Int J Clin Exp Pathol 2016;9(8):8448-8455
www.ijcep.com /ISSN:1936-2625/IJCEP0031053

Original Article

Personalized identification of differentially expressed pathways in colon cancer

Feng Gao[1,2*], Lei Nie[2*], Rui Chen[3], Jun-Sheng Kang[2], Feng-Jun Liu[1]

[1]Department of General Surgery, Qilu Hospital, Shandong University, Jinan, Shandong, China; [2]Department of General Surgery, The 88th Hospital of PLA, Taian, Shandong, China; [3]Orthopaedic Rehabilitation Hospital of Laiwu, Laiwu, Shandong, China. [*]Equal contribututors.

Received April 22, 2016; Accepted June 17, 2016; Epub August 1, 2016; Published August 15, 2016

Abstract: Studies of colon cancer (CC) have shown that hundreds of pathways are differentially expressed in tumors when compared to normal tissue samples. A simple approach to identify individual's differentially expressed pathways is to compare tumor data with accumulated normal samples. The rationale behind is to quantify the aberrance of an individual sample's pathway. In this study, a novel approach-the individualized pathway aberrance score (iPAS) for the personalized identification of differentially expressed pathways was performed in CC, making special use of accumulated normal data. Combination of the differential co-expression network (DCEN) and pathway analysis was used to select accurate differentially expressed genes (DEGs) and differentially expressed pathways. There are 129 DEG in 387 differential pathways selected by the iPAS and the construction of DCEN, including 59 up-regulated genes and 70 down-regulated genes. These pathways and DEG might play an important role in the development of CC and could be served as therapy targets. Our data concludes that iPAS can provide a sensitive measure for clinical features of patients and can be useful to identify CC. We hope the novel way can be helpful in the individual interpretation of tumor data and can be a useful tool of customized pharmacy in the future.

Keywords: Individualized pathway aberrance score, differentially expressed pathways, accumulated normal tissue data, co-expression network, colon cancer

Introduction

The colon cancer (CC) is a frequently occurring disease of the digestive tract with high mortality, representing 13% of all malignancies [1]. Studies of CC have shown that thousands of genes are differentially expressed in tumors when compared to normal tissue samples [2]. Gene expression data have been used to classify tumor phenotypes as well as evaluate CC tumors [3-5], while gene expression research is limited in the ability to distinguish individual genes that are differentially expressed in tumors [6]. Meanwhile, existing pathway analysis techniques mainly pay attention to the detection of altered pathways between two phenotype groups, which are not suitable for identifying differentially expressed pathways that may occur in an individual sample. Therefore the analysis of differentially expressed pathways in an individual with cancer is efficient to command disease condition and suggest personalized therapeutic strategies.

Khatri et al. [7] classified current pathway methods into three kinds: overrepresentation analysis (ORA), functional class scoring (FCS) and pathway topology (PT), which added the network topology information. ORA and FCS methods are focused on developing the new way, implementing and extending each for personalized pathway analysis.

The individualized pathway aberrance score (iPAS) is outstanding to screen molecular aberrances, which based on the comparison of one cancer sample with a lot of accumulated normal samples (ANS) [8]. This is a biologically intuitive guideline to interpret a single sample, which is absolutely different from previous studies.

The new method provides analysis steps including four parts: data processing, gene-level sta-

tistics, iPAS and a significance test. The iPAS is expendable to interpret a patient in the context of many published or user-defined pathway gene sets [8]. Recently it was reported that iPAS was a sensible and efficient way to capture biological and clinical information for CC and lung cancer [8]. In this study, iPAS was used to explore differentially expressed genes (DEGs) and differentially expressed pathways of CC.

Material and methods

Gene expression data

The transcription profile was obtained from EMBI-EBI Array Express [9]. Gene expression profiling of 111 colon tissues were collected from E-GEOD-44861 [10]. Samples came from individuals were used from 56 colon tumor tissues and 55 adjacent noncancerous tissues of A-AFFY-113. The platform is A-AFFY-113-Affymetrix GeneChip HT Human Genome U133A HT_HG-U133A.

Identification of DEG

Data of the gene chip was read in the affy package [11]. The Linear Models for Microarray Data (LIMMA) was then used to preprocess data [12]. Background adjustment and quantile data normalization were performed by robust multi-array average (RMA) [13]. To protect against outlier probes we use a robust procedure, median polish, to estimate model parameters. The average value of a gene symbol with multiple probes was calculated and 12493 genes were obtained. $P \leq 0.01$ and $|\log \text{fold change (FC)}| \geq 2$ were set as the threshold levels for the identification of DEG.

Construction of differential co-expression network (DCEN)

Co-expression analysis was performed based on the expression profile of DEG we got in 111 microarray data of colon tumor and adjacent noncancerous tissues. The identification and exhibition of co-expressed gene pairs used EBcoexpress at http://www. bioconductor.org/packages/release/bioc/html/EBcoexpress.html [14]. For the tissue transcriptome data, the degree, clustering coefficient and betweenness value within each set were calculated to construct topology attribute of the DCEN.

Pathway data

Gene expressing information representing biological pathways is obtained from Reactome Pathway Database (http://www.reactome.org/) [15], which are also provided in the Molecular Signature Database [16]. Pathways with large number of genes metabolize more complicated. Therefore, pathways of which gene set size is >100 are filtered. On account of some differences among data from different platforms, pathways are filtered when the intersection of their genes and 12493 got genes is 0. Then 1004 pathways remained after filtering including 4269 genes.

Individualized analysis

ANS obtained from the gene expression omnibus (GEO) database of NCBI (www. ncbi. nlm. nih.gov/geo/) [17]. Microarray data of adjacent noncancerous tissues of patients with CC were served as the ANS. Totally 55 ANS were collected for identifying individual pathways.

Data preprocessing and gene-level statistics

Expression level was defined using the robust multichip average [13]. For individual tumor cases,

$$\text{proj}_d q_k = \left(\frac{1}{n} \sum_{j=1}^{n} qkj, \ldots, \frac{1}{n} \sum_{j=1}^{n} qkj \right) \quad (1)$$

as quantile normalization was performed after combining the single tumor data [18].

Pathway-level statistics

Average Z method is a biologically valid modification of differentially expressed pathway analysis techniques for iPAS, enabling us to test an individual CC pathway aberrance using the ANS [8].

Standardizing the gene expression by mean and standard deviation (SD) from datasets is often used in microarray analysis. A vector $Z = (z_1, z_2, \ldots, z_n)$ denotes the expression status of a pathway where z_i symbolizes the standardized expression value of i-th gene, where the number of genes belonging to the pathway is n. Gene level statistics of every gene from every sample:

Table 1. Top ten up- and down-regulated DEGs

DEGs	Log FC	*P*-value
Up-regulated		
IL8	5.28	1.66E-11
SPP1	4.94	1.11E-09
CEMIP	4.85	3.25E-13
PHLDA1	4.74	7.43E-15
KRT23	4.65	1.89E-09
SULF1	4.43	5.77E-11
AZGP1	4.32	4.44E-10
INHBA	4.26	8.07E-14
COL11A1	4.25	5.85E-10
NEBL	4.20	6.33E-12
Down-regulated		
GCG	-7.06	1.68E-16
CA1	-6.92	1.52E-15
AQP8	-6.89	6.57E-19
CA4	-6.81	1.71E-17
GUCA2B	-6.79	1.47E-19
MS4A12	-6.66	1.88E-15
CLCA4	-6.20	5.72E-12
CHGA	-6.09	1.48E-17
CHP2	-5.93	7.09E-18
ZG16	-5.92	1.88E-13

$$z_i = \frac{g_{Ti} - \text{mean}(g_{\text{nRef}})}{\text{stdev}(g_{n\text{Ref}})} \quad (2)$$

Pathway statistics of every pathway:

$$iPAS = \frac{\sum_i^n z_i}{n} \quad (3)$$

z_i represents the standardized value of 1-*i* gene and the number of genes belonging to the pathway is *n*. Pathway statistics from tumor and normal sample tissues were tested in pairs [19]. Since the test might induce false positive results, we used the method of Benjamini et al. [20], which can adjust the raw *P*-values into False discovery rate (FDR) to circumvent the problem. The FDR <0.05 and |log FC| >1 were used as the cut-off criteria.

Results

DEGs in CC

According to the criteria outlined (|log FC| ≥2; P≤0.01), a total of 485 DEGs were identified in CC, of which 194 were up-regulated and 291 were down-regulated. The top ten up and down-regulated DEGs are listed in **Table 1**. A total of 245 DEGs were enriched in pathways which benefited from the intersection between all the genes of 1004 pathways and 485 DEGs.

Construction of DCEN

DCEN was constructed in order to understand connections among 485 DEGs and explore the molecular mechanism. Co-expression analysis was performed based on the expression profile of 485 DEGs in 111 microarray data set. A total of 4612 pairs of co-expression, involving 480 DEGs were identified (data not shown). The DCEN resembles a tree-like network due to the intrinsic properties of co-expression networks. The circle nodes denote genes; meanwhile edges indicate the linked genes are differentially co-expressed.

The DCEN has a lower clustering coefficient and average shortest path length in contrast to other DCEN [21]. Top 10 degree DEGs in the DCEN are as follows: *QPCT, SFN, SPINT1, GOT1, SLC22A18, CFI, PTBP3, FUT3, SSX2IP* and *PRSS8* (**Table 2**). They are testified to be related with cancers except PTBP3 and SSX2IP. These results suggest that the differential co-expressed links typically span multiple pathways. Moreover, the linked pathways have clear interdependent functional relationships.

Differentially expressed pathways analysis

Differentially expressed pathways were selected from normal and tumor pathways, respectively, whose P<0.01 were considered as the cut-off criterion. Then 387 pathways are obtained, which are considered to play a significant role in the development of tumors. Main pathways inside are Ketone body metabolism, Ethanol oxidation, Mitochondrial fatty acid beta-oxidation of saturated fatty acids and Formation of the active cofactor, UDP-glucuronate. They linked complicatedly by differentially co-expressed genes, which may have synergistic effects. They were united to observe expression status of the same DEG in different tissue samples (**Figure 1**). Cluster analysis of using Average Z as the iPAS method identified 4 sample clusters (S1, S3, S4; S2 is from the ANS). Sample clusters S1, S3 and S4 represent well the differentiation status of CC. It is a rational result that clusters S1 is close to the ANS. This assures us that unbiased cluster-

Table 2. Top 10 degree DEGs in different co-expression network

DEGs	Average Shortest Path Length	Betweenness Centrality	Closeness Centrality	Clustering Coefficient	Degree
QPCT	1.87	0.0852	0.535	0.0387	127
SFN	1.86	0.0606	0.539	0.0368	126
SPINT1	1.90	0.0386	0.527	0.0374	105
GOT1	2.05	0.0300	0.487	0.0477	97
SLC22A18	1.95	0.0307	0.513	0.0741	94
CFI	1.96	0.0331	0.511	0.0597	90
PTBP3	2.02	0.0405	0.496	0.0220	89
FUT3	1.99	0.0198	0.502	0.0377	87
SSX2IP	1.96	0.0360	0.511	0.0388	82
PRSS8	2.04	0.0169	0.492	0.0438	81

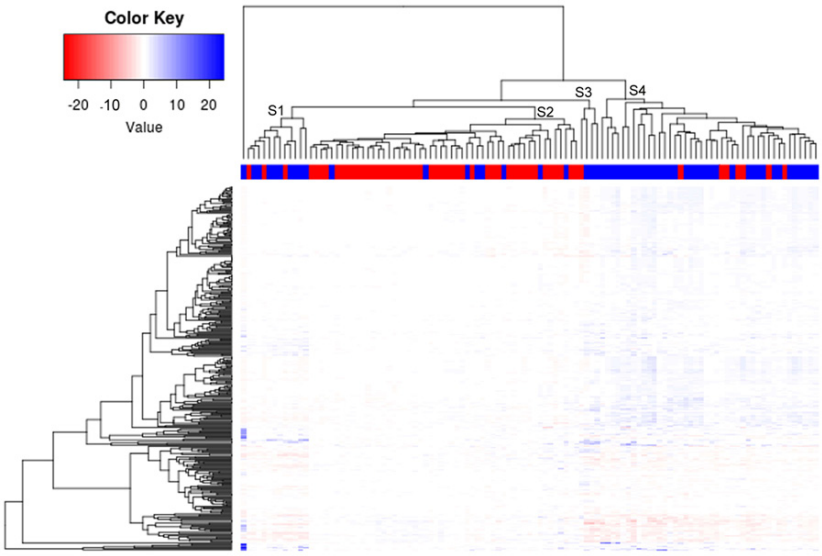

Figure 1. Heat map of differential pathways in normal and tumor tissues. Color bar was at top left corner. Samples were clustered with the abscissa and pathways were clustered with the ordinate. Blue indicates up-regulated pathways and red indicates down-regulated ones; and the expression level is proportional to the brightness of the color. White indicates no difference in expression level between the two tissue types and lighter coloring indicates lower overall expression.

ing based on iPAS has enough sensitivity to capture clinically important associations. The result is consistent with prior report that well-differentiated lung adenocarcinoma patients may have better prognosis [22].

DEGs enriched in differential pathways were mapped into the DCEN, and their interaction connections were obtained in **Figure 2**. There are 129 DEGs in the differential pathways, including 59 up-regulated genes and 70 down-regulated genes. Among them, 118 DEGs contact complicatedly except *FABP6, NR1H4, ASCC3, PTGS2, CPT2, NAT2, CXCL2, CXCL1, UGT2B17, SLC35D1* and *UGT2B15*. The top 20 degree genes of co-expression network are as follows: *SFN, GOT1, CFI, STAM, VDR, PAPSS2, SLCO2A1, MMP3, DGKA, FOXO1, PRKAR2B, AKR1C1, DHDDS, CD44, ACACB, RAE1, ECT2, ADH1B, AKR1C2* and *CDK1*. Among them, *SFN, GOT1, CFI, STAM, VDR, MMP3, DGKA, FOXO1, PRKAR2B, AKR1C1, DHDDS, CD44, ACACB, ADH1B, AKR1C2* and *CDK1* are key genes which are related to some cancers. These overlapped DEGs might play more important roles in the development of CC.

Discussion

Gene-expression profiling of human diseased tissues may provide better insights into molecular mechanisms and eventually lead to the identification of novel therapeutic targets [23]. The identification of potential DEG may assist in improved CC diagnosis. In this study, among totally 485 DEGs identified in CC, the top one up-regulated DEG is IL8, which is often treated as variables in expression of tumor necrosis factor [24]. The top one down-regulated DEG is *GCG*, which is testified to relate to various intestinal diseases [25]. Thus, these DEGs may identify further molecular alterations and provide new diagnostic biomarkers in the survival therapy of CC.

Differential co-expression analysis is emerging as a valid complement to conventional differential gene expression research in diseases. The

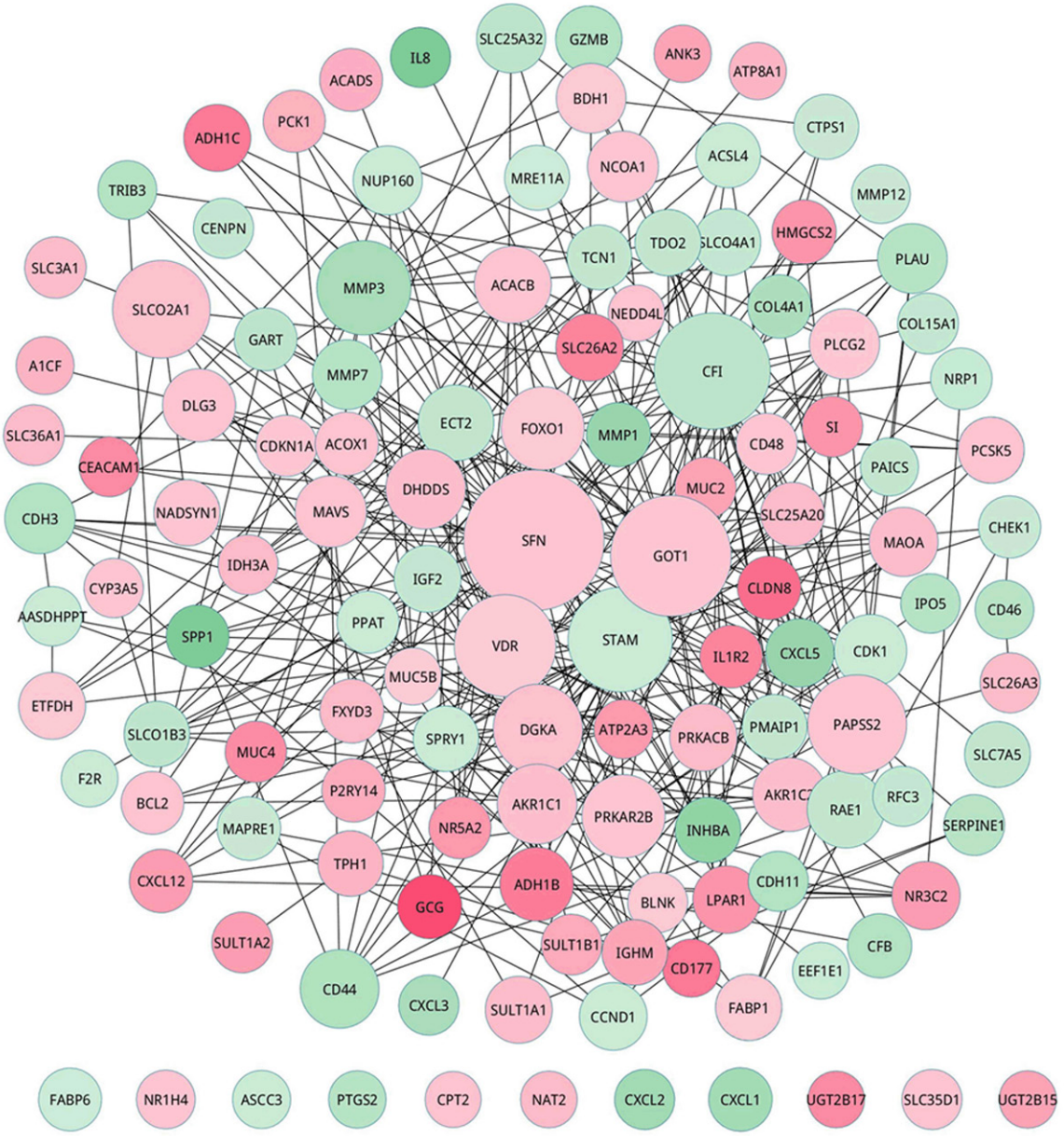

Figure 2. Co-expression links of DEGs in DCEN. The size of nodes represented degree of the DEG; green: up-regulated gene, red: down-regulated gene.

identified differential co-expression links can be assembled into a DCEN in response to genetic changes [21]. Co-expression analysis using transcriptome datasets generated by high-throughput microarray transcript profiling produces correlations that have often been considered to imply functional relationships [26, 27]. Several reports have revealed that the architecture of a molecular network can be massively rewired during a cellular response and demonstrated the power of differential network analyses for elucidating biological mechanisms [28, 29]. Among 4612 pairs of co-expression, *GPA33* and *FCGBP, AGT* and *S100A2, KRT23* and *CEMIP, KRT20* and *PAPSS2* have the strongest relations in the DCEN we constructed, which share the score of 0.9999999997. Most of DEGs are testified to be related with some cancers. The top two degree DEG is *QPCT* and *SFN*, the former is

densely methylated in >95% of uncultured melanoma tumor samples [30], and the latter is dramatically low in two cell lines derived from human mammary carcinoma [31]. It indicates these DEGs screened accurately in the DCEN might play an important role in the development of CC, which are considered to be served as therapy targets.

Khatri et al. [7] considered that pathway analysis has become the first choice for extracting and explaining the underlying biology for high throughput molecular measurements. Identification of differential expressed pathways in individuals is significant for understanding disease mechanisms and for the futu re application of custom therapeutic decisions. Current pathway analysis methods are not suitable to identify the pathway aberrance that may occur in an individual sample [8]. Therefore, we propose the novel method iPAS for the personalized identification of differentially expressed pathways. A key innovation of the method is the iPAS using ANS in cancer. Ahn et al. [8] proved Average Z method had the best statistical power when identifying a previously known survival-related pathway and the best averaged validation rate for CC. As the data repository is developing rapidly, it is expected that ANS data will be available for more and more diseases in the upcoming days. Ketone body metabolism is screened to be the most significant pathway, where *HMGCL, HMGCS2, ACAT1, OXCT1* and *BDH1* were enriched in. Among them, *HMGCS2* is a target gene expressed in differentiated cell of human colonic epithelium and down-regulated in CC [32]. Sawai et al. [33] reported *OXCT1* expression was examined in relation to the growth of gastric cancer. Therefore, these differentially expressed pathways are associated with survival of tumors and can provide broader carcinogenic insight [6].

Gene expression profiling has been widely used for cancer research. Coupled with individual pathway analysis, the construction of DCEN has been explored in many types of cancer. Here, the combination of above two is a significant progress to CC: we combine the advantages of the above to obtain more accurate DEGs and pathways from large-scale gene expression profiles in response to different situations. DEGs enriched in differential pathways are mapped into the DCEN, and their interaction connections are supposed to play important role in molecular mechanism and pathology process of CC.

Eventually 129 DEGs are obtained, of which 118 DEGs contact closely in the DCEN. Among them, *SFN, GOT1* are the top two degree genes, both of which are down-regulated genes. *SFN* is a maker of gastric adenocarcinoma precursor lesions [34], which is the top second degree gene of the DCEN. It is enriched in pathways of Chk1/Chk2 (Cds1) mediated inactivation of Cyclin B: Cdk1 complex, G2/M DNA damage checkpoint, Activation of BH3-only proteins, Intrinsic Pathway for Apoptosis and G2/M Checkpoints. Grenier et al. [35] reported *GOT1* in Schwann cells differed from that in hepatoma cells, suggesting a cell-specific regulation, which enriched in pathways of Gluconeogenesis, Metabolism of polyamines, amino acid synthesis and interconversion (transamination) and Glucose metabolism. These DEGs and differentially expressed pathways show the functional mechanism of CC precisely.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Feng-Jun Liu, Department of General Surgery, Qilu Hospital, Shandong University, 107 Wenhua Xi Road, Jinan 250012, Shandong, China. E-mail: fengjunliu@126.com

References

[1] Dinu D, Birla R, Caragui A, Constantinoiu S. Therapeutic strategies in colonic cancer. Chirurgia (Bucur) 2014; 109: 741-746.

[2] Birkenkamp-Demtroder K, Christensen LL, Olesen SH, Frederiksen CM, Laiho P, Aaltonen LA, Laurberg S, Sorensen FB, Hagemann R, TF OR. Gene expression in colorectal cancer. Cancer Res 2002; 62: 4352-4363.

[3] Budinska E, Popovici V, Tejpar S, D'Ario G, Lapique N, Sikora KO, Di Narzo AF, Yan P, Hodgson JG, Weinrich S, Bosman F, Roth A, Delorenzi M. Gene expression patterns unveil a new level of molecular heterogeneity in colorectal cancer. J Pathol 2013; 231: 63-76.

[4] Burgess DJ. Gene expression: colorectal cancer classifications. Nat Rev Cancer 2013; 13: 380-381.

[5] Sanz-Pamplona R, Berenguer A, Cordero D, Riccadonna S, Sole X, Crous-Bou M, Guino E, Sanjuan X, Biondo S, Soriano A, Jurman G, Capella G, Furlanello C, Moreno V. Clinical value of prognosis gene expression signatures in colorectal cancer: a systematic review. PLoS One 2012; 7: e48877.

[6] Slattery ML, Herrick JS, Mullany LE, Gertz J, Wolff RK. Improved survival among colon cancer patients with increased differentially expressed pathways. BMC Med 2015; 13: 75.
[7] Khatri P, Sirota M, Butte AJ. Ten years of pathway analysis: current approaches and outstanding challenges. PLoS Comput Biol 2012; 8: e1002375.
[8] Ahn T, Lee E, Huh N, Park T. Personalized identification of altered pathways in cancer using accumulated normal tissue data. Bioinformatics 2014; 30: i422-429.
[9] Parkinson H, Kapushesky M, Shojatalab M, Abeygunawardena N, Coulson R, Farne A, Holloway E, Kolesnykov N, Lilja P, Lukk M, Mani R, Rayner T, Sharma A, William E, Sarkans U, Brazma A. Array Express--a public database of microarray experiments and gene expression profiles. Nucleic Acids Res 2007; 35: D747-750.
[10] Ryan BM, Zanetti KA, Robles AI, Schetter AJ, Goodman J, Hayes RB, Huang WY, Gunter MJ, Yeager M, Burdette L, Berndt SI, Harris CC. Germline variation in NCF4, an innate immunity gene, is associated with an increased risk of colorectal cancer. Int J Cancer 2014; 134: 1399-1407.
[11] Gautier L, Cope L, Bolstad BM, Irizarry RA. affy--analysis of Affymetrix GeneChip data at the probe level. Bioinformatics 2004; 20: 307-315.
[12] Armougom F, Moretti S, Poirot O, Audic S, Dumas P, Schaeli B, Keduas V, Notredame C. Expresso: automatic incorporation of structural information in multiple sequence alignments using 3D-Coffee. Nucleic Acids Res 2006; 34: W604-608.
[13] Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 2003; 4: 249-264.
[14] Dawson JA, Ye S, Kendziorski C. R/EBcoexpress: an empirical Bayesian framework for discovering differential co-expression. Bioinformatics 2012; 28: 1939-1940.
[15] Croft D, O'Kelly G, Wu G, Haw R, Gillespie M, Matthews L, Caudy M, Garapati P, Gopinath G, Jassal B, Jupe S, Kalatskaya I, Mahajan S, May B, Ndegwa N, Schmidt E, Shamovsky V, Yung C, Birney E, Hermjakob H, D'Eustachio P, Stein L. Reactome: a database of reactions, pathways and biological processes. Nucleic Acids Res 2011; 39: D691-697.
[16] Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005; 102: 15545-15550.
[17] Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, Marshall KA, Phillippy KH, Sherman PM, Holko M, Yefanov A, Lee H, Zhang N, Robertson CL, Serova N, Davis S, Soboleva A. NCBI GEO: archive for functional genomics data sets--update. Nucleic Acids Res 2013; 41: D991-995.
[18] Bolstad BM, Irizarry RA, Astrand M, Speed TP. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics 2003; 19: 185-193.
[19] Rosner B, Glynn RJ, Lee ML. Incorporation of clustering effects for the Wilcoxon rank sum test: a large-sample approach. Biometrics 2003; 59: 1089-1098.
[20] Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I. Controlling the false discovery rate in behavior genetics research. Behav Brain Res 2001; 125: 279-284.
[21] Hsu CL, Juan HF, Huang HC. Functional Analysis and Characterization of Differential Coexpression Networks. Sci Rep 2015; 5: 13295.
[22] Barletta JA, Yeap BY, Chirieac LR. Prognostic significance of grading in lung adenocarcinoma. Cancer 2010; 116: 659-669.
[23] Chen ZH, Kim HP, Ryter SW, Choi AM. Identifying targets for COPD treatment through gene expression analyses. Int J Chron Obstruct Pulmon Dis 2008; 3: 359-370.
[24] Weiss DJ, Evanson OA, de Souza C, Abrahamsen MS. A critical role of interleukin-10 in the response of bovine macrophages to infection by Mycobacterium avium subsp paratuberculosis. Am J Vet Res 2005; 66: 721-726.
[25] Meier JJ, Nauck MA, Pott A, Heinze K, Goetze O, Bulut K, Schmidt WE, Gallwitz B, Holst JJ. Glucagon-like peptide 2 stimulates glucagon secretion, enhances lipid absorption, and inhibits gastric acid secretion in humans. Gastroenterology 2006; 130: 44-54.
[26] Movahedi S, Van Bel M, Heyndrickx KS, Vandepoele K. Comparative co-expression analysis in plant biology. Plant Cell Environ 2012; 35: 1787-1798.
[27] Han X, Chen C, Hyun TK, Kumar R, Kim JY. Metabolic module mining based on Independent Component Analysis in Arabidopsis thaliana. Mol Cells 2012; 34: 295-304.
[28] Bisson N, James DA, Ivosev G, Tate SA, Bonner R, Taylor L, Pawson T. Selected reaction monitoring mass spectrometry reveals the dynamics of signaling through the GRB2 adaptor. Nat Biotechnol 2011; 29: 653-658.
[29] Bandyopadhyay S, Mehta M, Kuo D, Sung MK, Chuang R, Jaehnig EJ, Bodenmiller B, Licon K,

Copeland W, Shales M, Fiedler D, Dutkowski J, Guenole A, van Attikum H, Shokat KM, Kolodner RD, Huh WK, Aebersold R, Keogh MC, Krogan NJ, Ideker T. Rewiring of genetic networks in response to DNA damage. Science 2010; 330: 1385-1389.

[30] Muthusamy V, Duraisamy S, Bradbury CM, Hobbs C, Curley DP, Nelson B, Bosenberg M. Epigenetic silencing of novel tumor suppressors in malignant melanoma. Cancer Res 2006; 66: 11187-11193.

[31] Prasad GL, Valverius EM, McDuffie E, Cooper HL. Complementary DNA cloning of a novel epithelial cell marker protein, HME1, that may be down-regulated in neoplastic mammary cells. Cell Growth Differ 1992; 3: 507-513.

[32] Camarero N, Mascaro C, Mayordomo C, Vilardell F, Haro D, Marrero PF. Ketogenic HMGCS2 Is a c-Myc target gene expressed in differentiated cells of human colonic epithelium and down-regulated in colon cancer. Mol Cancer Res 2006; 4: 645-653.

[33] Sawai M, Yashiro M, Nishiguchi Y, Ohira M, Hirakawa K. Growth-inhibitory effects of the ketone body, monoacetoacetin, on human gastric cancer cells with succinyl-CoA: 3-oxoacid CoA-transferase (SCOT) deficiency. Anticancer Res 2004; 24: 2213-2217.

[34] Cunningham SC, Kamangar F, Kim MP, Hammoud S, Haque R, Iacobuzio-Donahue CA, Maitra A, Ashfaq R, Hustinx S, Heitmiller RE, Choti MA, Lillemoe KD, Cameron JL, Yeo CJ, Schulick RD, Montgomery E. Claudin-4, mitogen-activated protein kinase kinase 4, and stratifin are markers of gastric adenocarcinoma precursor lesions. Cancer Epidemiol Biomarkers Prev 2006; 15: 281-287.

[35] Grenier J, Tomkiewicz C, Trousson A, Rajkowski KM, Schumacher M, Massaad C. Identification by microarray analysis of aspartate aminotransferase and glutamine synthetase as glucocorticoid target genes in a mouse Schwann cell line. J Steroid Biochem Mol Biol 2005; 97: 342-352.