippella M, Cavallo LM, Di Somma C, Cappabianca P, Colao A, Annunziato L, Lombardi G (2004) Dopamine receptor expression and function in clinically nonfunctioning pituitary tumors: comparison with the effectiveness of cabergoline treatment. J Clin Endocrinol Metab 89(4):1674–1683
4. Yoshida D, Kim K, Yamazaki M, Teramoto A (2005) Expression of hypoxia-inducible factor 1α and cathepsin D in pituitary adenomas. Endocr Pathol 16(2):123–131. doi:10.1385/ep:16:2:123
5. Thapar K, Scheithauer BW, Kovacs K, Pernicone PJ, Laws ERJ (1996) p53 expression in pituitary adenomas and carcinomas: correlation with invasiveness and tumor growth fractions. Neurosurgery 38(4):765–771
6. Lee HK, Hsu AK, Sajdak J, Qin J, Pavlidis P (2004) Coexpression analysis of human genes across many microarray data sets. Genome Res 14(6):1085–1094. doi:10.1101/gr.1910904
7. McInturff JE, Modlin RL, Kim J (2005) The role of toll-like receptors in the pathogenesis and treatment of dermatological disease. J Investig Dermatol 125(1):1–8
8. KA M, AJ K, M X, K-V K, MG E, M G, BK K-D KOL, ME W (2011) Identification of growth arrest and DNA-damage-inducible gene beta (GADD45beta). Endocrinology 152(10):3603–3613. doi:10.1210/en2011-0109
9. Liu BH, Yu H, Tu K, Li C, Li YX, Li YY (2010) DCGL: an R package for identifying differentially coexpressed genes and links from gene expression microarray data. Bioinformatics 26(20):2637–2638. doi:10.1093/bioinformatics/btq471
10. Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I (2001) Controlling the false discovery rate in behavior genetics research. Behav Brain Res 125(1–2):279–284
11. Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RA (2003) DAVID: database for annotation, visualization, and integrated discovery. Genome Biol 4(5):P3
12. de la Fuente A (2010) From 'differential expression' to 'differential networking'—identification of dysfunctional regulatory networks in diseases. Trends Genet 26(7):326–333. doi:10.1016/j.tig.2010.05.001
13. Altenberger T, Bilban M, Auer M, Knosp E, Wolfsberger S, Gartner W, Mineva I, Zielinski C, Wagner L, Luger A (2006) Identification of DLK1 variants in pituitary- and neuroendocrine tumors. Biochem Biophys Res Commun 340(3):995–1005. doi:10.1016/j.bbrc.2005.12.094
14. Cheunsuchon P, Zhou Y, Zhang X, Lee H, Chen W, Nakayama Y, Rice KA, Tessa Hedley-Whyte E, Swearingen B, Klibanski A (2011) Silencing of the imprinted DLK1-MEG3 locus in human clinically nonfunctioning pituitary adenomas. Am J Pathol 179(4):2120–2130. doi:10.1016/j.ajpath.2011.07.002

15. Seemann N, Kuhn D, Wrocklage C, Keyvani K, Hackl W, Buchfelder M, Fahlbusch R, Paulus W (2001) CDKN2A/p16 inactivation is related to pituitary adenoma type and size. J Pathol 193(4):491–497. doi:10.1002/path.833
16. Frost SJ, Simpson DJ, Clayton RN, Farrell WE (1999) Transfection of an inducible p16/CDKN2A construct mediates reversible growth inhibition and G1 arrest in the AtT20 pituitary tumor cell line. Mol Endocrinol 13(11):1801–1810
17. Tani Y, Inoshita N, Sugiyama T, Kato M, Yamada S, Shichiri M, Hirata Y (2010) Upregulation of CDKN2A and suppression of cyclin D1 gene expressions in ACTH-secreting pituitary adenomas. Eur J Endocrinol 163(4):523–529. doi:10.1530/EJE-10-0245EJE-10-0245
18. Chang E, Park DI, Kim YJ, Kim BK, Park JH, Kim HJ, Cho YK, Sohn CI, Jeon WK, Kim BI, Kim HD, Kim DH, Kim YH (2010) Detection of colorectal neoplasm using promoter methylation of ITGA4, SFRP2, and p16 in stool samples: a preliminary report in Korean patients. Hepato-Gastroenterology 57(101):720–727
19. Saramäki OR, Porkka KP, Vessella RL, Visakorpi T (2006) Genetic aberrations in prostate cancer by microarray analysis. Int J Cancer 119(6):1322–1329. doi:10.1002/ijc.21976
20. Katoh M, Katoh M (2004) Identification and characterization of human MPP7 gene and mouse Mpp7 gene in silico. Int J Mol Med 13(2):333–338
21. Kon S, Tanabe K, Watanabe T, Sabe H, Satake M (2008) Clathrin dependent endocytosis of E-cadherin is regulated by the Arf6GAP isoform SMAP1. Exp Cell Res 314(7):1415–1428. doi:10.1016/j.yexcr.2007.11.006
22. Thalappilly S, Suliman M, Gayet O, Soubeyran P, Hermant A, Lecine P, Iovanna JL, Dusetti NJ (2008) Identification of multi-SH3 domain-containing protein interactome in pancreatic cancer: a yeast two-hybrid approach. Proteomics 8(15):3071–3081. doi:10.1002/pmic.200701157