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RESEARCH

Screening of Hub Genes and Pathways in Colorectal Cancer with Microarray Technology

Yonggang Wang · Tianying Zheng

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Abstract Here we intend to identify key genes and pathways in the pathogenesis of colorectal cancer (CRC) through analyzing microarray data with bioinformatic tools. The gene expression profile dataset GSE23878 was downloaded from Gene Expression Omnibus and differentially expressed genes (DEGs) were screened out using Student's *t*-test. GO function and KEGG pathway enrichment analyses were performed for these DEGs with the DAVID online tool. Interaction network was constructed among the over-represented pathways based on the protein-protein interactions within the pathways. Besides, the protein interaction information obtained from HPRD database were applied to constructed protein-protein interaction networks among the DEGs and hub genes and function module were screened out. A total of 2,296 DEGs were obtained and they were enriched in 34 pathways. An interaction network was constructed among 32 pathways, in which p53 signaling pathway acted as the hub pathway as it showed the highest node degree. The protein-protein interaction network comprised 1,481 interaction relationships among 332 genes which included 40 DEGs. Further analysis revealed that theses DEGs formed 7 function modules and many genes, such as PDGFRB, MET, FZD2, CCND1, PRKCB, ARHGEF6, JUP, WNT2, WNT5A and WNT11 were key genes in the networks. The DEGs and disturbed biological functions uncovered in present study may play important roles in the development of CRC and can contribute to the understanding on molecular mechanisms of CRC. Further these DEGs we obtained can be acted as potential biomarkers for diagnosis and therapy of CRC.

Y. Wang · T. Zheng (✉)
Department of Chemotherapy, Cancer Center, Qilu Hospital, Shandong University, NO. 107 West Wenhua Road, Jinan, Shandong Province 250012, China
e-mail: zhentianyingzh@hotmail.com

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Introduction

Colorectal cancer (CRC), described as uncontrolled cell growth in colon, rectum or appendix, is one of the most common cancer in the world that brings great challenge for human health. It is estimated that more than 1 million cases of CRC are diagnosed and 600,000 patients die of this cancer every year [1]. Survival rate is closely associated with stage of cancer. The 5-year survival rate of patients with stage I CRC is about 93 %, which decreases to about 80 % for patients with stage II CRC and to 60 % for patients with stage III CRC [2]. Therefore, detection of CRC at early stage appears to be critical measure to reduce the mortality, and many CRC specific-deaths can be avoided by early diagnosis and timely therapy.

During the last decade, gene expression profiling has displayed great promising in diagnosis as well as targeted therapy of cancers [3]. Multiple studies have been focused on the molecular changes of CRC and a range of genetic alterations were demonstrated to be involved in the development of CRC. Chromosomal instability (CIN) and microsatellite instability (MSI) are two main pathways that induce the occurrence of CRC [4]. Loss of heterozygosity (LOH) centring around APC (adenomatous polyposis coli) in chromosomes 5q, TP53 (tumor protein 53) in 17p and SMAD4 (SMAD family member 4) in 18q, which result in CIN, have been found in CRC at high frequencies [5–8]. MSI is caused by defects in the DNA mismatch repair (MMR) system [9]. Germ-line mutations of MMR genes, such as MSH2 (MutS protein homolog 2), MSH6, MLH1 (MutL homolog 1) and PMS2 (mismatch repair endonuclease PMS2), are associated

with hereditary non-polyposis colorectal cancer (HNPCC), the main inherited predisposition to colorectal cancer [10].

Microarray technology is an effective tool to disclose the global molecular changes occurred at the onset and during the development of cancer [11]. Besides, it allows the measure of the expression levels of thousands of genes simultaneously that helps the investigators to recognize the nature of cancer comprehensively. In this study, we analyzed the differentially expressed genes (DEGs) between CRC samples and healthy controls based on the microarray data using bioinformatics method. Further researches were performed to screen the critical genes and pathways associated with the pathogenesis of CRC. We aimed to explore the molecular mechanism of CRC through bioinformatics method.

Materials and Methods

Microarray Data

The gene expression profile dataset GSE23878 [12] was downloaded from Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/), including 35 CRC samples and 24 healthy controls. The probe level data in the original files were converted into corresponding gene symbols based on the annotation information in the platform GPL 570. Then the raw data were normalized with RMA (quantile) method [13, 14] and subjected to logarithmic transformation. Nonspecific probes were filtered. While for a given gene with multiple corresponding probes, we took the average expression value of all probes as the final expression value of this gene. Finally, the expression profiles of total 20,283 genes were obtained.

Screening of Differentially Expressed Genes

The Student's *t* test was applied to identify the differentially expressed genes (DEGs) between CRC samples and control samples. After multiple testing correction based on Benjamini & Hochberg method [15], the FDR<0.05 and fold-change> 1.5 were selected as the threshold.

Functional Enrichment Analysis

DAVID (Database for Annotation, Visualization and Integrated Discovery) is an online tool that provides a comprehensive set of functional annotation for large list of genes [16]. Gene Ontology (GO) enrichment analysis and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis were conducted for the DEGs with DAVID. The EASE method was used to identify over-represented GO categories or KEGG pathways and EASE score<0.1 was set as the cut-off criterion.

Analysis of Interaction Between Pathways

Two pathways will be considered as interactive if a protein within one pathway presents significant interactions with proteins within the other pathway. A cumulative hypergeometric probability model was chosen to test the significance of interactions between pathways. The formula of this model was shown as below.

$$p = 1-\sum_{k=0}^{m-1} \frac{\binom{M}{k}\binom{N-M}{n-k}}{\binom{N}{n}}$$

Table 1 Significantly over-represented pathways in CRC

Term	Count	*P* value
hsa03030:DNA replication	16	4.89E-05
hsa04060:Cytokine-cytokine receptor interaction	61	6.24E-05
hsa04640:Hematopoietic cell lineage	25	5.99E-04
hsa04110:Cell cycle	31	0.002018
hsa00280:Valine, leucine and isoleucine degradation	15	0.002144
hsa04672:Intestinal immune network for IgA production	16	0.002317
hsa00071:Fatty acid metabolism	14	0.002477
hsa00982:Drug metabolism	18	0.004444
hsa05310:Asthma	11	0.004777
hsa04115:p53 signaling pathway	19	0.005214
hsa04610:Complement and coagulation cascades	19	0.006149
hsa00980:Metabolism of xenobiotics by cytochrome P450	17	0.007617
hsa04621:NOD-like receptor signaling pathway	17	0.010598
hsa00500:Starch and sucrose metabolism	13	0.011208
hsa00410:beta-Alanine metabolism	8	0.027476
hsa00983:Drug metabolism	12	0.033282
hsa03430:Mismatch repair	8	0.034824
hsa00350:Tyrosine metabolism	12	0.038919
hsa00680:Methane metabolism	4	0.040559
hsa00480:Glutathione metabolism	13	0.042547
hsa00140:Steroid hormone biosynthesis	12	0.052114
hsa00230:Purine metabolism	30	0.057721
hsa05330:Allograft rejection	10	0.058786
hsa04940:Type I diabetes mellitus	11	0.064004
hsa00830:Retinol metabolism	13	0.071088
hsa04514:Cell adhesion molecules (CAMs)	26	0.074652
hsa04020:Calcium signaling pathway	33	0.077077
hsa00360:Phenylalanine metabolism	7	0.079582
hsa04614:Renin-angiotensin system	6	0.080634
hsa05332:Graft-versus-host disease	10	0.090282
hsa00062:Fatty acid elongation in mitochondria	4	0.091405
hsa00910:Nitrogen metabolism	7	0.095379
hsa00650:Butanoate metabolism	9	0.097549
hsa00040:Pentose and glucuronate interconversions	6	0.099215

N: the total number of interactions between proteins within pathway A and B; M: the number of interactions for pathway A excluding those within the pathway; n: the number of interactions for pathway B excluding those within the pathway; m: the number of interactions between the two pathways. *P* value<0.01 was chosen as the cut-off criterion. Then an interaction network was constructed based on the relationships among pathways and visualized with cytoscape.

Construction of Protein-Protein Interaction Network

In order to observe the distribution of the DEGs in whole network, a protein-protein interaction network was constructed using information from human protein reference database (HPRD, http://www.hprd.org/) [17]. Clique Percolation method [18] in cfinder software [19] was used to identify modules in the network.

Results

Differentially Expressed Genes

Based on the dataset GSE23878, a total of 2,296 DEGs with FDR<0.05 and fold-change>1.5 were identified between CRC and control samples, including 939 up-regulated and 1,357 down-regulated genes.

Functional Enrichment Analysis of DEGs

In order to evaluate the disordered biological functions in CRC, KEGG pathway enrichment analysis was performed for all DEGs and 34 terms were significantly over-represented among these genes (Table 1). The data revealed that DEGs were mainly associated with the following groups of pathways: (1) proliferation and apoptosis (e.g. cell cycle, p53 signaling pathway); (2) DNA replication and repair (e.g. DNA replication, mismatch repair); (3) signal transduction (e.g. calcium signaling pathway); (4) immune system and immune disease (e.g. hematopoietic cell lineage, intestinal immune network for IgA production, complement and coagulation cascades, NOD-like receptor signaling pathway, asthma, allograft rejection and graft-versus-host disease); (5) metabolism (fatty acid metabolism, drug metabolism, metabolism of xenobiotics by cytochrome P450, amino acid metabolismand etc.). Among them, DNA replication is the most significantly over-represented pathway.

GO enrichment analysis was also carried out for DEGs to validate above results. Finally a total of 41 significantly over-

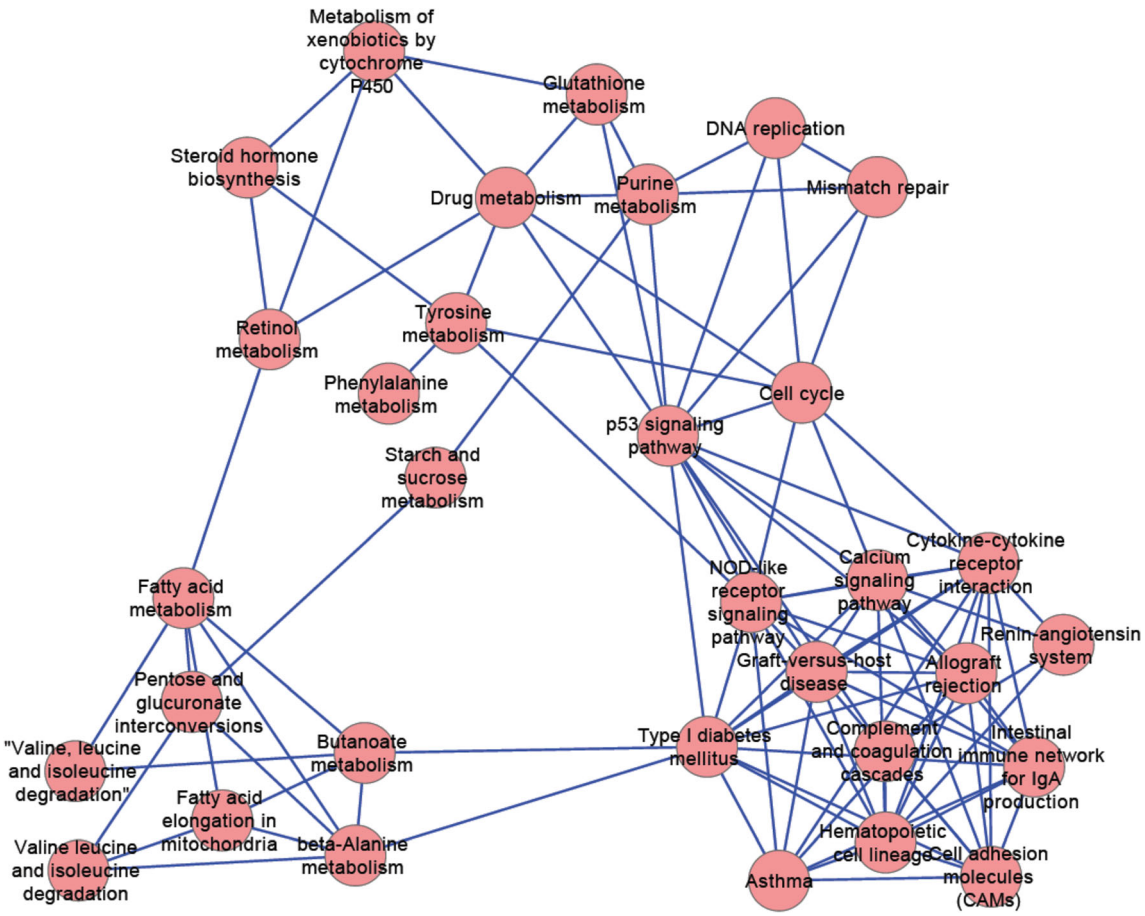


Fig. 1 Interaction network for CRC-related pathways. *Red circles* represent pathways and edges indicate interactions

represented GO terms were obtained with FDR<0.05 as the cut-off value. Terms associated with cell cycle, DNA replication, inflammatory response and immune response were enriched among the DEGs, which was similar with the results obtained from KEGG enrichment analysis.

Interaction Network for CRC-Related Pathways

To explore interactions among the CRC-related pathways, networks were constructed among genes within the 34 pathways obtained above. Then the probability of relationships between pathways was evaluated by the cumulative hypergeometric probability model. A total of 103 interactions with p value less than 0.01 were identified among 32 enriched pathways and an interaction network were constructed based on them (Fig. 1). Then the degree was calculated for each node in the network to screen out hub nodes, which were generally thought to play critical roles in the development of disease. From Table 2 we found that the degree of "P53 signaling pathway" was 13, which ranked first among all 32 pathways. The following were "cytokine-cytokine receptor interaction", "type I diabetes mellitus", "calcium signaling pathway" and "NOD-like receptor signaling pathway" which were all connected with "P53 signaling pathway". Besides, the "cell cycle", "mismatch repair" and "DNA replication" were also displayed links with this pathway (Fig. 1).

Interaction Network for CRC-Related Genes

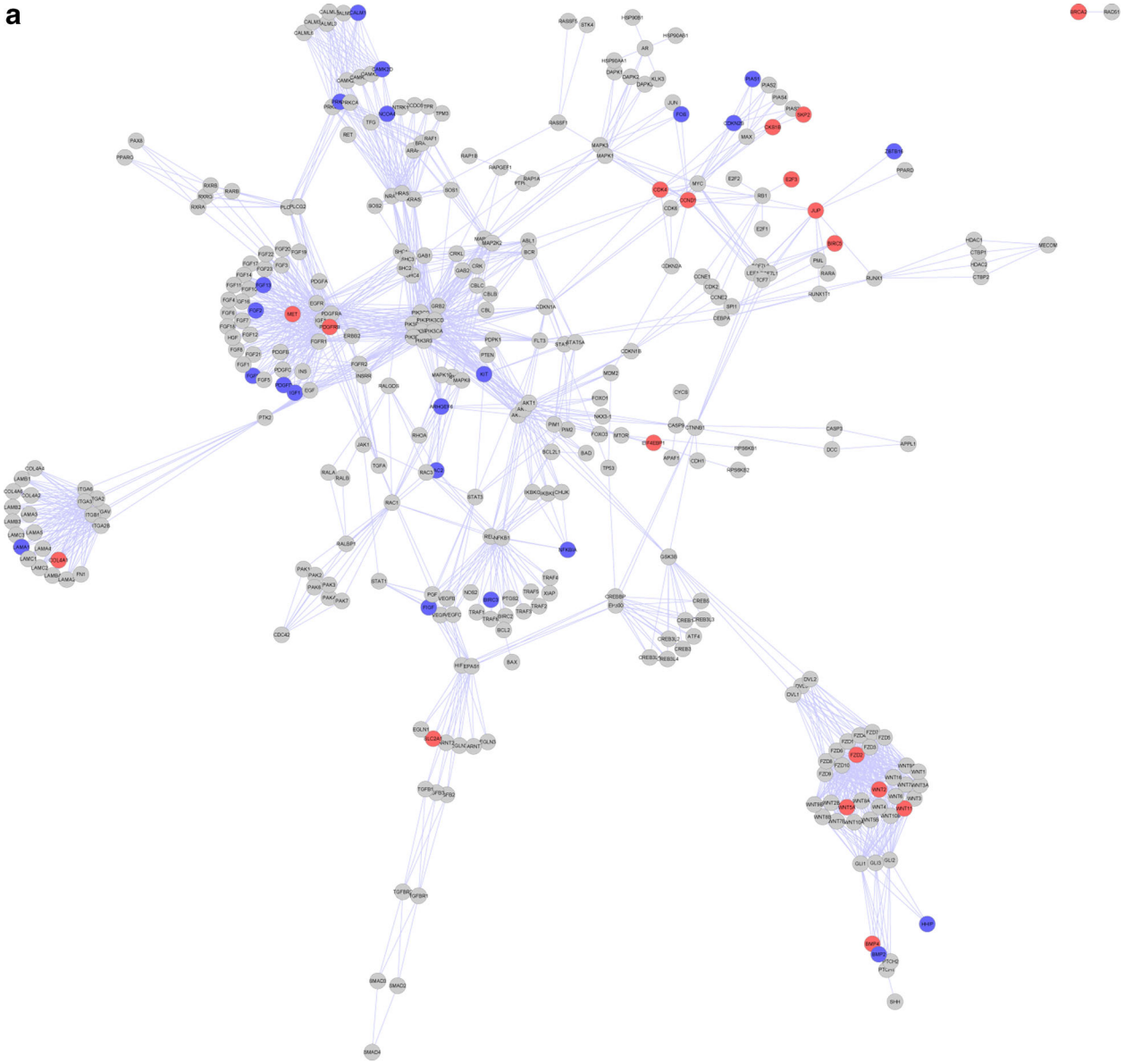
In order to identify key genes and function modules in pathogenesis of CRC, an interaction network was established among the DEGs related to the over-represented KEGG pathways and GO biological processes. The network was consisted of 1,481 interaction relationships among 332 genes, in which there were 40 DEGs including 23 down-regulated and 17 up-regulated genes (Fig. 2). Among these DEGs, genes FZD2 (frizzled-2), WNT2 (wingless-type MMTV integration site family member 2), WNT5A and WNT11, which were all up-regulated in CRC samples, formed a function module. While genes PRKCB (protein Kinase C, beta), CALM1 (calmodulin 1) and CAMK2D (calcium/calmodulin-dependent protein kinase type II delta chain), which were all up-regulated, formed another function module. Besides, there were interaction relationships among PDGFD (platelet-derived growth factor D), PDGFRB (PDGF receptor, beta polypeptide), MET (met proto-oncogene), FGF2 (fibroblast growth factor 2), FGF9, FGF13, and IGF1 (insulin-like growth factor 1), of which PDGFRB and MET were two core genes in the network. Another seven DEGs, CCND1 (cyclin D1), CDK4 (cyclin-dependent kinase 4), CDKN2B (cyclin-dependent kinase 4 inhibitor B), FOS (Oncogene FOS), JUP (junction plakoglobin), ZBTB16 (zinc finger and BTB domain-containing protein 16) and PIAS1 (protein Inhibitor of Activated STAT-1), were also formed a function module (Fig. 2b).

The degree of each DEGs in the network was then calculated and the top 11 ones were PDGFRB (46), MET (31), FZD2 (22), CCND1 (18), PRKCB (14), ARHGEF6 (14), JUP (13), WNT2 (13), WNT5A (13) and WNT11 (13). These genes might be of great importance in the whole network.

Table 2 Degrees for the 32 KEGG pathway terms

Name	Degree
p53 signaling pathway	13
Cytokine-cytokine receptor interaction	12
Type I diabetes mellitus	12
Calcium signaling pathway	11
NOD-like receptor signaling pathway	11
Hematopoietic cell lineage	11
Allograft rejection	10
Graft-versus-host disease	10
Cell adhesion molecules (CAMs)	9
Intestinal immune network for IgA production	9
Cell cycle	8
Asthma	8
Drug metabolism	7
Fatty acid metabolism	6
Purine metabolism	6
beta-Alanine metabolism	6
Tyrosine metabolism	5
Butanoate metabolism	5
Pentose and glucuronate interconversions	4
Fatty acid elongation in mitochondria	4
Glutathione metabolism	4
Retinol metabolism	4
Metabolism of xenobiotics by cytochrome P450	4
DNA replication	4
Mismatch repair	4
Complement and coagulation cascades	4
Renin-angiotensin system	4
Steroid hormone biosynthesis	3
Valine leucine and isoleucine degradation	3
Valine, leucine and isoleucine degradation	2
Starch and sucrose metabolism	2
Phenylalanine metabolism	1

Fig. 2 Interaction network for CRC-related DEGs. **a**: the whole network; **b**: part of the network. Up-regulated genes are in *red* and down-regulated genes are in *blue*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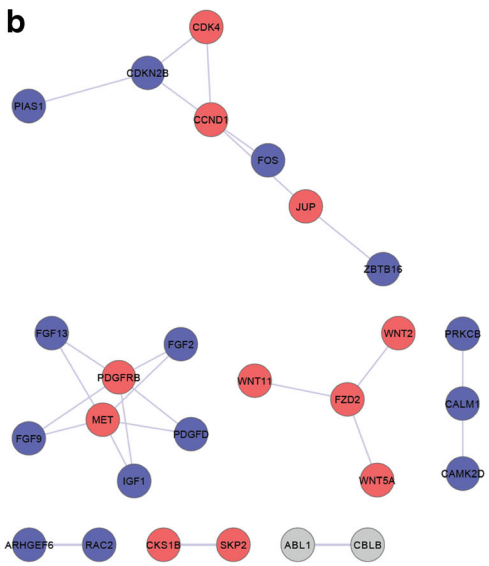
b
CDK4
CDKN2B
PIAS1
CCND1
FOS
JUP
ZBTB16
FGF13
FGF2
PDGFRB
MET
FGF9
PDGFD
IGF1
WNT11
WNT2
FZD2
WNT5A
PRKCB
CALM1
CAMK2D
ARHGEF6
RAC2
CKS1B
SKP2
ABL1
CBLB

Discussion

In present study, gene expression profiles of CRC samples were compared with those of healthy controls to identify DEGs. Functional enrichment analysis revealed a variety of pathways that were closely related with CRC.

DNA replication was the most significantly over-represented pathway. Additionally, relevant pathways like cell cycle and mismatch repair were also enriched. DNA replication is a key step in cell cycle, while mismatch repair is a mechanism -that affects the fidelity of DNA replication. DNA damage during the course of replication and cell cycle deregulation are common features of human cancer [20, 21]. The close relationships between DNA damage due to mismatch repair and CRC have been reported in several studies [22–24]. DNA must be duplicated precisely once per cycle which is monitored and controlled by a set of proteins such as CDKs and CDCs [25]. Alterations of protein level or amino acid sequence in these proteins really result in disturbance of normal biological functions, which subsequently bring about tumorigenesis [26]. In this study, we found the aberrant expression of CCND1, CDK4 and CDKN2B, suggesting that they may play important roles in the development of CRC.

Other pathways associated with immune system and immune disease were also listed in Table 1. Immune response undoubtedly has a significant impact on the tumorigenesis, which is highlighted by the clear associated between chronic inflammatory conditions and subsequent malignant transforming in the inflamed tissue [27]. Moreover, evidence has already existed linking immunologic response-related genes to CRC tumorigenesis [28]. Several xenobiotic metabolism pathways were also enriched, which was in accordance with the fact that colonic cells respond to various environmental factors associated with metabolic pathways [29].

Interactions between pathways were then analyzed and p53 signaling pathway was identified as the hub node. Connections between p53 signaling pathway and cell cycle, mismatch repair and DNA replication were also observed, which further confirmed the critical position of p53 signaling pathway in the whole regulatory mechanisms. Numerous studies have indicated that p53 signaling pathway plays an important role in development of cancers and it is also the target for therapy of cancer [30]. The p53 is a tumor suppressor gene and it mediates the cell cycle arrest after DNA damage [31]. It also contributes to chromosome stability [32] as well as mitochondrial genetic stability [33], which are critical for suppression of tumorigenesis [34, 35]. Moreover, mutation of p53 has been widely found in patients with CRC [36].

Biomarkers discovery is always the focus in cancer research as they can be utilized for diagnosis, treatment or prognosis. Therefore, protein interaction network analysis was performed for the CRC-related DEGs to identify hub genes. A number of genes with high degree were obtained, such as PDGFRB, MET and FZD2.

PDGFRB is a cell surface tyrosine kinase receptor for members of the platelet-derived growth factor family. Its expression level correlates with the metastatic behavior of human colorectal cancer [37] and the potential therapeutic application of it for CRC has been demonstrated in mice [38]. According our analysis, MET connected with PDGFRB and other genes including FGF9, FGF13, FGF2, PDGFD and IGF1 formed a function module. MET is a proto-oncogene and it encodes hepatocyte growth factor receptor with tyrosine-kinase activity. Aberrant expression of MET and its physiological ligand hepatocyte growth factor/scatter factor (HGF/SF) have been found in different types of cancers including CRC [39–41]. Down-regulation of MET displayed inhibitory effect on the growth of CRC cells [42]. FGF9, FGF13 and FGF2 are three fibroblast growth factors that showed down-regulation in CRC samples in our study. FGF9 closely interacts with PDGFRB [43] and thus may take a part in the development of CRC. In addition, several genes participating in Wnt signaling pathway were obtained: FZD2, WNT2, WNT5A and WNT11. All of them were up-regulated in CRC and formed a module, implying the involvement of Wnt signaling pathway in the pathogenesis of CRC. In fact, it is reported that aberrant Wnt pathway signaling is an early progression event in 90 % of colorectal cancers [44]. Modulation of the Wnt pathway remains to be an attractive therapeutic possibility for CRC [45].

Overall, we identified a number of DEGs between CRC and healthy controls by comparing the gene expression profiles of them. Further functional enrichment analysis and interaction networks analysis revealed several hub pathways and hub genes that may be involved in the pathogenesis of CRC. These findings can enhance our understanding on the molecular mechanism of CRC. Besides, the genes identified in current study may be acted as potential biomarkers for diagnosis and therapy of CRC.

References

1. WHO. Cancer, fact sheet N°297, World Health Organization, reviewed January 2013 http://wwwwhoint/mediacentre/factsheets/fs297/en/indexhtml
2. O'Connell JB, Maggard MA, Ko CY (2004) Colon cancer survival rates with the new American Joint Committee on Cancer sixth edition staging. J Natl Cancer Inst 96(19):1420–1425
3. Ramaswamy S, Tamayo P, Rifkin R, Mukherjee S, Yeang C-H, Angelo M, Ladd C, Reich M, Latulippe E, Mesirov JP (2001) Multiclass cancer diagnosis using tumor gene expression signatures. Proc Natl Acad Sci 98(26):15149–15154
4. Lanza G, Ferracin M, Gafà R, Veronese A, Spizzo R, Pichiorri F, C-g L, Calin GA, Croce CM, Negrini M (2007) mRNA/microRNA gene

expression profile in microsatellite unstable colorectal cancer. Mol Cancer 6(1):54
5. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Smits AM, Bos JL (1988) Genetic alterations during colorectal-tumor development. N Engl J Med 319(9):525–532
6. Law DJ, Olschwang S, Monpezat J-P, Lefrancois D, Jagelman D, Petrelli NJ, Thomas G, Feinberg AP (1988) Concerted nonsyntenic allelic loss in human colorectal carcinoma. Science 241(4868):961–965
7. Vogelstein B, Fearon ER, Kern SE, Preisinger A, Nakamura Y, White R (1989) Allelotype of colorectal carcinomas. Science 244(4901): 207–211
8. Thiagalingam S, Lengauer C, Leach FS, Schutte M, Hahn SA, Overhauser J, Willson JK, Markowitz S, Hamilton SR, Kern SE (1996) Evaluation of candidate tumour suppressor genes on chromosome 18 in colorectal cancers. Nat Genet 13(3):343–346
9. Peltomäki P (2003) Role of DNA mismatch repair defects in the pathogenesis of human cancer. J Clin Oncol 21(6):1174–1179
10. Lynch HT, Lynch J (2000) Lynch syndrome: genetics, natural history, genetic counseling, and prevention. J Clin Oncol 18(suppl 1):19s–31s
11. DeRisi J, Penland L, Brown PO, Bittner ML, Meltzer PS, Ray M, Chen Y, Su YA, Trent JM (1996) Use of a cDNA microarray to analyse gene expression patterns in human cancer. Nat Genet 14(4): 457–460. doi:10.1038/ng1296-457
12. Uddin S, Ahmed M, Hussain A, Abubaker J, Al-Sanea N, AbdulJabbar A, Ashari LH, Alhomoud S, Al-Dayel F, Jehan Z, Bavi P, Siraj AK, Al-Kuraya KS (2011) Genome-wide expression analysis of Middle Eastern colorectal cancer reveals FOXM1 as a novel target for cancer therapy. Am J Pathol 178(2):537–547
13. Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP (2003) Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 4(2):249–264
14. Bolstad BM, Irizarry RA, Astrand M, Speed TP (2003) A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics 19(2):185–193
15. Hochberg Y, Benjamini Y (1990) More powerful procedures for multiple significance testing. Stat Med 9(7):811–818
16. da Huang W, Sherman BT, Lempicki RA (2009) Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 37(1):1–13
17. Peri S, Navarro JD, Amanchy R, Kristiansen TZ, Jonnalagadda CK, Surendranath V, Niranjan V, Muthusamy B, Gandhi TK, Gronborg M, Ibarrola N, Deshpande N, Shanker K, Shivashankar HN, Rashmi BP, Ramya MA, Zhao Z, Chandrika KN, Padma N, Harsha HC, Yatish AJ, Kavitha MP, Menezes M, Choudhury DR, Suresh S, Ghosh N, Saravana R, Chandran S, Krishna S, Joy M, Anand SK, Madavan V, Joseph A, Wong GW, Schiemann WP, Constantinescu SN, Huang L, Khosravi-Far R, Steen H, Tewari M, Ghaffari S, Blobe GC, Dang CV, Garcia JG, Pevsner J, Jensen ON, Roepstorff P, Deshpande KS, Chinnaiyan AM, Hamosh A, Chakravarti A, Pandey A (2003) Development of human protein reference database as an initial platform for approaching systems biology in humans. Genome Res 13(10):2363–2371
18. Palla G, Derenyi I, Farkas I, Vicsek T (2005) Uncovering the overlapping community structure of complex networks in nature and society. Nature 435(7043):814–818
19. Adamcsek B, Palla G, Farkas IJ, Derenyi I, Vicsek T (2006) CFinder: locating cliques and overlapping modules in biological networks. Bioinformatics 22(8):1021–1023
20. Malumbres M, Barbacid M (2009) Cell cycle, CDKs and cancer: a changing paradigm. Nat Rev Cancer 9(3):153–166
21. Kastan MB, Bartek J (2004) Cell-cycle checkpoints and cancer. Nature 432(7015):316–323
22. Molaei M, Yadollahzadeh M, Almasi S, Shivarani S, Fatemi SR, Zali MR (2011) Sporadic colorectal polyps and mismatch repair proteins. Indian J Pathol Microbiol 54(4):725–729
23. Tajima A, Iwaizumi M, Tseng-Rogenski S, Cabrera BL, Carethers JM (2011) Both hMutSalpha and hMutSss DNA mismatch repair complexes participate in 5-fluorouracil cytotoxicity. PLoS One 6(12): e28117
24. Ramirez-Ramirez MA, Sobrino-Cossio S, de la Mora-Levy JG, Hernandez-Guerrero A, Macedo-Reyes Vde J, Maldonado-Martinez HA, Alonso-Larraga JO, Ramirez-Solis ME (2012) Loss of expression of DNA mismatch repair proteins in aberrant crypt foci identified in vivo by magnifying colonoscopy in subjects with hereditary nonpolyposic and sporadic colon rectal cancer. J Gastrointest Cancer 43(2):209–214. doi:10.1007/s12029-011-9303-z
25. Nishitani H, Lygerou Z (2002) Control of DNA replication licensing in a cell cycle. Genes Cells 7(6):523–534
26. Bandura JL, Calvi BR (2002) Duplication of the genome in normal and cancer cell cycles. Cancer Biol Ther 1(1):8–13
27. Macarthur M, Hold GL, El-Omar EM (2004) Inflammation and Cancer II. Role of chronic inflammation and cytokine gene polymorphisms in the pathogenesis of gastrointestinal malignancy. Am J Physiol Gastrointest Liver Physiol 286(4):G515–G520
28. Theodoropoulos G, Papaconstantinou I, Felekouras E, Nikiteas N, Karakitsos P, Panoussopoulos D, Ch Lazaris A, Patsouris E, Bramis J, Gazouli M (2006) Relation between common polymorphisms in genes related to inflammatory response and colorectal cancer. World J Gastroenterol 12(31):5037
29. Allen JI Molecular biology of colon polyps and colon cancer. In: Seminars in surgical oncology, 1995. Wiley Online Library, pp 399–405
30. Stegh AH (2012) Targeting the p53 signaling pathway in cancer therapy—the promises, challenges and perils. Expert Opin Ther Targets 16(1):67–83. doi:10.1517/14728222.2011.643299
31. Bunz F, Dutriaux A, Lengauer C, Waldman T, Zhou S, Brown JP, Sedivy JM, Kinzler KW, Vogelstein B (1998) Requirement for p53 and p21 to sustain G2 arrest after DNA damage. Science 282(5393): 1497–1501
32. Tong WM, Hande MP, Lansdorp PM, Wang ZQ (2001) DNA strand break-sensing molecule poly(ADP-Ribose) polymerase cooperates with p53 in telomere function, chromosome stability, and tumor suppression. Mol Cell Biol 21(12):4046–4054. doi:10.1128/MCB.21.12.4046-4054.2001
33. Achanta G, Sasaki R, Feng L, Carew JS, Lu W, Pelicano H, Keating MJ, Huang P (2005) Novel role of p53 in maintaining mitochondrial genetic stability through interaction with DNA Pol gamma. EMBO J 24(19):3482–3492. doi:10.1038/sj.emboj.7600819
34. Liu G, Parant JM, Lang G, Chau P, Chavez-Reyes A, El-Naggar AK, Multani A, Chang S, Lozano G (2004) Chromosome stability, in the absence of apoptosis, is critical for suppression of tumorigenesis in Trp53 mutant mice. Nat Genet 36(1):63–68. doi:10.1038/ng1282
35. Iglesias P, Salas A, Costoya JA (2012) The maintenance of mitochondrial genetic stability is crucial during the oncogenic process. Commun Integr Biol 5(1):34–38
36. Lengauer C, Kinzler KW, Vogelstein B (1997) Genetic instability in colorectal cancers. Nature 386(6625):623–627. doi:10.1038/386623a0
37. Wehler TC, Frerichs K, Graf C, Drescher D, Schimanski K, Biesterfeld S, Berger MR, Kanzler S, Junginger T, Galle PR, Moehler M, Gockel I, Schimanski CC (2008) PDGFRalpha/beta expression correlates with the metastatic behavior of human colorectal cancer: a possible rationale for a molecular targeting strategy. Oncol Rep 19(3):697–704
38. Kuwai T, Nakamura T, Sasaki T, Kitadai Y, Kim J-S, Langley R, Fan D, Wang X, Do K-A, Kim S-J, Fidler I (2008) Targeting the EGFR, VEGFR, and PDGFR on colon cancer cells and stromal cells is

required for therapy. Clin Exp Metastasis 25(4):477–489. doi:10.1007/s10585-008-9153-7

39. Di Renzo MF, Olivero M, Giacomini A, Porte H, Chastre E, Mirossay L, Nordlinger B, Bretti S, Bottardi S, Giordano S (1995) Overexpression and amplification of the met/HGF receptor gene during the progression of colorectal cancer. Clin Cancer Res 1(2): 147–154
40. Fukuura T, Miki C, Inoue T, Matsumoto K, Suzuki H (1998) Serum hepatocyte growth factor as an index of disease status of patients with colorectal carcinoma. Br J Cancer 78(4):454
41. Gherardi E, Birchmeier W, Birchmeier C, Woude GV (2012) Targeting MET in cancer: rationale and progress. Nat Rev Cancer 12(2):89–103
42. Herynk MH, Stoeltzing O, Reinmuth N, Parikh NU, Abounader R, Laterra J, Radinsky R, Ellis LM, Gallick GE (2003) Down-regulation of c-Met inhibits growth in the liver of human colorectal carcinoma cells. Cancer Res 63(11):2990–2996
43. Frontini MJ, Nong Z, Gros R, Drangova M, O'Neil C, Rahman MN, Akawi O, Yin H, Ellis CG, Pickering JG (2011) Fibroblast growth factor 9 delivery during angiogenesis produces durable, vasoresponsive microvessels wrapped by smooth muscle cells. Nat Biotechnol 29(5):421–427
44. Suzuki H, Watkins DN, Jair KW, Schuebel KE, Markowitz SD, Chen WD, Pretlow TP, Yang B, Akiyama Y, Van Engeland M, Toyota M, Tokino T, Hinoda Y, Imai K, Herman JG, Baylin SB (2004) Epigenetic inactivation of SFRP genes allows constitutive WNT signaling in colorectal cancer. Nat Genet 36(4):417–422. doi:10.1038/ng1330
45. Segditsas S, Tomlinson I (2006) Colorectal cancer and genetic alterations in the Wnt pathway. Oncogene 25(57):7531–7537