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ORIGINAL ARTICLE

Identification of disrupted pathways in ulcerative colitis-related colorectal carcinoma by systematic tracking the dysregulated modules

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Summary

Purpose: *The aim of this study was to identify the altered biological pathways associated with ulcerative colitis (UC)-related colorectal carcinoma (CRC) by systematic tracking the dysregulated modules from re-weighted protein-protein interaction (PPI) networks based on the expression profiles from normal, UC and various stages of CRC.*

Methods: *We firstly recruited the UC- and CRC-related microarray data from ArrayExpress database, and obtained 8 expression profiles which contained 5 conditions (normal, UC, early stage CRC, stage II CRC and stage III CRC). Then, the PPI networks of normal and different disease stages were constructed and re-weighted using Pearson correlation coefficient (PCC). Next, the condition-specific modules were extracted from 5 PPI networks via clique-merging algorithm, and altered modules were captured on the basis of module correlation density (MCD). Subsequently, the gene compositions of altered modules and gene differential expressions in different disease stages were identified to screen the dysregulated genes. Finally, pathways enrichment analyses for the genes in altered modules and differentially expressed genes (DEGs) were implemented.*

Results: *The extensive changes of gene correlations existed in 5 condition-specific PPI networks, which made different MCDs among different disease stages. The same number of modules (N=1952) were explored in 5 PPI networks. By comparing with normal condition, there were 463, 791, 1060 and 345 altered modules in UC, early stage CRC, stage II and III CRC, respectively. Overall, 77, 110, 170 and 110 common genes were identified between genes of altered modules and DEGs in UC, early stage CRC, stage II CRC and stage III CRC, respectively. Functional enrichment analyses indicated that cell cycle and oocyte meiosis were the common and most significant pathways in colonic diseases.*

Conclusions: *Tracking the altered modules from PPI networks is useful to uncover disrupted pathways in colonic diseases. Cell cycle and oocyte meiosis might be associated with the pathophysiological background of colonic diseases.*

Key words: *altered modules, colorectal carcinoma, module correlation density, Kyoto Encyclopedia of Genes and Genomes, ulcerative colitis*

Introduction

Globally, CRC is the second most frequent cancer in women and the third in men [1]. It is predicted that nearly 376,300 new cases will be diagnosed and 191,000 will die from CRC in China in 2015. Despite advances in diagnosis and treatment in the last decades, the prognosis of patients with CRC is still poor, and about half of the cases after treatment die from this disease [2,3]. The

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outcomes depend on disease stage, resistance to chemotherapy, and so on. According to American Joint Committee on Cancer, 5-year survival rate is more than 80% in early-stage patients, yet decreases to less than 10% in patients with metastasized cancer (stage IV) [4]. This raised a query if some of such deaths could be avoided when these cases were diagnosed in early stage. Thus, understanding the local pathophysiological alterations is very important.

Several lines of evidence have demonstrated that inflammatory bowel disease (IBD) is a high risk factor for developing CRC [5]. UC, the most common clinical form of IBD, is a chronic relapsing inflammatory condition of the gastrointestinal tract, which is caused by an inappropriate and continuous inflammatory response to microbes in genetically susceptible hosts [6]. In patients with UC, about 16% develop CRC over 30 years [7]. Hence, targeting inflammatory mediators can decrease the incidence of CRC. Nevertheless, suppressing inflammation in the therapeutic or preventing process is in its infancy. Exploring the molecular pathways involved in cancer-related inflammation could contribute to illuminate the underlying mechanism of CRC.

High throughput microarray technology, as a powerful approach for expression profiling, has played important roles in the molecular research of various diseases. Previous researches have produced a considerable amount of microarray data associated with colonic disease, including UC [8-10] and CRC [11-14], and focused on the features in a specific condition of colonic disease. However, IBDs predisposing to CRC have been discussed less in gene expression level. So far, comparative microarray analyses of samples from all these colonic disorders have not been reported in the literature. Complementarily, comprehensive analyses across UC and different CRC stages in parallel were conducted to determine conditional-specific and significant pathways involved in UC-related CRC in the present paper. Importantly, this examination is helpful to find significant alterations with lower risk of unspecificity [13].

As we all know, protein interactions play broad roles in understanding the cellular functions. With the advert of high-throughput techniques, significant amounts of protein interactions are generated. Nevertheless, it is noteworthy that protein interactions identified with high throughput techniques can give high false positive and negative rates [15]. Thus, the method for evaluating the reliability of protein interactions is very important. Liu and colleagues devised an iterative scoring approach to evaluate the reliability of interactions [16]. Moreover, this method has been demonstrated to be better than the FSWeight method proposed by Chua et al. [17]. But it is difficult to study the different stages of cancer synchronously, and genes involved in different cancer stages may play different roles. Importantly, Srihari et al. [18] offered a way to track the genes and modules behavior across specific conditions.

In the current study, with the aim of further revealing the mechanism of the initiation and progression of CRC, we conducted a systematic tracking of altered modules from the re-weighted PPI networks to extract the significant and disturbed pathways among UC, early-stage CRC, stage II CRC and stage III CRC. Our results will provide further insights into the pathogenesis of colonic diseases. Moreover, the identified pathways might enforce the theoretical basis of drug discovery for CRC.

Methods

Data acquisition and preprocessing

A total of 8 gene expression datasets, including E-GEOD-9452 [8], E-GEOD-13367 [9], E-GEOD-36807 [10], E-GEOD-9348 [11], E-GEOD-37892 [12], E-GEOD-60697, E-GEOD-4183 [13] and E-GEO-23878 [14], were recruited from ArrayExpress database based on the platform of Affymetrix Human Genome U133 Plus 2.0 Array. E-GEOD-9452 microarray profile contained 8 UC samples with macroscopic signs of inflammation, 13 UC without macroscopic signs of inflammation and 5 control subjects. E-GEOD-13367 included 8 active UC, 9 quiescent UC and 10 controls. A total of 15 UC samples and 7 control samples were obtained from E-GEOD-36807. E-GEOD-9348 included 70 early-stage CRC patients and 12 controls. E-GEOD-37892 included 73 CRC patients with stage II, 57 with stage III CRC, and Gene profile E-GEOD-60697 contained 20 CRC cases with stage III. We obtained 15 cases with CRC, 15 with IBD, 15 with adenoma and 8 healthy controls from E-GEOD-4183. E-GEOD-23878 included 35 CRC and 24 normal samples. Then, we extracted and merged the samples across 8 datasets. The merged data were divided into 5 parts based on disease status, including normal, UC, early-stage CRC, stage II CRC and stage III CRC.

Prior to analysis, data preprocessing was conducted to eliminate undesired batch effects. Preprocessing procedures included background correction with robust multichip average method, normalization by means of quartile function, and perfectmatch/mismatch probe correction using micro array suite 5.0. Then, the probe data filtration was carried out over FeatureFilter meth-

od. Finally, a total of 20,102 genes were obtained.

Conditional-specific PPI networks

The Search Tool for the Retrieval of Interacting Genes (STRING) database provides all information about functional links between proteins, and the interactions are offered with a combine-score in STRING [19]. In the current study, all human protein interactions (N=1,048,576) with combine-scores were recruited from STRING database. To minimize false positive rate, only interactions with combine-score ≥ 0.7 were remained, including 9745 nodes and 63,869 interactions. By intersecting with the expression data, the PPI sub-network, including 9117 nodes and 58494 interactions, was constructed.

Subsequently, we constructed and re-weighted the conditional-specific PPI networks by Pearson's correlation coefficient (PCC), a well-established measure of correlation between two variables. H_S, H_U, H_E, H_{II} and H_{III} were defined as the conditional-specific PPI sub-network of normal, UC, early-stage CRC, stage II CRC and stage III CRC, respectively. Meanwhile, PCC value of each interaction in normal and different stages of colonic disease and the changed values in different stages relative to normal condition were computed. The calculation of PCC was based on the formula described previously [20].

Modules from conditional-specific PPI sub-networks

From 5 conditional-specific PPI sub-networks, the conditional-specific modules were inferred using a clique-merging algorithm [21]. Firstly, all maximal cliques with node size more than 5 in PPI sub-networks were screened by means of fast depth-first method. Subsequently, the weighted interaction density (WID) of each maximal clique was computed and then ranked in descending sort. The formula of WID was as follows:

$$score(C)=\frac{\sum_{u\in C,v\in C} w(u,v)}{|C|\cdot(|C|-1)}$$

where score(C) meant the WID score of the clique C; w(u,v) represented the weight of the interaction between u and v.

A number of maximal cliques might overlap with each other, because there were numerous cliques in the PPI network. To reduce the result size, the overlapped maximal cliques should be removed or merged. To achieve this, the weighted inter-connectivity (WIC) between 2 cliques was calculated to determine whether these 2 overlapped cliques were merged or not based on the following formula:

$$score(C_1,C_2)=\sqrt{\frac{\sum_{u\in(C_1-C_2)}\sum_{v\in C_2} w(u,v)}{|C_1-C_2|\cdot|C_2|}\cdot\frac{\sum_{u\in(C_2-C_1)}\sum_{v\in C_1} w(u,v)}{|C_2-C_1|\cdot|C_1|}}$$

The cliques mentioned above were sorted in descending order based on their WIC,named as clique $[C_1]$, $[C_2]$, $[C_3]$..... $[C_n]$. The specific steps of removing and merging the highly overlapped cliques were as follows: For every maximal clique $[C_i]$ in the list, we repeatedly checked whether a maximal clique $[C_j]$ existed. If the ratio of the overlap between maximal clique $[C_i]$ and maximal clique $[C_j]$ was more than t_0 which was a predefined overlap-threshold. The t_0 was set as 0.5 in our study. If there was such a maximal clique $[C_j]$, the WIC was calculated between the non-overlapping proteins of $[C_i]$ and $[C_j]$. When WIC was higher than tm, a predefined merge-threshold (tm was set as 0.25 in the present study), the maximal clique $[C_j]$ was merged into maximal clique $[C_i]$ to develop a module. Otherwise, maximal clique $[C_j]$ was abandoned.

Altered modules in different disease stages

H_S, H_U, H_E, H_{II} and H_{III} stood for the PPI sub-network of normal, UC, early stage CRC, stage II CRC and stage III CRC, respectively. The sets of modules $S = \{S_1, S_2, ..., S_h\}$, $U = \{U_1, U_2, ..., U_l\}$, $E = \{E_1, E_2, ..., Ek\}$, $M = \{M_1, M_2, ..., M_m\}$, and $T = \{T_1, T_2, ..., T_n\}$ were indentified from H_S, H_U, H_E, H_{II} and H_{III} , respectively. For the module S, the module correlation density (MCD) was computed as follows:

$$d_{cc}(S_i)=\frac{\sum_{u,v\in S_i} PCC((u,v),N)}{|S_i|(|S_i|-1)}$$

Similarly, the MCDs of modules in the other conditions were also computed. Subsequently, randomization test was used to measure the set of altered module pairs based on the values of MCD in 5 conditions. P value was adjusted using false discovery rate (FDR) which was computed using the Benjamini & Hochberg method [22]. The altered modules were identified based on FDR less than 0.01.

Differentially expressed genes in different disease stages

It is indicated that the difference of gene expression levels could reflect the disease propensity. In this work, in order to identify important genes at different disease stages, DEGs in different disease stages were screened based on the expression profiles using linear models for microarray data (LIMMA) package [23]. The values of |logFoldChange| > 2 and $p < 0.01$ were chosen as the cutoff threshold.

Table 1. Differentially expressed genes in ulcerative colitis and different stages of colorectal carcinoma

	Total DEGs	*Up-regulated DEGs*	*Down-regulated DEGs*
UC	1104	679	425
Early stage CRC	1090	451	639
Stage II CRC	1073	714	359
Stage III CRC	1644	1022	622

DEGs: differentially expressed genes, UC: ulcerative colitis, CRC: colorectal carcinoma

Pathway enrichment analysis

Database for Annotation, Visualization and Integrated Discovery (DAVID) is an analytic tool to investigate the biological meaning for a mass of genes [24]. In our study, DAVID was utilized for KEGG pathway enrichment analysis for genes in altered modules of different disease stages. Similarly, pathway enriched analyses were performed for DEGs of each disease stage. A p value <0.01 was applied to extract the significant terms.

Results

Expression data

By merging 8 expression datasets, all samples were divided into 5 parts based on disease status, including 85 normal, 28 UC, 70 early-stage CRC, 73 stage II CRC and 77 stage III CRC. After data preprocessing, a total of 20,102 genes were extracted among these 5 groups. We further calculated the differential expression levels of genes in different disease stages relative to normal condition. Under the threshold values of |logFoldChange| > 2 and p<0.01, a total of 1104,1090, 1073 and 1644 DEGs were identified in UC, early-stage CRC, stage II CRC and stage III CRC, respectively. The details are shown in Table 1.

Disruptions in PPI sub-networks

H_S, H_U, H_E, H_{II} and H_{III} stood for the re-weighted PPI sub-networks of normal, UC, early-stage CRC, stage II CRC and stage III CRC, respectively. The H_S, H_U, H_E, H_{II} and H_{III} PPI sub-networks exhibited the same number of nodes (N=9,117) and interactions (N=58,494), however, the PCC frequency distributions were different across the 5 conditions (Figure 1). Their mean scores of PCC were 0.145, 0.170, 0.100, 0.142 and 0.134, respectively. Measuring these interactions more carefully, the scores of 36,309 interactions in early-stage CRC network were higher relative to other 4 networks, especially higher comparing with normal and UC networks. Similarly, the scores of 20,605 interactions in the UC network were higher than in the other 4 networks, especially higher relative to early-stage, stage II and III CRC networks. The interactions with score changes more than 0.6 were extracted for further analysis, which included 5,937, 1,314, 4,781 and 3,945 interactions in UC, early-stage CRC, stage II and III CRC, respectively.

Disruptions in altered modules in different disease stages

Clique-merging approach was utilized to extract modules from the PPI sub-networks of normal, UC and different stages of CRC. Based on the threshold of nodes >5, a total of 5,850 maximal cliques were screened for module analysis. Subsequently, we conducted extensively analyses among normal and disease modules to comprehend the disruption or alteration of modules. As exhibited in Table 2, we explored the same number of modules (N=1,952) in the PPI sub-networks of normal, UC, early-stage CRC, stage II CRC and stage III CRC. The mean module size in normal condition was a little larger than that in the other 4 conditions. While the average MCD in normal condition was smaller than that in UC, it was greater than that in different stages of CRC. Meanwhile, modules in different conditions presented different frequency distribution of MCD (Figure 2). Next, by comparing module MCD in disease conditions with that in normal condition, a total of 463, 791, 1060 and 345 altered modules were identified in

Table 2. Properties of modules in normal and different disease stages

Module set	*No. of modules*	*Average module size*	*Correlation*		
			Max	*Min*	*Average*
Normal	1952	27.829	0.508	-0.0986	0.175
UC	1952	27.809	0.644	-0.0960	0.191
Early stage CRC	1952	27.809	0.411	-0.0580	0.103
Stage II CRC	1952	28.271	0.508	-0.101	0.157
Stage III CRC	1952	28.271	0.481	-0.0949	0.147

UC: ulcerative colitis, CRC: colorectal carcinoma

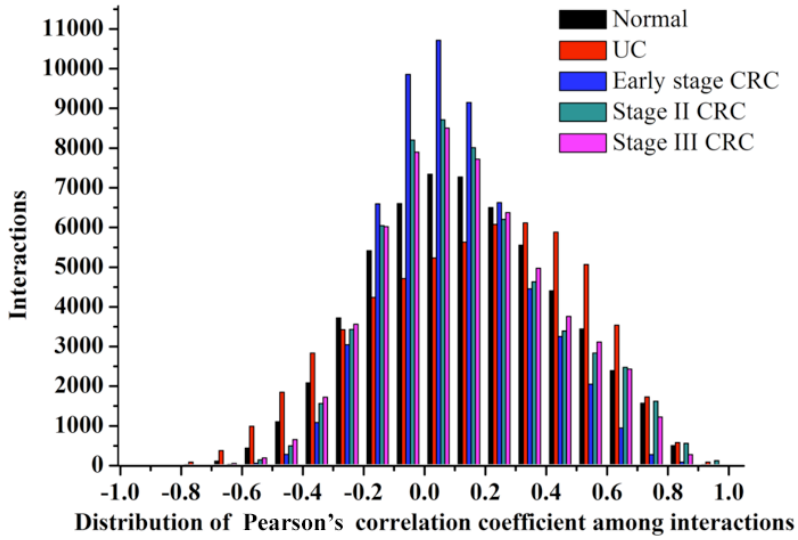

Figure 1. The expression correlationwise frequency distribution of interactions in normal, UC and different stages of CRC.

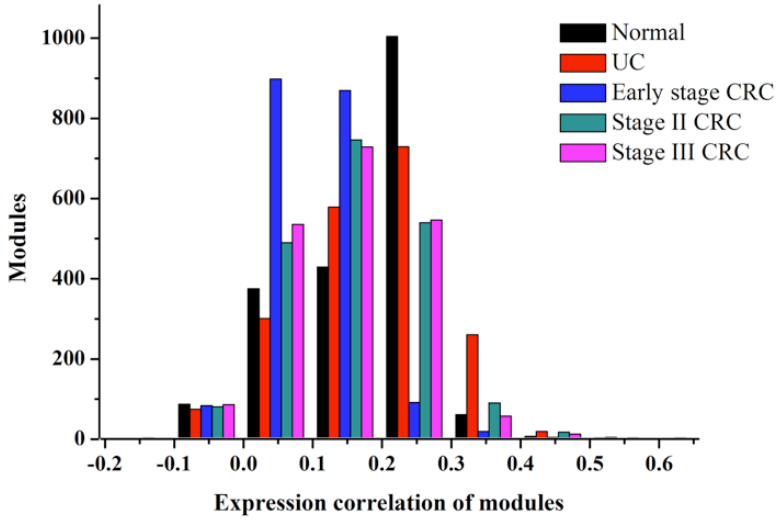

Figure 2. The correlationwise frequency distribution of module correlation density in normal, UC and different stages of CRC.

UC, early-stage CRC, stage II and III CRC, respectively.

Pathway enrichment analysis

To further explore the dysregulated biological functions in disease condition, pathway analysis was conducted based on the genes in altered modules and DEGs. Based on p value < 0.01, genes in altered modules were enriched in 17, 26, 33 and 19 significant pathways in UC, early-stage CRC, stage II CRC and stage III CRC, respectively. The top 10 significant pathways in each stage are shown in Table 3. Among these, the enriched pathways in these altered modules across different disease stages were similar. Importantly, progesterone-mediated oocyte maturation pathway was disturbed only in the altered module of stage III CRC. Moreover, the pathways of purine metabolism and RNA polymerase were mutual in different stages of CRC. Collectively, the common top 3 pathways were cell cycle, ribosome and oocyte meiosis in the altered pathways of colonic diseases.

Among these genes in altered modules, a total of 77, 110, 170 and 110 genes were differen-

Table 3. The top 10 pathways of genes in altered modules based on p<0.01

	Term name	*Count*	*p value*
UC	Proteasome	36	4.43E-35
	Cell cycle	54	5.67E-35
	Huntington's disease	62	1.22E-33
	Oxidative phosphorylation	53	9.00E-33
	Parkinson's disease	52	4.88E-32
	Alzheimer's disease	51	3.15E-25
	Ribosome	37	2.13E-23
	DNA replication	24	6.84E-21
	Oocyte meiosis	31	7.91E-14
	Mismatch repair	14	2.60E-11
Early stage CRC	Ribosome	64	1.17E-46
	Cell cycle	64	7.43E-33
	Proteasome	37	2.34E-28
	Purine metabolism	60	3.73E-23
	Huntington's disease	65	8.29E-23
	Pyrimidine metabolism	45	3.02E-21
	Nucleotide excision repair	30	4.85E-20
	RNA polymerase	24	1.00E-19
	Parkinson's disease	50	3.37E-19
	Oxidative phosphorylation	50	7.26E-19
Stage II CRC	Cell cycle	78	6.07E-38
	Ribosome	64	1.63E-37
	Oxidative phosphorylation	66	6.65E-25
	Proteasome	37	3.19E-23
	Huntington's disease	72	1.24E-19
	Nucleotide excision repair	32	2.29E-18
	Parkinson's disease	57	3.94E-18
	Purine metabolism	62	2.91E-17
	RNA polymerase	24	1.89E-16
	Alzheimer's disease	62	1.34E-15
Stage III CRC	Ribosome	63	1.84E-57
	Cell cycle	58	2.52E-37
	Proteasome	36	1.55E-33
	RNA polymerase	19	6.85E-16
	Pyrimidine metabolism	32	1.77E-15
	Spliceosome	33	1.54E-12
	DNA replication	18	4.79E-12
	Oocyte meiosis	30	6.67E-12
	Purine metabolism	33	3.87E-10
	Progesterone-mediated oocyte maturation	21	1.40E-07

UC: ulcerative colitis, CRC: colorectal carcinoma

tially expressed in UC, early-stage CRC, stage II CRC and stage III CRC, respectively. Subsequently, pathway-enriched analyses were performed for these DEGs in altered modules, and a total of 4, 6, 7 and 4 significant pathways were identified in UC, early-stage CRC, stage II CRC and stage III CRC, respectively, based on the cutoff-value of p<0.01 as shown in Table 4. Among these, cell cycle and oocyte meiosis were the mutual pathways in UC and different stages of CRC. Importantly, DNA replication and adherence junction only existed in early-stage CRC and stage III CRC group, respec-

Table 4. The significant pathways analyses of differentially expressed genes in altered modules in different stages based on p<0.01

	Term name	*Count*	*p value*
UC	Cell cycle	12	7.20E-12
	Oocyte meiosis	6	1.98E-04
	Progesterone-mediated oocyte maturation	5	8.58E-04
	Huntington's disease	6	1.88E-03
Early stage CRC	Cell cycle	15	1.91E-13
	Oocyte meiosis	9	1.61E-06
	Huntington's disease	8	4.29E-04
	DNA replication	4	2.69E-03
	Progesterone-mediated oocyte maturation		4.38E-03
	Prostate cancer		4.96E-03
Stage II CRC	Cell cycle	17	1.22E-12
	Oocyte meiosis	11	8.47E-07
	Small cell lung cancer	7	5.92E-04
	Focal adhesion	10	8.20E-04
	Huntington's disease	9	1.71E-03
	ECM-receptor interaction	6	3.88E-03
	Oxidative phosphorylation	7	5.54E-03
Stage III CRC	Cell cycle	15	2.84E-13
	Oocyte meiosis	7	2.17E-04
	Prostate cancer	6	6.69E-04
	Adherence junction	5	3.22E-03

UC: ulcerative colitis, CRC: colorectal carcinoma, ECM: extracellular matrix

tively. Moreover, the pathways of small cell lung cancer, focal adhesion, ECM-receptor interaction and oxidative phosphorylation were unique in stage II CRC.

Discussion

It is broadly accepted that inflammation exerts a critical function in CRC initiation, promotion and progression, while the molecular mechanisms of CRC progression are still unclear. With the aim of explaining the pathomechanism of UC-related CRC, we determined the altered biological pathways of 4 different stages by systematical tracking the altered modules from re-weighted PPI networks. In this paper, a total of 463, 791, 1,060 and 345 altered modules were identified in UC, early-stage CRC, stage II and III CRC, respectively. In these altered modules, a total of 77, 110, 170 and 110 DEGs were identified in UC, early-stage CRC, stage II CRC and stage III CRC. Pathway enrichment analyses for genes in altered modules and DEGs in different stages of disease were conducted. The results exhibited 2 disrupted pathways (cell cycle and oocyte meiosis), which were the most significant pathways across UC and different CRC stages.

In various organs, long-term chronic inflammation is a widely well known risk factor for cancer development [25]. UC is a chronic relapsing inflammatory condition of the gastrointestinal tract, which confers a raised risk for CRC [26]. In the current study, cell cycle was found to be the mutual altered pathway in UC and different CRC stages. Cell cycle is a series of coordinated procedures, which exerts important roles of integrating the environment signal pathways with cell proliferation and cell growth [27]. The cell division cycle is a key event, by which several internal organs are updated. In the intestine, inflammatory responses cause abnormal differentiation and proliferation of the epithelial cells, and lead to cells loss. Colon

cells replicate at a comparatively high rate with 10^{10} epithelial cells being displaced each day [28]. This high rate of replication contributes to the susceptibility of the epithelium to mutation, and further to result in the CRC formation [29]. Importantly, inflammation can cause overproduction of reactive oxygen and nitrogen species which further contribute to the initiation of CRC in UC patients via mediating pro-mitogenic activities [30]. In inflammatory cells and premalignant cells, NF-κB signaling is activated [31]. Aberrant activation of NF-κB was observed in more than 50% of CRC by increasing cell proliferation, regulating cell cycle progression as well as promoting cell invasion and metastasis [32,33]. Hence, we infer that cell cycle might be a marker pathway for identification of high CRC-risk patients within the UC.

Genomic instability is a definitive characteristic in tumor progression, and chromosomal instability is one of the forms of genomic instability, resulting from defects in chromosomal segregation, DNA damage response, and telomere stability. Chronic inflammatory microenvironment in the colon can increase oxidative stress which results in DNA damage, further contributing to CRC initiation [34]. Moreover, chromosome segregation in oocyte meiosis is controlled by kinetochores, which guarantee the fidelity of segregation [35]. Abnormal function of kinetochore causes the gains or losses of large portions of chromosomes, which leads to karyotypic variability [36]. Additionally, telomere instability is demonstrated to be related with cancer and tolemere shortening might be the earlier risk of CRC within UC [7]. Significantly, the chromosomal instability is a distinct pathway in CRC pathogenesis [37]. It is worth noting that mammalian oocytes are notorious because of high rates of chromosomal abnormalities [38]. In the current study, oocyte meiosis was the common pathway across UC and different CRC stages. Accordingly, oocyte meiosis might play important roles in the initiation, development and progression of UC-related CRC.

In conclusion, this integration-based analysis has several advantages. Unlike publications of individual disease or individual disease stage, we focused on the common mechanisms among UC, early-stage CRC, stage II and III CRC, thus providing a global understanding of the pathogenesis of intestinal disorders. Our results indicate that pathways of cell cycle and oocyte meiosis play crucial roles in initiation, development and progression of UC-related CRC. Although our study lacked experimental validations *in vivo* or *in vitro*, our findings provided some preliminary evidence to uncover potential candidate therapeutic strategies for CRC. Making use of specific blockage-related pathways in different stages of CRC will shed new insights for therapeutic and preventive methods.

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