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Research Article

Bioinformatics Analysis of Colorectal Cancer Risk Related Genes and Candidate Pathways Based on GEO Database

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Abstract

Colorectal cancer (CRC) is one of the most common digestive tract cancers in the world. The incidence rate of cancer is increasing year by year around the world. At present, the exact mechanism of colorectal cancer has not been fully elucidated leading to poor diagnosis and treatment methodologies, therefore, it is necessary to understand the molecular mechanism of colorectal cancer. Applying bioinformatics to analyze the differentially expressed genes derived from CRC tissues or tissues adjacent to carcinoma has been a popular research methodology for investigating the underlining pathology of cancers. In this study, we downloaded the CRC related microarray data set (GSE32323) from the Gene Expression Omnibus (GEO). 33 samples were analyzed by microarray and 410 genes were up-regulated and 499 genes were down-regulated inCRC. Using gene chip prediction analysis (PAM) method and gsea-msigdb resource, the expressions of 1135 genes were compared and analyzed. Among them, prkcb, pik3cg, camk2d, CCND1, Cdk6, CDK4, CDKN1A were found to be potential biomarkers for CRC diagnosis. In addition, the PAM method revealed a better resolution of calculation, which provides confirmative data mining results for CRC related diagnostic marker prediction. these results will provide the basis for new research projects in clinical practice, and provide the basis for rapid risk assessment of colorectal cancer by microarray gene expression analysis.

Key words: bioinformatics analysis, colorectal cancer, risk related genes, GEO database.

Introduction

Colorectal cancer (CRC) refers to the malignant tumor occurring in the rectum or colon lumen, mainly originated from the inner wall of the colorectal, more than 95% of which are adenocarcinoma (1,2). With the formation of blood vessels and the growth of lymphatic vessels in the tumor, the lesions continue to invade the whole intestinal wall, and even spread to the abdominal cavity or cause distant organ metastasis through blood circulation (3-5). The early clinical manifestations were mainly the change of defecation habits and bloody stool. Colonoscopy is helpful for the early diagnosis of the disease. At present, the most effective treatment is surgery, but the prognosis of advanced patients is still poor.

The incidence rate of CRC in many countries including China is increasing year by year (1,2). although the level of diagnosis and treatment of CRC has improved, the is still very poor. Therefore, further elucidation of the exact molecular mechanism of CRC development and progression attracts a lot attention. The 5-year survival rate for patients without CRC metastasis was 90.3%; For patients with the CRC metastasis, the rate has dropped to 70.4% when the metastasis spread to distant organs, the rate dropped to 14%. The exact mechanism of the occurrence and development of CRChas not been fully elucidated. A large number of studies have shown that it may be related to the imbalance of oncogenes, tumor suppressor genes, changes in cell signaling pathways, gene polymorphism and microarray instability. Inflammatory bowel disease (IBD) is an important risk factor in the development of CRC. Patients with IBD more than 10 years tend to have higher tendency of developing CRC. This suggests that there may be some evolutionary relationship or some common pathogenic factors between IBDand CRC. In recent years, studies on the role of enteric nervous system (ENS) in the pathogenesis of CRC have gradually clarified the role of ENS in the pathogenesis of CRC (6-9).

ENS is mainly composed of two parts: intestinal neurons (ENCS) and intestinal glial cells (EGCS), which are distributed in the whole intestine in the form of submucosal plexus and intermuscular plexus, and are considered to be the largest and most complex part of the peripheral nervous system. As an important part of ENS, EGCS are small cells with star-like appearance wrapped in the cell bodies and nerve bundles of intestinal neurons. This cell not only has nutritional and protective effects on intestinal neurons, but also participates in the integration and regulation of neural activities, as well as the regulation of intestinal motility and intestinal epithelial barrier (10-16). In 1998, the EGCS deficient mice induced by Bush TG et al. (17) showed the loss of mucosal barrier integrity, increased permeability, intestinal inflammation, bleeding and necrosis, which confirmed that EGCS played a regulatory role in intestinal epithelial homeostasis. Since then, savidge TC et al. (18) confirmed that EGCS can protect intestinal mucosal barrier and reduce inflammation by releasing S-nitrosoglutathione (snog).In addition, , it has been found that EGCS can not only secrete mediators that protect the intestinal mucosal barrier, such as snog, GDNF, nerve growth factor (NGF), TGF - β 1, but alsocan secrete iNOS / no, 15dpgj2 and other factors to inhibit the proliferation of intestinal epithelial cells and increase the permeability of intestinal mucosa. However, the effect of these two kinds of mediators from EGCS on the integrity of intestinal epithelial barrier, intestinal inflammation and tumor remains to be further elucidated (18).

Bioinformatics analysis has enabled genomic and transgenomic screening of CRC samples and helped to identify differentially expressed genes involved in its development and progression. Research shows that the application of microarray analysis in clinical practice is still facing challenges. Firstly, due to the technology related changes in the process of sample collection and different types of platforms and methods, there is a general lack of consistency between individual research results.

Second, lack of large-scale research can lead to the decline of statistical availablity. Third, identifying the most informative and useful data for the development of reliable clinical applications has always been a challenge. Microarray analysis can be used to obtain information of thousands of genes at the same time, and provide clear insight into the genomic changes related to colorectal carcinogenesis, tumor growth and metastasis. The results of microarray analysis can identify gene features for diagnosis, molecular characterization, prognosis analysis and treatment prediction. To overcome these challenges, one approach is to use the online gene expression integrated database (GEO), which can help to increase the sample size, sample heterogeneity, and statistical power. Several methods can be used to analyze the changes of gene expression between colorectal cancer and normal mucosa to screen important cancer-related genes. In this study, we applied the method of microarray predictive analysis (PAM) to screen the potential CRC related genes for early cancer detection. In addition, Gene Ontology (go) approach and gene set enrichment analysis (GSEA) were used to validate the function of candidate genes and their association with factors of CRC(19-21).

The purpose of this study is to collect and analyze the gene expression microarray of CRC related tissues by bioinformatics method, to find out the gene difference between CRC tissue and normal tissue, so as to explore the molecular mechanism of intestinal glial cells on the occurrence and development of CRC, and to find out biomarkers for early diagnosis and prognosis of CRC.

Materials and methods

2.1 Selection of gene expression microarray in CRC

In this study, The data of gse32323 (t = 17, n = 16) (4) were selected From geo database (https://www.ncbi.nlm.nih.gov/geo/). Gene expression database (GEO, http://www.ncbi.nlm.nih.gov/geo) The NCBI was retrieved to identify the relevant microarray data sets. The selection of microarray data set follows the following rules: samples were strictly to human derived CRC tissueS; the sample size was maintained above 5; the CRC patients selected must not receive any special treatments, including radiotherapy and chemotherapy; the research type of data set is expression profile research. We excluded non-human studies, CRC cell related studies and comprehensive analysis of expression profiles. Data mining was performed under strictly concervation. informations relating to Geographic accession number, sample type, platform, number of cases and controls, references, and gene expression data were collected.

2.2 Differential expression analysis of CRC microarray data extraction

The series matrix files are obtained from geo database. The microarray data were grouped according to tumor tissue and normal tissue. The limma function in R language (x643.5.1 version) was used for differential expression analysis. The parameters were |log2 FC|> 1, FDR < 0.05. The differentially expressed genes between tumor tissue and normal tissue were obtained. Using image GP(http://www.ehbio.com/ImageGP/index.php/Home/Index/index.html)The clustering heat map is generated. The results of differential expression analysis of the above three microarray data were analyzed with Venne diagram (http://bioinfogp.cnb.csic.es /tools/venny/index.html). After integration, the same genes in the differential expression data of the three chips were obtained.

2.3 Analysis of KEGG signaling pathway

The same gene was screened by David 6.7 (https://david.ncifcrf.gov/) KEGG signal pathway analysis was performed to obtain the signal pathway of CRC compared with normal tissue.

In R Affy software package, robust multi array analysis algorithm is used to normalize the original data in cel file. The data were divided into two

groups: CRC group and control group. T-test was performed with limma software package of R statistical software to estimate DEGs of cancer tissue and normal tissue. Genes showing at least 2-fold changes, corresponding to a false detection rate of less than 0.05, were selected as significant differences.

2.4 Functional enrichment and pathway analysis of DEGs

In order to screen out the biological processes involved in the pathogenesis of CRC, the online software database of annotation, visualization and comprehensive discovery was used to annotate the gene ontology (go) of CRC and analyze the pathway appying Kyoto gene genome Encyclopedia (KEGG). These analyses were performed by hypergeometric distribution test with $P < 0.05$ as the cut-off value. We also built the pathway relationship network of DEGs, and identify the relationship between these pathways.

2.5 Protein-protein interaction (PPI) network analysis

In order to determine the function of the proteins they encode, we import DEGs into a PPI network constructed based on biogrid(http://thebiogrid.org/)and the network is visualized using Cytoscape software(http://www.cytoscape.org/). The PPI network of DEGs was screened in the whole genome, and there were DEGs at both ends. Network construction method was based on the genome context and structure information.

2.6 Analysis of gene co-expression network

In order to further identify the genes that might play key roles in CRC, we used gcbi online program to select DEGs with significant expression in GO annotation and KEGG pathway(https://www.gcbi.com.cn/gclib/html/index)。 Using the intersection of GO annotation and KEGG pathway analysis to construct gene co-expression network, we mapped DEGs into a large known network database, and screened significant gene-gene interactions using gcbi online program. The correlation between genes in the network is determined by weighting the GO terms (biological processes). In order to construct the co-expression network, the Spearman rank correlation of all expression vector pairs was calculated, and the correlation matrix was established by modularization analysis with Louvain method. $P < 0.05$ was significant.

Result

3.1 Differential gene expression results of both CRC and normal tissues

Microarray analysis of 33 samples from gse32323 data set showed that in gse32323 chip, 410 genes were up-regulated and 499 genes were down regulated in CRC tissues (Fig. 1a); in gse44861 chip, 480 genes were up regulated and 655 genes were down regulated in colorectal cancer tissues (Fig. 1b). Using Venne map to integrate the above results, the results showed that there were 148 identical genes in the differentially expressed genes of the two chips, suggesting that these genes are closely related to the occurrence and development of CRC (Fig. 2).

3.2 KEGG signaling pathway analysis of differentially expressed genes

The KEGG signaling pathway of the selected genes was analyzed by using DAVID6.7 Compared with normal tissues, CRC tissues had significant changes in nitrogen metabolism , aldosterone regulated sodium reabsorption, mineral absorption and other signaling pathways (Fig. 3, table 1).

3.3 Identification of DEGs microarray data sets

The original data of microarray data set was converted and standardized by log2 to make the mean value and unit variance zero. According to the robust rank aggregate (RRA) algorithm, we identified 1135 DEG based on the p value and FC

level of microarray data set. There were 480 up-regulated DEG and 655 down-regulated DEG (Fig. 1).

3.4 Functional annotation of DEGs and KEGG pathway

In order to further understand the biological role of DEGs, we carried out GO taxonomic enrichment analysis. The GO function and KEGG pathway enrichment of total DEGs are listed in Table 2. We found that the most abundant GO terms in biological processes are: p53 signaling pathway (P = 2.71e-05), cell cycle (P = 5.26e-05) and chemokine signaling pathway (P = 1.07e-04); Wnt signaling pathway (P = 2.43e-04), gap junction (P = 8.34e-4) and PPAR signaling pathway (P = 4.93e-4); glioma (P = 3.45e-4), GnRH signaling pathway (P = 1.11e-3). In the KEGG pathway enrichment analysis of DEGs, we found that glioma (P = 3.45e-4) was the most important pathway in KEGG analysis. In addition, p53 signaling pathway (P = 2.71e-05) and cell cycle (P = 5.26e-05) were also highly abundant.

In order to further evaluate the relationship between these pathways, we constructed the pathway relationship network of DEGs. We found that p53 signaling pathway (degree = 12), cell cycle (degree = 17) and cancer signaling pathway (degree = 27) were in the top three. We also found that gene expression was up-regulated only in apoptosis, cell cycle and JAK STAT signaling pathways, while the remaining pathways included up-regulated and down regulated genes (Fig. 1).

3.5 PPI network analysis of DEGs

The PPI network of DEGs was constructed, and the most significant module was obtained by Cytoscape-GO analysis. The module showed significant enrichment of RNA polymerase II promoter transcription in p53 signaling pathway (P = 2.71e-05), cell cycle (P = 5.26e-05) and intestinal glial cell stimulation response and negative regulation. Using online database string and Cytoscape software to complete PPI network diagram between differentially expressed genes (Fig. 4), set confidence > 0.4, degree > 5 as cut-off criteria, take the top 6 differentially expressed genes of clustering coefficient expression as target genes, up-regulated DEGs are: phorbol-12-myristate-13-acetate-induced protein 1 (PMAIP1), cyclin D1 (CCND1), and, Checkpoint kinase 1 (chek1), recombinant cyclin dependent kinase 6 (Cdk6), cyclin dependent kinase 1 (CDK1), CDK4; down regulated DEGs were: phosphatidylinositol-4,5-bisphosphonate 3-kinase, catalytic subunit gamma (pik3cg), protein kinase C, beta (prkcb), tumor protein p53, inducible protein 3 (tp53i3), stratifin (SFN), thrombospondin 1(THBS1), Fas cell surface death receptor(FAS)，cyclin G2(CCNG2), cyclin-dependent kinase inhibitor 1A (CDKN1A)。

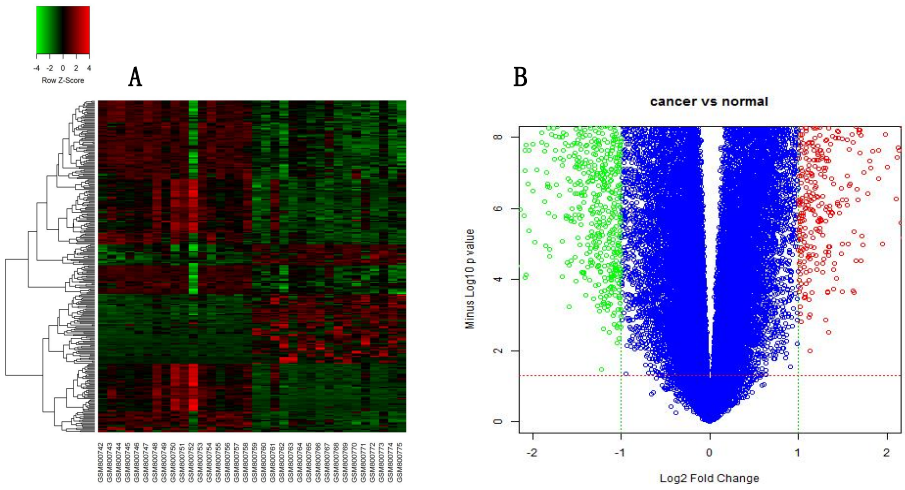

Figure1. Heatmap of gene expression differences by gene coexpression network analysis.

(A). Hierarchical cluster tree maps of gene expression. Red is up, green is down. The darker red indicates that the expression is higher, while the darker green indicates that the expression is lower. It can be concluded that the samples can be divided into general clusters: false injury control group and injury experimental group. (B). Gene coexpression network analysis of differential heat maps of gene expression. Blue dots represent genes that have statistically significant differences in expression. The blue dot represents an increase in gene expression, while the blue dot on the left represents a decrease in gene expression. Black indicates that there is no statistically significant difference in gene expression. The larger the longitudinal coordinate value, the corresponding point is, the greater the difference in gene expression corresponding to this point. Similarly, the greater the absolute value of the transverse coordinate corresponding to the point, the greater the difference in gene expression corresponding to the point.

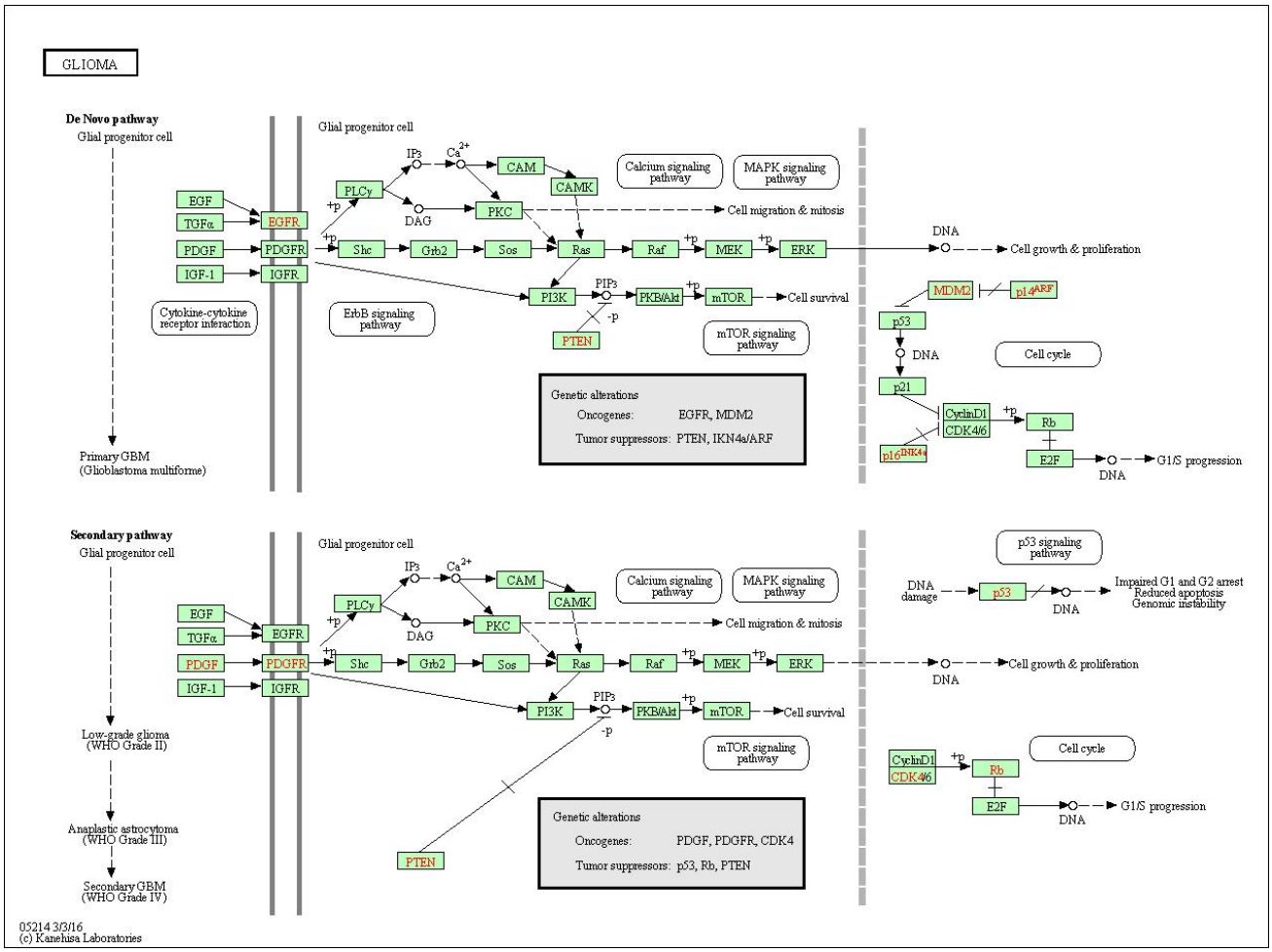

Figure 2. Differentially expressed genes related to midgut glial cells in colorectal cancer.
Red markers are up-regulated genes.

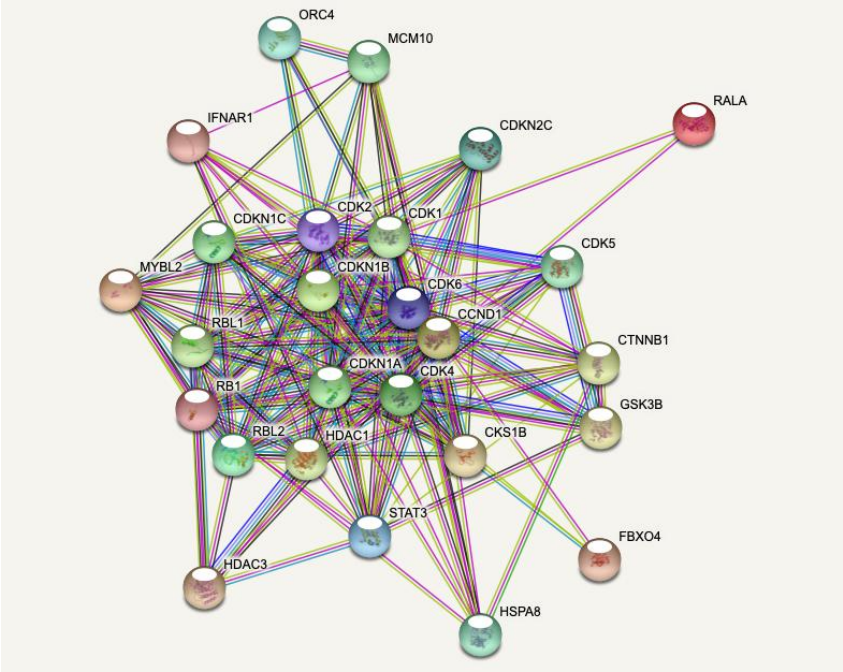

Figure3. Protein protein interaction of up-regulated genes in colorectal cancer.

Network statistics: the number of nodes is 26, and the number of sides is 173. This network includes seven cell cyclin genes, including CDK2, CDK1, CDK4, Cdk6, ccng2, CDKN1A, CDKN1B and Stat3. PPI enrichment p-value: < 1.0e-16.

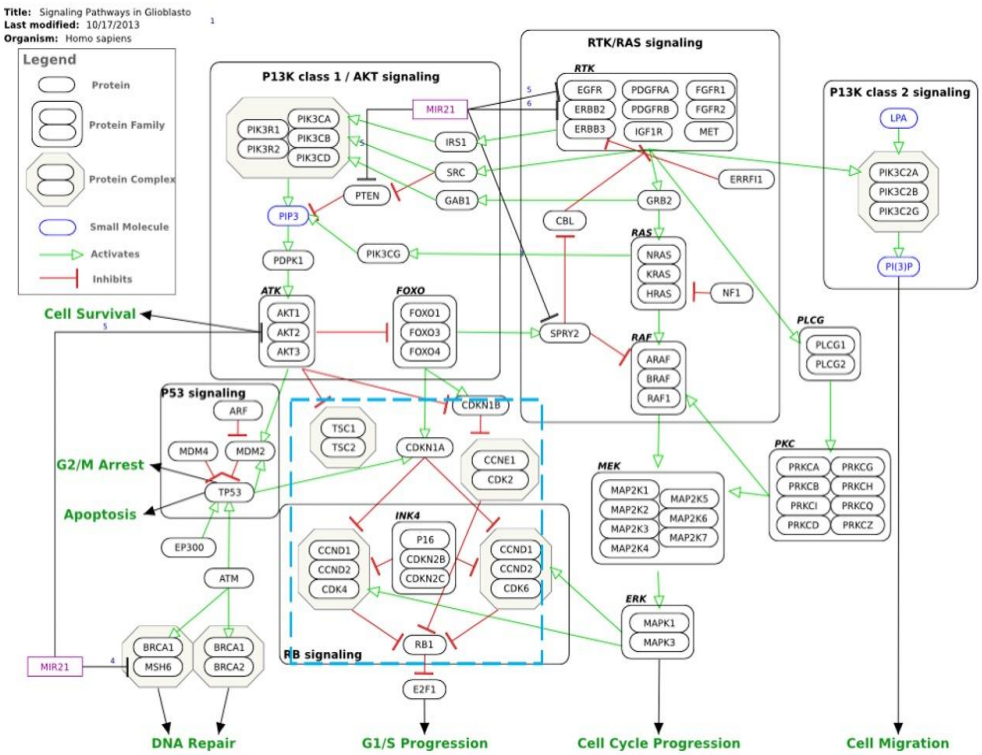

Figure 4. Colorectal cancer is a candidate biomarker and upstream regulator of intestinal glial cells.

This pathway showed the relationship between candidate biomarkers TP53, CDKN1A, CCND1, Cdk6, CDK1, CDK4 and their upstream regulator p53.

Table 1. GO and KEGG pathway enrichment analysis of hub genes.

Symbol	Description	Category	GIFtS	GC id	Score
BRCA2	BRCA2 DNA Repair Associated	Protein Coding	49	GC13P032315	224.51
TP53	Tumor Protein P53	Protein Coding	54	GC17M007661	185.15
PTEN	Phosphatase And Tensin Homolog	Protein Coding	52	GC10P087863	125.97
EGFR	Epidermal Growth Factor Receptor	Protein Coding	54	GC07P055019	114.34
CDKN2A	Cyclin Dependent Kinase Inhibitor 2A	Protein Coding	51	GC09M021967	100.96
CCND1	Cyclin D1	Protein Coding	52	GC11P069641	77.64
CDK4	Cyclin Dependent Kinase 4	Protein Coding	54	GC12M057743	70.91
CDKN1A	Cyclin Dependent Kinase Inhibitor 1A	Protein Coding	50	GC06P047460	67.2
CDKN1B	Cyclin Dependent Kinase Inhibitor 1B	Protein Coding	48	GC12P012716	59.32
CDKN2B	Cyclin Dependent Kinase Inhibitor 2B	Protein Coding	47	GC09M022002	52.27

The color depth of nodes refers to the P-value. The size of nodes refers to the numbers of genes. (a) GO BP terms. (b) GO CC terms. (c) GO MF terms. (d) KEGG Pathway of hub genes.

Table 2. GO function and KEGG pathway enrichment of the total DEGs.

GO ID	GO name	Diff gene counts in GO	Gene amount in GO	Enrichment score	P	FDR
GO:0044421	extracellular region part	198	462	1.32	1.11E-11	7.63E-09
GO:0005829	cytosol	59	136	2.05	3.50E-09	1.20E-06
GO:0043230	extracellular organelle	140	462	1.35	1.90E-08	4.35E-06
GO:1903561	extracellular vesicle	140	462	1.35	1.90E-08	3.26E-06
GO:0070062	extracellular exosome	140	462	1.35	1.90E-08	2.61E-06
GO:0031982	vesicle	159	464	1.31	5.48E-08	6.28E-06
GO:0005576	extracellular region	78	461	1.48	1.59E-07	1.56E-05
GO:0005615	extracellular space	89	461	1.43	3.48E-07	2.99E-05
GO:0044444	cytoplasmic part	156	251	1.29	3.96E-06	3.02E-04
GO:0009986	cell surface	33	348	1.77	1.17E-04	8.08E-03
GO:0043227	membrane-bounded organelle	112	155	1.28	1.97E-04	1.23E-02
GO:0042641	actomyosin	5	138	5.96	4.16E-04	2.38E-02

Discussion

The occurrence and development of CRC is a multi-stage and multi-step process, involving a series of molecular events, manifested as the complex network integration between genes or gene signal transduction pathways. IBD is an important risk factor in the development of CRC. The incidence rate of CRC is significantly higher in the population with more than 10 years of inflammatory bowel disease. This suggests that there may be some evolutionary relationship or some common pathogenic factors between inflammatory bowel disease and CRC (22-26). In recent years, the role of ENS in the pathogenesis of CRC has been gradually clarified. Among them, intestinal glial cells (EGCS), distributed in the form of submucosal plexus and intermuscular plexus, are considered to be the largest and most complex part of the peripheral nervous system. However, the role of EGCS derived mediators in intestinal epithelial barrier integrity, intestinal inflammation and tumor remains to be further elucidated.

Intestinal glial cell related pathway is one of the most concerned signaling pathways in CRC research in recent years, and constitutes a complex network regulatory system in tumor cells with important signaling pathways such as p53 and cell

cycle (27). In this paper, the expression of PMAIP1, CCND1, chek1, Cdk6, CDK1 and CDK4 in CRC tissues and tumor adjacent tissues was analyzed by bioinformatics method, and the expression level was correlated with prognosis. As for signal pathways, we found that p53 signaling pathway and cell cycle pathway are highly enriched, indicating that DEGs of microarray data are mainly involved in these signal pathways. In addition, we found that CCND1, Cdk6, CDK1, CDK4 genes are most closely related to glial signaling pathway. In addition, as shown in Figure 1, we also found that the role of glial signaling pathway in the development of CRC has been clearly defined. Apoptosis and cell cycle are important processes of normal cell carcinogenesis. These signalling pathway interactions in colorectal carcinogenesis provide more important evidence. We predict that these genes play an important role in the occurrence and development of CRC, and have the potential to be used as markers for the diagnosis, prognosis and therapeutic targets of CRC.

In conclusion, this comprehensive analysis provides a comprehensive perspective for understanding the molecular mechanism of CRC, and identifies some genes and pathways of intestinal glial cells. The genes and pathways of intestinal glial cells may be potential targets for the treatment of CRC. However, further research is still necessary to unravel the pathogenesis of CRC.

Declarations

1) ***Consent to publication***
We declare that all authors agreed to publish the manuscript at this journal based on the signed Copyright Transfer Agreement and followed publication ethics.
2) ***Ethical approval and consent to participants***
Not applicable.
3) ***Disclosure of conflict of interests***
We declare that no conflict of interest exists.
4) ***Funding***
None
5) ***Availability of data and material***
We declare that the data supporting the results reported in the article are available in the published article.
6) ***Acknowledgement***
None.
7) ***Authors 'contribution***
Authors contributed to this paper with the design (TW, WJ), dada analysis (JHL, BB, HZ). writing (JHL, BB, HZ), revision (JHL, BB, HZ, TXX, SJ, SR, TW, WJ), editing (JHL, BB, HZ) and final approval (TW, WJ).
8) ***Authors' biography***
None.

References

(1)Liu X, Liu L, Dong Z, Li J, Yu Y, et al. Expression patterns and prognostic value of. m(6)A-related genes in colorectal cancer. Am J Transl Res. 2019 Jul 15;11(7):3972-3991.

(2)Gröne J, Weber B, Staub E, Heinze M, Klaman I, Pilarsky C, Hermann K, Castanos- Velez E, Röpcke S, Mann B, Rosenthal A, Buhr HJ. Differential expression of genes encoding tight junction proteins in colorectal cancer: frequent dysregulation of claudin-1, -8 and -12. International Journal of Colorectal Disease, 2007;22(6):651–659.

(3) Shuwei Li, Lisheng Xie,Lei He, Zhimin Fan, Junhua Xu， et al.Plasma esothelin as. a Novel Diagnostic and Prognostic Biomarker in Colorectal Cancer. Journal of Cancer 2017,8(8): 1355-1361.

(4)Kang D, Park JM, Jung CK, Lee B-I, Oh ST, Choi M-G. Prognostic impact of 。 membranous ATP-binding cassette sub-family G member 2 expression in patients with colorectal carcinoma after surgical resection. Cancer Biology & Therapy, 2015;16(10):1438–1444.

(5)Kim YH, Lee HC, Kim SY, Yeom YI, Ryu KJ, Min BH, Kim DH, Son HJ, Rhee。PL, Kim JJ, Rhee JC, Kim HC, Chun HK, Grady WM, Kim YS. Epigenomic analysis of aberrantly methylated genes in colorectal cancer identifies genes commonly

affected by epigenetic alterations. Annals of Surgical Oncology, 2011 Aug;18(8):2338-47.

(6)Bahram Tahmasbi, Ghasem Abedi, Mahmood Moosazadeh, Ghasem Janbabai, Fereshteh Farshidi, et al. Determining the Survival Rate of Colorectal Cancer in Iran: A Systematic Review and Meta-Analysis. Asian Pac J Cancer Prev. 2018; 19(11): 3009–3018.

(7) Noha M.Ragab, Khaled E. Soliman, Omar Shebl Zahra. Clinicopathological. Significance of Mesothelin Expression In Colorectal Cancer.International Journal of Advanced Research. 2016, 4(1): 219- 228.

(8)Kuan JC, Wu CC, Sun CA, Chu CM, Lin FG, Hsu CH, Kan PC, Lin SC, Yang T, Chou YC. DNA methylation combinations in adjacent normal colon tissue predict cancer recurrence: evidence from a clinical cohort study. PLOS ONE 10(3): 2015;e0123396.

(9) Mitchell SM, Ross JP, Drew HR, Ho T, Brown GS, Saunders NFW, Duesing KR, Buckley MJ, Dunne R, Beetson I, Rand KN, McEvoy A, Thomas ML, Baker RT, Wattchow DA, Young GP, Lockett TJ, Pedersen SK, LaPointe LC, Molloy PL. 2014. A panel of genes methylated with high frequency in colorectal cancer. BMC Cancer. 2014 Jan 31;14:54.

(10)Emilie Duchalais, Christophe Guilluy, Steven Nedellec, Melissa Touvron, Anne. Bessard,et al. Colorectal Cancer Cells Adhere to and Migrate along the Neurons of the Enteric Nervous System. Cell Mol Gastroenterol Hepatol. 2018; 5(1): 31–49.

(11)Nannini M, Pantaleo MA, Maleddu A, Astolfi A, Formica S, Biasco G. Gene. expression profiling in colorectal cancer using microarray technologies: results and perspectives. Cancer Treatment Reviews, 2009. 35:201–209.

(12)Godlewski J, Kmiec Z. Colorectal Cancer Invasion and Atrophy of the Enteric. Nervous System: Potential Feedback and Impact on Cancer Progression. Int J Mol Sci. 2020 May 11;21(9):3391.

(13)Turtoi A, Blomme A, Debois D, Somja J, Delvaux D, Patsos G, di Valentin E, Peulen O, Mutijima EN, De Pauw E, Delvenne P, Detry O, Castronovo V. Organized proteomic heterogeneity in colorectal cancer liver metastases and implications for therapies. Hepatology , 2014.59(3):924–934.

(14)Vaes N, Lentjes MHFM, Gijbels MJ, Rademakers G, Daenen KL,et al. NDRG4, an.early detection marker for colorectal cancer, is specifically expressed in enteric neurons. Neurogastroenterol Motil. 2017 Sep;29(9).

(15)Tuy HD, Shiomi H, Mukaisho KI, Naka S, Shimizu T, Sonoda H, Mekata E, Endo。Y, Kurumi Y, Sugihara H, Tani M, Tani T. ABCG2 expression in colorectal ade- nocarcinomas may predict resistance to irinotecan. Oncology Letters， 2016. 12:2752–2760.

(16)Xie ZY, Lv K, Xiong Y, Guo WH. ABCG2-meditated multidrug resistance 。 And. tumor-initiating capacity of side population cells from colon cancer. Oncology Research and Treatment , 2014. 37(11):666–670.

(17)T G Bush, T C Savidge, T C Freeman, H J Cox, E A Campbell,et al.Fulminant jejuno-ileitis following ablation of enteric glia in adult transgenic mice.Cell. 1998 Apr 17;93(2):189-201.

(18)Julie Cabarrocas 1 , Tor C Savidge, Roland S Liblau.Role of enteric glial cells in. inflammatory bowel disease.lia. 2003 Jan;41(1):81-93.

(19)Tao Z, Shi A, Li R, Wang Y, Wang X, Zhao J.Microarray bioinformatics in cancer- a review.J BUON. 2017 Jul-Aug;22(4):838-843.

(20)Huang X, Liu S, Wu L, Jiang M, Hou Y.High Throughput Single Cell RNA. Sequencing, Bioinformatics Analysis and Applications.Adv Exp Med Biol. 2018;1068:33-43.

(21)Guo Y, Bao Y, Ma M, Yang W.Identification of Key Candidate Genes and Pathways. in Colorectal Cancer by Integrated Bioinformatical Analysis.Int J Mol Sci. 2017 Mar 28;18(4):722.

(22)Bujko M, Kober P, Mikula M, Ligaj M, Ostrowski J, Siedlecki JA. Expression. changes of cell–cell adhesion-related genes in colorectal tumors. Oncology Letters, 2015. 9:2463–2470.

(23)Cardoso J, Boer J, Morreau H, Fodde R. Expression and genomic profiling of. colorectal cancer. Biochimica et Biophysica Acta (BBA)–Reviews on Cancer , 2007. 1775(1):103–137.
(24)Chan SK, Griffith OL, Tai IT, Jones SJ. Meta-analysis of colorectal cancer gene. expression profiling studies identifies consistently reported candidate biomarkers. Cancer Epidemiology, Biomarkers & Prevention, 2008. 17:543–552.
(25)Chang YT, Huang CS, Yao CT, Su SL, Terng HJ, Chou HL, Chou YC, Chen KH, Shih YW, Lu CY, Lai CH, Jian CE, Lin CH, Chen CT, Wu YS, Lin KS, Wetter T, Chang CW, Chu CM. Gene expression profile of peripheral blood in colorectal cancer. World Journal of Gastroenterology. 2014;20(39):14463–14471.
(26)Chang YT, Yao CT, Su SL, Chou YC, Chu CM, Huang CS, Terng HJ, Chou HL, Wetter T, Chen KH, Chang CW, Shih YW, Lai CH. Verification of gene expression profiles for colorectal cancer using 12 internet public microarray datasets. World Journal of Gastroenterology, 2014. 20(46):17476–17482.
(27)Valès S, Bacola G, Biraud M, Touvron M, Bessard A,et al.Tumor cells hijack. enteric glia to activate colon cancer stem cells and stimulate tumorigenesis.EBioMedicine. 2019 Nov;49:172-188.