MEDICAL SCIENCE MONITOR

CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2019; 25: 3549-3557
DOI: 10.12659/MSM.916181

Received: 2019.03.12
Accepted: 2019.04.09
Published: 2019.05.13

Impact of Colon-Specific DNA Methylation-Regulated Gene Modules on Colorectal Cancer Patient Survival

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Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
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Source of support: This work was supported by the project of Heilongjiang Provincial Health Planning Commission (2017-374)

Background: Colorectal cancer (CRC) is the second most commonly diagnosed cancer in females and the third in males worldwide. Although existing evidence explained some critical functions of the single genetic abnormality in the pathogenesis of CRC, the function of interactors involved in the colon-specific regulatory network, especially DNA methylation regulated network is still poorly understood.

Material/Methods: In this work, matched gene expression and DNA methylation samples of CRC patients were retrieved. Differential gene expression and methylation analyses were performed. In addition, gene expression and DNA methylation were integrated into a colon-specific regulatory gene network, detecting the epigenetically regulated gene modules which drive CRC through an underlying epigenetic mechanism. Finally, the colon-specific DNA methylation-regulated gene modules were validated using an independent set of CRC patients.

Results: Differential gene expression analysis demonstrated the upregulation of the cell cycle and DNA replication and downregulation of cGMP-PKG signaling pathway and calcium signaling pathway in CRC. Differentially methylated regions (DMRs) showed the different levels of methylation in promoters, CpG islands, and genes in CRC. In addition, gene expression and DNA methylation were integrated into a colon-specific regulatory gene network, detecting 8 epigenetically regulated gene modules which drive CRC through an underlying epigenetic mechanism. Interestingly, 2 of the colon-specific DNA methylation-regulated gene modules showed a significant predictive ability for the survival of an independent set of CRC patients.

Conclusions: The results of this study could open a new era and aid the development of novel therapeutic targets for the treatment of CRC patient.

MeSH Keywords: **Colorectal Neoplasms • DNA Methylation • Gene Expression • Organ Specificity • Survival Analysis**

Abbreviations: **CRC** – colorectal cancer; **CV** – chaos vector; **DAVID** – Database for Annotation, Visualization and Integrated Discovery; **DMR** – differentially methylated regions; **FDR** – false discovery rate; **FEM** – functional epigenetic modules; **KEGG** – Kyoto Encyclopedia of Genes and Genomes; **RRBS** – reduced representation bisulfite sequencing; **TF** – transcription factor

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/916181

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Background

Colorectal cancer (CRC) is the second most commonly diagnosed cancer in females and the third in males, accounting for over 1.8 million new cases and 862 000 deaths worldwide in 2018 [1]. Despite therapy advancing and prognosis improving, the treatment for CRC has still shown less benefit. The identification of clinical biomarkers is therefore critical to guide the treatment of CRC, which may lead to the development of novel therapeutic targets and further lower the risk of death in patients with CRC.

With the advancement of next-generation high-throughput sequencing, almost all transcript sequence and expression information of a specific tissue at a certain state can be rapidly generated and comprehensively studied using RNA-seq technology, describing the entire process of transforming genetic information carried by genes into discernible phenotypes [2]. Genes express differentially in the same cell type in different tissues, besides, the same cell expresses a different set of genes in different states, especially for the disease states, such as CRC [3]. Differential expression provides a hint for understanding molecular mechanisms in biological processes, which has been widely used in clinical diagnosis and drug discovery.

Gene expression can be regulated directly or indirectly without sequence changes by DNA methylation, which is defined as the process by which methyl groups are added to the DNA molecule. As physical blockade of the binding of transcriptional proteins to the gene, age-related hyper-methylation was suggested to affect SP1 binding sites or tandem B1 elements in CRC [4]. As affecting the chromatin structure through histone modifications, inhibitors of EZH2 and DOT1L, histone-methylation enzymes, have been reported as promising therapeutic effects in preclinical CRC treatment [5]. However, the impact of either gene expression or DNA methylation alone is rarely sufficient to lead to CRC, which can spread along the links of the complex intracellular network to alter the activity of gene products, especially the colon-specific gene regulatory network. These interactors might participate in similar function or cell processes, which is robust to provide valuable insights to decipher the molecular mechanisms of CRC [6].

In this work, 20 gene expression and DNA methylation samples from 10 CRC patients were integrated into a colon-specific gene regulatory network to study the potential biological mechanisms in CRC. The impact of single evidence, i.e., gene expression or DNA methylation, was first to be studied. The significantly upregulated and downregulated genes were enriched to cell cycle, DNA replication and cGMP-PKG signaling pathway, calcium signaling pathway, respectively. Differentially methylated regions (DMRs), including promoters, CpG islands, and genes, were identified, demonstrating the different levels of methylation in these DMRs in CRC. Then, the impact of the integration was studied, identifying 8 epigenetically regulated gene modules that drive CRC through an underlying epigenetic mechanism. Biomarkers that have been significantly associated with patient survival have become potential interests in helping diagnosis and treat cancers [7,8]. Thus, the 8 colon-specific DNA methylation-regulated gene modules were further validated by an independent set of CRC patients. Interestingly, 2 of these modules showed a significant predictive ability for the survival of an independent set of CRC patients. These results suggested that integrating gene expression and DNA methylation based on the network helped to explain the survival of colorectal cancer patients, which may open a new era in the development of novel therapeutic targets for the treatment of CRC patients.

Material and Methods

Gene expression, DNA methylation profiles, and regulatory network

Reduced representation bisulfite sequencing (RRBS) and RNA sequencing datasets of 20 samples from 10 CRC patients were downloaded from the GEO database (database no. GSE95656) [9], involving 10 CRC samples and 10 matched normal samples collected from the margins on either side of the resected tumor. RRBS libraries were sequenced using HiSeq 2500 (Illumina, San Diego, CA, USA), generating a minimum of 14 million 100 bp paired-ends reads per sample. The transcriptome was sequenced using HiSeq 2500 (Illumina, San Diego, CA, USA), generating a minimum of 40 million 40 bp paired-ends reads per sample. RRBS and RNA-seq reads were subsequently mapped onto the human genome (hg19). Additionally, gene expression quantification and clinical data of a cohort of 382 individuals, including 39 normal samples and 393 CRC samples (including metastatic, primary tumor and recurrent tumor) from the TCGA repository (*http://cancergenome.nih.gov/*) were used as an independent external dataset to validate the identified modules. Patient characteristics of the 382 normal or CRC individuals are listed in Table 1.

Tissue-specific regulatory networks of 394 cell types were inferred by Marbach et al. [10] from integrating transcription factor (TF) sequence motifs with promoter and enhancer activity data from the FANTOM5 project [11]. This work used one such tissue-specific regulatory network from the colon of adults, involving 1 215 042 non-redundant regulations from 15 057 genes with a weight of regulations inferred using TF binding motifs and tissue-specific expression of target elements [10]. The top 10 000 regulations from 1909 genes were used to construct a colon-specific regulatory network in this work.

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Table 1. Patient characteristics.

Characteristics	Number of patients (N=382)
Sex	
Male	202 (52.9%)
Female	178 (46.6%)
Age at diagnosis	
Median	67.5
Range	31.2–90.1
Stage	
Stage I	65 (17.0%)
Stage II	149 (39.0%)
Stage III	102 (26.7%)
Stage IV	53 (13.9%)

Differential expression and methylation analysis

Differentially expressed genes were identified using the R/Bioconductor package DESeq2 v 1.22.1 [12]. Wald test and false discovery rate (FDR) were used to calculate the statistical *P*-value and the adjusted *P*-value, respectively. Significantly differential expression genes were defined as FDR <0.01 and the absolute value for $\log_2$(fold-change) more than 1.5. Differentially methylated sites and regions, including CpG islands, genes, and promoters were detected using the R/Bioconductor package RnBeads v 2.0.0 [13]. Linear modeling and FDR were used to calculate the statistical *P*-value and the adjusted *P*-value, respectively. Significantly differentially methylated sites were defined as FDR <0.01, while significantly differentially methylated regions (DMRs) were defined as FDR <0.1. Heatmaps were generated using the R/Bioconductor package gplots v 3.0.1 [14], representing the gene expression or methylation levels using a color-coding system.

Identification of epigenetically regulated gene modules using functional epigenetic modules (FEM) algorithm

Epigenetically regulated gene modules which drive disease through an underlying epigenetic mechanism were detected using functional epigenetic modules (FEM) algorithm [15], by which the interactome hotspots of differential gene expression and differential DNA promoter methylation were identified. As the levels of methylation are normally anti-correlated with gene expression, a module with more genes that possessed a higher negative correlation between gene expression and DNA methylation had a higher module score [15]. Monte Carlo randomization procedure was used to calculate the *P*-value of the modules. In case of obtaining stable results, permutation times were set to 10 000.

Pathway enrichment and annotation analysis

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was performed using Database for Annotation, Visualization and Integrated Discovery (DAVID) v 6.8 [16,17], which used the hypergeometric test to calculate the statistical *P*-values. Significantly enriched pathways were defined as FDR <0.2. Pathway annotation analysis was performed using the R/Bioconductor packages clusterProfiler v 3.8.1 [18] and DOSE v 3.6.1 [19]. Annotated pathways with FDR <0.2 were shown. Boxplots that represented the significance of the listed pathways were generated using the R/Bioconductor package ggplot2 v 3.1.0 [20].

Survival analysis

Survival curves were estimated by the Kaplan-Meier method and compared with the log-rank test. For each candidate module, we calculated a chaos vector (CV) for each sample using the Pearson correlation coefficient of the module gene expression levels with normal samples [21]. Then, these vectors were used to cluster samples into different subgroups to assess its predictive ability as a predictor of survival [22].

Results

Identification of CRC-related pathways using differential expression analysis

As shown in Figure 1A, 473 and 1767 genes were respectively detected as significantly upregulated and downregulated genes (FDR <0.01, $|\log_2$(fold-change)$|$ >1.5), while 1843 genes were detected as marginally significant regulated genes (FDR <0.01, $|\log_2$(fold-change)$|$ <1.5). These genes showed different expression patterns in CRC and normal samples (Figure 1B). Functional enrichment analysis of these 473 upregulated genes demonstrated that cell cycle (FDR=1.31e-13), progesterone-mediated oocyte maturation (FDR=1.23e-03), oocyte meiosis (FDR=2.15e-03), and DNA replication (FDR=4.23e-03) were upregulated in CRC (Figure 1C). Involved in cell cycle, for example, *CDK1* was identified as upregulated (FDR=3.52e-10), which was reported as upregulated in CRC tissues and cell lines [23]. For another example, the increased expression of *CCNB1* was observed in CRC as previously reported [24,25], which was also identified as an upregulated gene in CRC in this study (FDR=1.49e-09). On the other hand, functional enrichment analysis of the 1767 downregulated genes showed the close relation with cGMP-PKG signaling pathway (FDR=2.32e-09), calcium signaling pathway (FDR=2.91e-09), circadian entrainment (FDR=7.09e-09), vascular smooth muscle contraction (FDR=2.02e-08), ECM-receptor interaction (FDR=1.94e-06), and focal adhesion (FDR=6.97e-06) etc. As in the existing finding,

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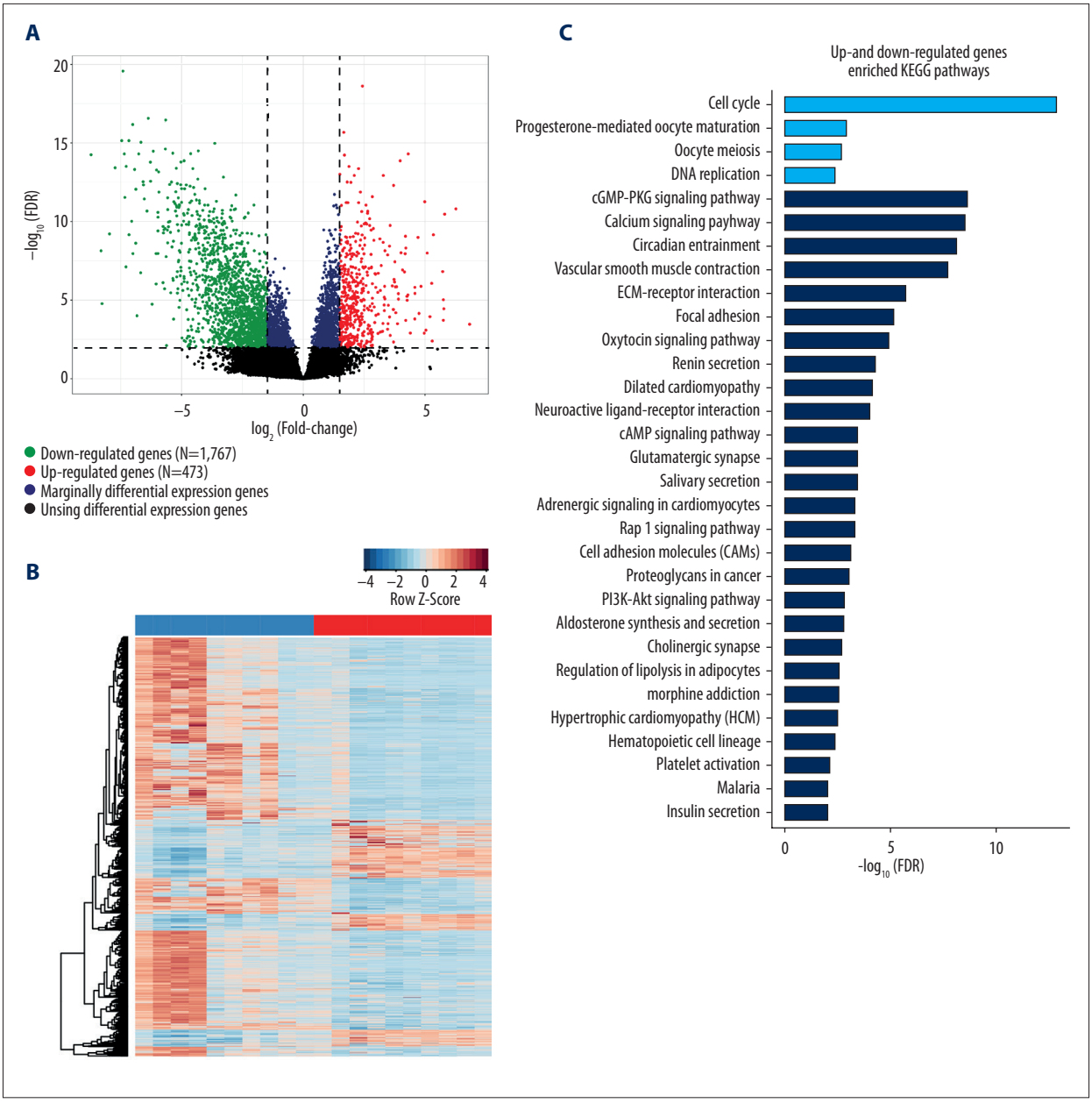

Figure 1. Differential expression analysis of CRC samples. (**A**) Volcano plot of $\log_2$(fold-change) versus $-\log_{10}$ (FDR) for all 18 891 genes in CRC versus normal. Downregulated and upregulated genes were respectively highlighted in green and red (FDR <0.01, $|\log_2$(fold-change) >1.5|). (**B**) Heatmap of the differentially expressed genes. (**C**) Significantly enriched KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways (FDR <0.2) of downregulated and upregulated genes in CRC.

therapeutic activation of cGMP/PKG was reported as offering a promising avenue for the prevention of CRC [26]. Taken together, differential expression analysis showed the consistency with the known CRC-related differentially expressed genes and pathways.

Different levels of methylation in three categories of DMRs in CRC

Then, differential methylation analysis was performed for 20 methylation samples of the 10 CRC patients. There were 13 958 and 6455 sites respectively detected as significantly hyper- and hypo-methylated sites (FDR <0.01, $|\log_2$(fold-change)| >1.5), while 3062 sites were detected as marginally significant differential methylation sites (FDR <0.01, $|\log_2$(fold-change)| <1.5).

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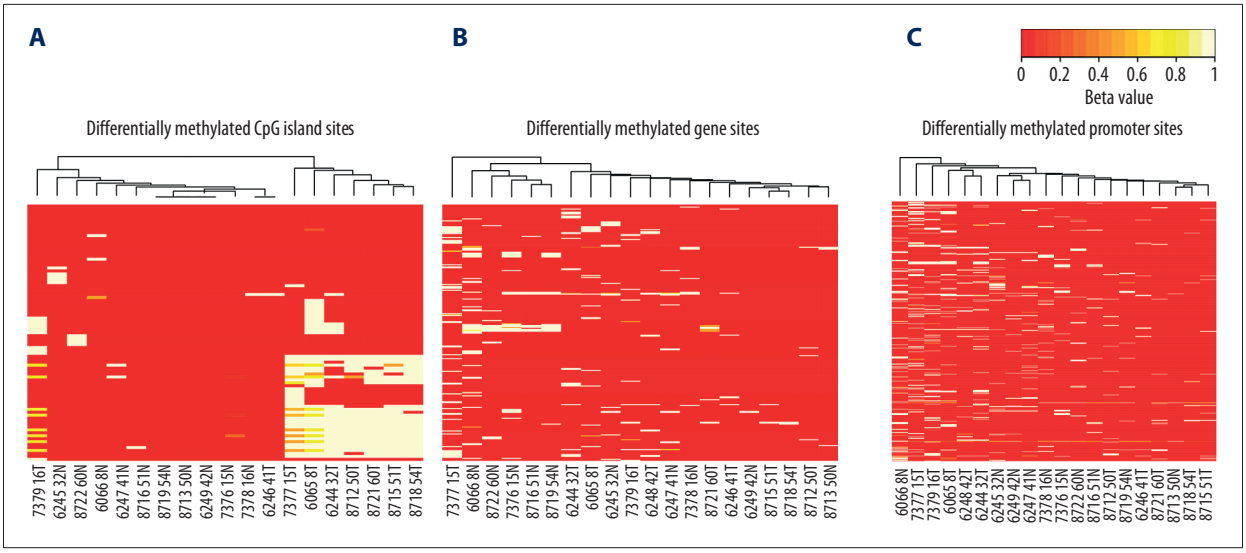

Figure 2. Heatmaps of the sites from differentially methylated regions. (**A**) Differentially methylated CpG island sites. (**B**) Differentially methylated gene sites. (**C**) Differentially methylated promoter sites.

Next, 141 regions were identified as DMRs (FDR <0.1), including 6 CpG islands, 35 genes, and 100 promoters, among which 3 CpG islands, 31 genes, and 71 promoters were identified as hyper-methylated regions, respectively. These 141 DMRs, including 87 CpG island sites, 262 gene sites, and 637 promoter sites, showed different levels of methylation in the 3 DMR categories in CRC (Figure 2A–2C). Hyper-methylation was observed in some CpG island sites in most CRC samples (Figure 2A). Taken together, different levels of methylation in CpG islands, genes, and promoter implied the different mechanisms of these DMRs in CRC.

Detection of colon-specific DNA methylation-regulated gene modules

Next, 8 colon-specific DNA methylation-regulated gene modules, including 194 genes, were detected using FEM algorithm as described in Materials and Methods section (*P*-value <0.05; Figure 3A, 3B, and Figure 4A–4F). Eight out of the 194 genes were detected in at least 2 modules, including *BHLHA15*, *CEBPD*, *CEBPE*, *CLDN18*, *FOXA2*, *FOXK1*, *HAND2*, and *LEP*. Although no difference was found in gene expression and DNA methylation for *FOXK1* (Figure 3A), *FOXK1* was demonstrated as an oncogenic factor in CRC [27–29]. The ability for the identification of such non-significant genes indicated the comprehensiveness of these colon-specific DNA methylation-regulated gene modules. Besides, *TFAP2C* was identified as robustly upregulated in CRC tissues and cells in the previous study [30], indicating the potential role of serving as a novel prognostic factor in CRC patients. Despite only the slight upregulation in gene expression of *TFAP2C* in our study ($\log_2$(fold-change)=0.38), the methylation at the promoter of *TFAP2C* was identified as downregulated ($\log_2$(fold-change)=–2.53; Figure 3B), indicating the potential role of methylation in regulating the gene expression of *TFAP2C*. For another example, *ELF3* was found to be hypo-methylated in CRC (Figure 5A), the upregulated gene expression of which has been reported as being associated with poor prognosis in CRC [31], implying the possibility of DNA methylation-mediated gene regulation in CRC. In addition, hypo-methylation was identified in *UNC3* (Figure 5B), indicating the underlying role of methylation-regulated *UNC3* expression in CRC. Therefore, these modules are able to include genes with a slight difference in either gene expression or DNA methylation through the interactions with significantly differential genes. Besides, some genes in these modules showed a significant difference in gene expression and DNA methylation, which have not been noticed as CRC biomarkers. Notably, *CLDN18*, for example, was identified as downregulated in gene expression ($\log_2$(fold-change)=–1.13) while upregulated in DNA methylation ($\log_2$(fold-change)=4.18; Figure 3A), only being reported as associated with non-small-cell lung cancer [32]. Therefore, functional epigenetic module detections using tissue-specific regulatory network have the ability to provide a more comprehensive understanding of the characteristics of biomarkers in CRC.

Two colon-specific DNA methylation-regulated gene modules showed significant relationship to survival of CRC patients

In order to assess the predictive ability of the 8 colon-specific DNA methylation-regulated gene modules on colorectal cancer patient survival, an independent set of CRC patients from TCGA was used. As shown in Table 1, 52.9% of the patients were male, while 46.6% were female. The median age of diagnosis is 67.5 years, though it ranged from 31.2 to 90.1 years. The portions of the patients with stage I, II, III, and IV were

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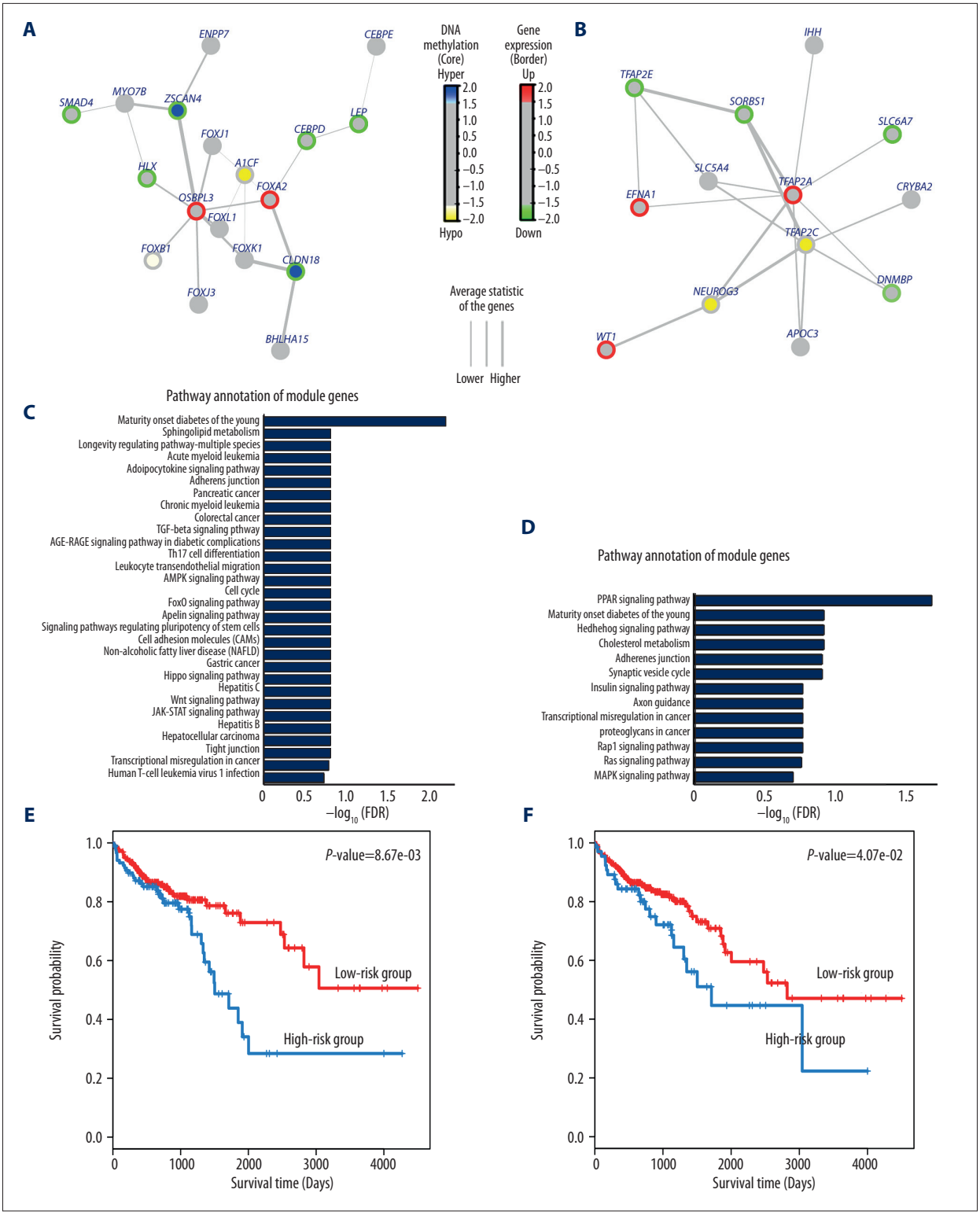

Figure 3. Two colon-specific DNA methylation-regulated gene modules associated with the survival of CRC patients. (**A, B**) The network structure of the 2 modules. Edge widths represented the average $\log_2$(fold-change) of the genes making up the edge. Core of the node represented the $\log_2$(fold-change) of DNA methylation. Border of the node represented the $\log_2$(fold-change) of gene expression. (**C, D**) Annotated pathways of the 2 modules (FDR <0.2). (**E, F**) Survival curves of the 2 modules in an independent set of CRC patients.

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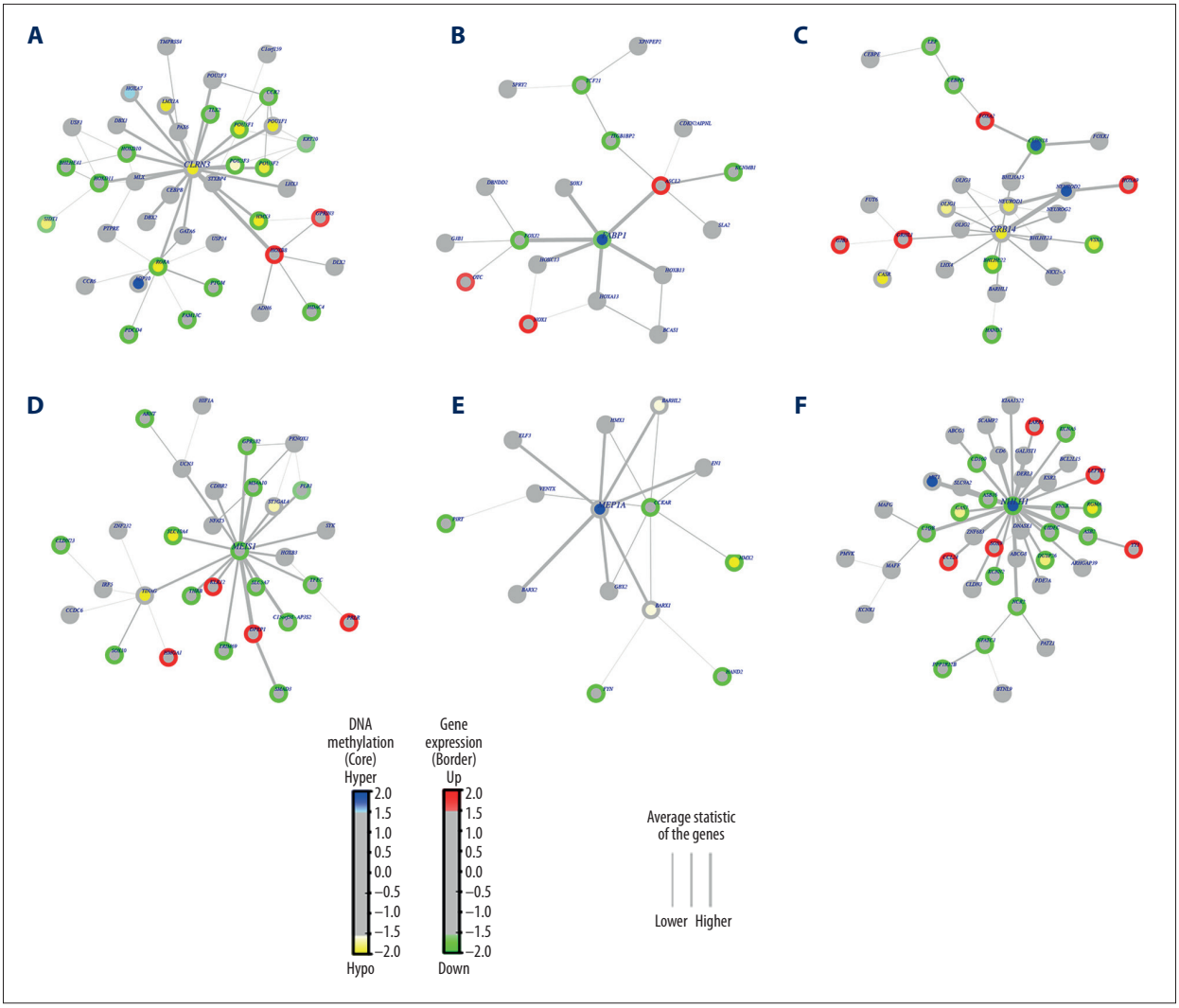

Figure 4. (**A–F**) Six colon-specific DNA methylation-regulated gene modules. Edge widths represented the average $\log_2$(fold-change) of the genes making up the edge. Core of the node represented the $\log_2$(fold-change) of DNA methylation. Border of the node represented the $\log_2$(fold-change) of gene expression.

17.0%, 39.0%, 26.7%, and 13.9%, respectively. Interestingly, 2 out of the 8 modules showed significant relation to survival of CRC patients using the log-rank test (*P*-value=8.67e-03, *P*-value=4.07e-02; Figure 3E, 3F). The patients with CVs that were more different from normal samples had a lower possibility of survival. The pathway annotation analysis demonstrated that these 2 modules showed a close relation to maturity onset diabetes of the young (Figure 3C,3D). Several studies have suggested that diabetes had a negative effect on CRC [33,34]. In addition to that, the 2 modules were annotated by the immune-related pathways, including AMPK signaling pathway [35], PPAR signaling pathway [36], and TGF-beta signaling pathway [37]. Taken together, these 2 colon-specific DNA methylation-regulated gene modules showed significant relation to survival of CRC patients, implying a novel direction of potential targets for the treatment of CRC.

Discussion

CRC is one of the most commonly diagnosed cancers worldwide with poor treatment outcomes. The aim of this work is to integrate gene expression and DNA methylation information into a colon-specific regulatory network to detect the colon-specific DNA methylation-regulated gene modules, which were further validated using an independent set of CRC patients. Following this, 473 and 1767 significantly upregulated and downregulated genes were identified which were enriched to cell cycle, DNA replication and cGMP-PKG signaling pathway, calcium signaling pathway, respectively. Besides, 6 differentially methylated CpG islands, 35 differentially methylated genes, and 100 differentially methylated promoters were detected. These DMRs suggested the different levels of methylation in these 3 categories of DMRs in CRC.

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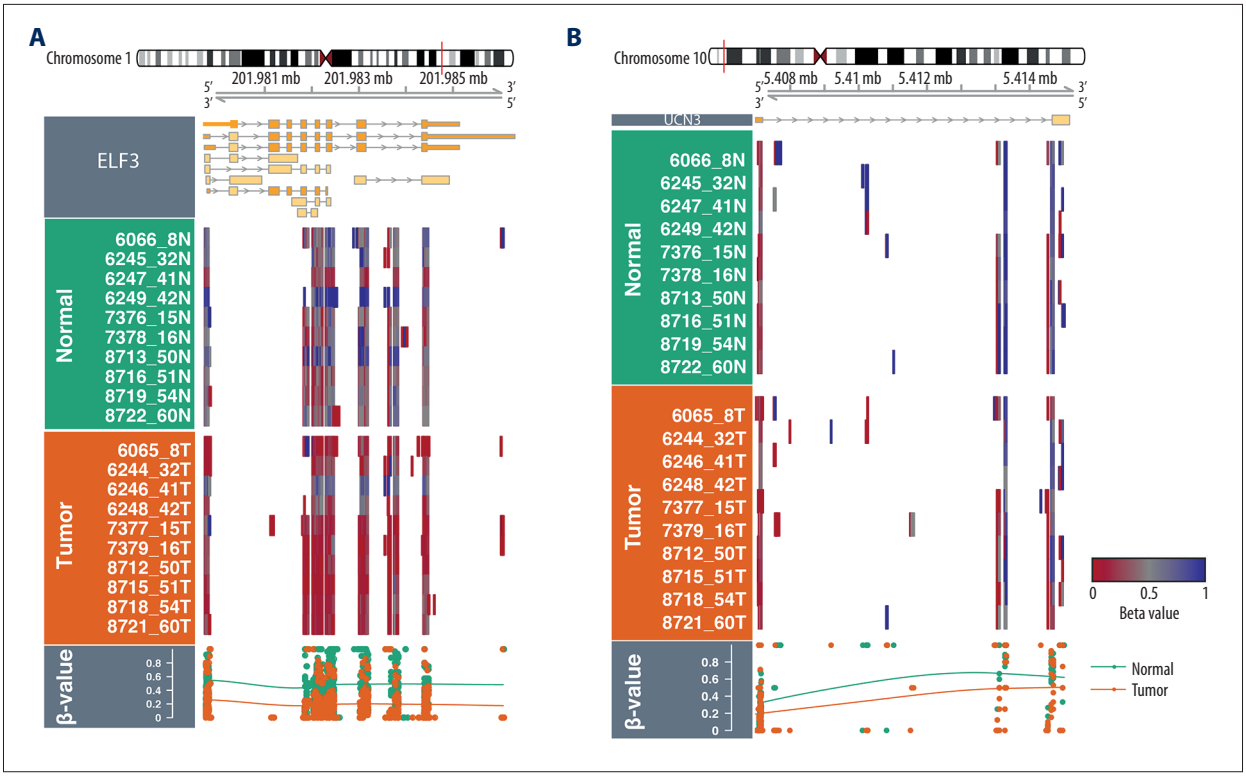

Figure 5. (**A, B**) Methylation level of *ELF3* and *UCN3* in CRC and normal samples. **The upper panel**, the genome coordinates of the genes. **The lower panel**, gene structures, heatmaps of the sample methylation levels, beta values of each sample and the fitted curves. The color represented different sample groups.

Unlike the traditional method that identifies the single biomarker, the integrated method proposed in this study detected the CRC biomarkers taking into consideration of gene expression, DNA methylation and colon-specific regulatory network, which could involve genes according to the interactions with significantly differential genes, despite of a slight difference in either gene expression or DNA methylation. Notably, 2 out of the 8 epigenetically regulated gene modules which drive CRC through an underlying epigenetic mechanism showed the significant predictive ability for the survival of the independent set of CRC patients. In addition to the immune-related pathways [35–37], pathway annotation analysis showed a close relationship between the two modules with maturity onset diabetes of the young, which has been suggested as a negative effect on CRC [33,34]. The cooperation of these module genes might have the potential to be the novel clinical biomarkers for CRC, despite the fact that experimental and clinical validations should be performed in the future. Drugs that effectively blockade the downregulation and hyper-methylation of *CLDN18*, along with the upregulation of *OSBPL3* and *FOXA2*, as well as the downregulation of *CEBPD* might contribute to the therapy of CRC patients. On the other hand, the stratification of the CRC patients in this work might suggest a different clinical treatment for patients in high-risk or low-risk group.

Conclusions

In conclusion, by integrating gene expression and DNA methylation data from matched samples of CRC patients into a colon-specific regulatory gene network, this work detected 8 colon-specific DNA methylation-regulated gene modules which drive CRC through an underlying epigenetic mechanism, 2 of which were further validated possessing the significant predictive ability for the survival of an independent set of CRC patients, contributing to the development of novel therapeutic targets for the treatment of CRC patients.

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References:

1. WHO. Cancer. 2018; Available from: *https://www.who.int/news-room/fact-sheets/detail/cancer*
2. Reuter JA, Spacek DV, Snyder MP: High-throughput sequencing technologies. Mol Cell, 2015; 58(4): 586–97
3. Jung M, Pfeifer GP: Aging and DNA methylation. BMC Biol, 2015; 13: 7
4. Lao VV, Grady WM: Epigenetics and colorectal cancer. Nat Rev Gastroenterol Hepatol, 2011; 8(12): 686–700
5. Huang T, Lin C, Zhong LL et al: Targeting histone methylation for colorectal cancer. Therap Adv Gastroenterol, 2017; 10(1): 114–31
6. Barabasi AL, Gulbahce N, Loscalzo J: Network medicine: A network-based approach to human disease. Nat Rev Genet, 2011; 12(1): 56–68
7. Bast RC Jr.: Molecular approaches to personalizing management of ovarian cancer. Ann Oncol, 2011; 22(Suppl. 8): viii5–15
8. Mok TS: Personalized medicine in lung cancer: What we need to know. Nat Rev Clin Oncol, 2011; 8(11): 661–68
9. Hanley MP, Hahn MA, Li AX et al: Genome-wide DNA methylation profiling reveals cancer-associated changes within early colonic neoplasia. Oncogene, 2017; 36(35): 5035–44
10. Marbach D, Lamparter D, Quon G et al: Tissue-specific regulatory circuits reveal variable modular perturbations across complex diseases. Nat Methods, 2016; 13(4): 66–70
11. Lizio M, Harshbarger J, Shimoji H et al: Gateways to the FANTOM5 promoter level mammalian expression atlas. Genome Biol, 2015; 16: 22
12. Love MI, Huber W, Anders S: Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol, 2014; 15(12): 550
13. Muller F, Scherer M, Assenov Y et al: RnBeads 2.0: comprehensive analysis of DNA methylation data. Genome Biol, 2019; 20(1): 55
14. Warnes GR, Bolker B, Bonebakker L et al: gplots: Various R programming tools for plotting data. R Package Version, 2009; 2(4): 1
15. Jiao Y, Widschwendter M, Teschendorff AE: A systems-level integrative framework for genome-wide DNA methylation and gene expression data identifies differential gene expression modules under epigenetic control. Bioinformatics, 2014; 30(16): 2360–66
16. Kanehisa M, Sato Y, Kawashima M et al: KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res, 2016; 44(D1): D457–62
17. Dennis G Jr., Sherman BT, Hosack DA et al: DAVID: Database for annotation, visualization, and integrated discovery. Genome Biol, 2003; 4(5): P3
18. Yu G, Wang L G, Han Y, He QY: clusterProfiler: An R package for comparing biological themes among gene clusters. OMICS, 2012; 16(5): 284–87
19. Yu G, Wang LG, Yan GR, He QY: DOSE: An R/Bioconductor package for disease ontology semantic and enrichment analysis. Bioinformatics, 2015; 31(4): 608–9
20. Wickham H: ggplot2: Elegant graphics for data analysis. Springer-Verlag New York, 2016
21. Wang H, Cai H, Ao L et al: Individualized identification of disease-associated pathways with disrupted coordination of gene expression. Brief Bioinform, 2016; 17(1): 78–87
22. Rousseeuw J, Silhouettes P: A graphical aid to the interpretation and validation of cluster analysis. Journal of Computational and Applied Mathematics, 1987; 20: 53–65
23. Gan W, Zhao H, Li T et al: CDK1 interacts with iASPP to regulate colorectal cancer cell proliferation through p53 pathway. Oncotarget, 2017; 8(42): 71618–29
24. Yan M, Song M, Bai R et al: Identification of potential therapeutic targets for colorectal cancer by bioinformatics analysis. Oncol Lett, 2016; 12(6): 5092–98
25. Yang IP, Tsai HL, Hou MF et al: MicroRNA-93 inhibits tumor growth and early relapse of human colorectal cancer by affecting genes involved in the cell cycle. Carcinogenesis, 2012; 33(8): 1522–30
26. Browning DD, Kwon IK, Wang R: cGMP-dependent protein kinases as potential targets for colon cancer prevention and treatment. Future Med Chem, 2010; 2(1): 65–80
27. Peng Y, Zhang P, Huang X et al: Direct regulation of FOXK1 by C-jun promotes proliferation, invasion and metastasis in gastric cancer cells. Cell Death Dis, 2016; 7(11): e2480
28. Shi X, Seldin DC, Garry DJ: Foxk1 recruits the Sds3 complex and represses gene expression in myogenic progenitors. Biochem J, 2012; 446(3): 349–57
29. Garry DJ, Meeson A, Elterman J et al: Myogenic stem cell function is impaired in mice lacking the forkhead/winged helix protein MNF. Proc Natl Acad Sci USA, 2000; 97(10): 5416–21
30. Wang X, Sun D, Tai J et al: TFAP2C promotes stemness and chemotherapeutic resistance in colorectal cancer via inactivating hippo signaling pathway. J Exp Clin Cancer Res, 2018; 37(1): 27
31. Wang JL, Chen ZF, Chen HM et al: Elf3 drives beta-catenin transactivation and associates with poor prognosis in colorectal cancer. Cell Death Dis, 2014; 5: e1263
32. Micke P, Mattsson JS, Edlund K et al: Aberrantly activated claudin 6 and 18.2 as potential therapy targets in non-small-cell lung cancer. Int J Cancer, 2014; 135(9): 2206–14
33. Zhu B, Wu X, Wu B et al: The relationship between diabetes and colorectal cancer prognosis: A meta-analysis based on the cohort studies. PLoS One, 2017; 12(4): e0176068
34. Gonzalez N, Prieto I, Del Puerto-Nevado L et al: 2017 update on the relationship between diabetes and colorectal cancer: Epidemiology, potential molecular mechanisms and therapeutic implications. Oncotarget, 2017; 8(11): 18456–85
35. Zulato E, Bergamo F, De Paoli A et al: Prognostic significance of AMPK activation in advanced stage colorectal cancer treated with chemotherapy plus bevacizumab. Br J Cancer, 2014; 111(1): 25–32
36. You M, Yuan S, Shi J, Hou Y: PPARdelta signaling regulates colorectal cancer. Curr Pharm Des, 2015; 21(21): 2956–59
37. Calon A, Espinet E, Palomo-Ponce S et al: Dependency of colorectal cancer on a TGF-beta-driven program in stromal cells for metastasis initiation. Cancer Cell, 2012; 22(5): 571–84

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]