Original Paper

Distinguishable Prognostic Signatures of Left- and Right-Sided Colon Cancer: a Study Based on Sequencing Data

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Key Words

Left- and right-sided • Colon cancer • Prognosis • TCGA

Abstract

Background/Aims: Left- and right-sided colon cancers are considered to be two different diseases and have altered outcomes. However, specific molecules to predict the prognosis of left- and right-sided colon cancers are currently lacking. **Methods:** Expression profiling of colon cancer were downloaded from The Cancer Genome Atlas (TCGA). Differentially expressed genes (DEGs) of left- and right-sided colon cancers were compared by DESeq analysis. The prognostic values of DEGs were assessed by univariate and multivariate Cox regression. Prognostic index models of two side colon cancers were conducted with prognostic values genes, respectively. Interaction of DEGs was then analyzed by the protein-protein interaction (PPI). Different biology function of two sides of colon cancer was assessed by Gene Set Enrichment Analysis (GSEA). **Results:** A total of 167 DEGs were identified between left- and right-sided colon cancers based on TCGA data. Using univariate COX regression analysis, five genes (PHACTR3, CKMT2, CYP2W1, ERFE, HOXC4) were related to overall survival in left-sided, and eight distinguishable genes (EREG, ERFE, HOXC6, SLC22A31, TFF1, GFI1, ZG16, RASL10B) in right-sided. Further, left-sided prognostic model was established with PHACTR3 and CKMT2 (HR=2.040; 95%CI=1.004-4.145; P=0.049). Distinguishable prognostic signature for right-sided colon cancer was established based on EREG, ERFE, GFI1, and RASL10B (HR=3.530; 95%CI: 1.934-6.444; P<0.001) in multivariate analysis. PPI analysis of 167 DEGs showed that CCL5, GNG4, GNLY, GZMH, DRD2, and FASLG genes were at the core of interaction network. In GSEA function analysis, four pathways, including antigen processing and presentation, natural killer cell mediated cytotoxicity, intestinal immune network for IgA production, and type I diabetes mellitus, were significantly enriched in the DEGs of the right-sided colon cancer. **Conclusions:** This study constructs a panel of potential prognostic model of left- and right-sided colon cancers, respectively. We also provide molecular biological alterations between left- and right-sided colon cancers.

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Introduction

Colon cancer has the enormously high rate among the cancers of the digestive system and is currently the third leading cause of morbidity and mortality worldwide [1-5]. Studies have verified that left- and right-sided colon cancers differ in embryonic origin, anatomy, physiology, and pathological type, as well as gender, age and other demographic characteristics [6-9]. In 1990, for the first time, Bufill discussed the alterations between left- and right-sided colon cancers regarding embryonic origin and genetic background. The right-sided colon comprises the ileocecum, the ascending colon, and the transverse colon. The left-sided colon contains the descending colon and the sigmoid colon [6]. According to the 2011 Surveillance, Epidemiology and End Results (SEER) database of the US National Cancer Institute, the occurrence of right-sided colon cancer increased by 25.26% during the 30 years from 1976 to 2005, with a mean annual growth rate of 0.68% [10]. Yahagi M et al. investigated the variation of the prognosis of left- and right-sided colon cancers using a systematic retrospective review and meta-analysis. Their results revealed that the location of the tumor was correlated with survival and prognosis, and the overall survival of right-sided colon cancer was pronouncedly poorer than that of left-sided colon cancer [11]. The National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology clearly declare that the therapeutic sensitivity of molecular targeted drugs differs for tumors in different locations. Treatment with EGFR inhibitors, such as cetuximab, panitumumab, or irinotecan can significantly prolong the survival of patients with left-sided colon cancer [12]. For RAS wild-type colon cancer, left-sided tumors had longer overall survival than right-sided tumors treated with bevacizumab. It may be due to the random distribution of various molecular subtypes in the colon, and the biological differences between right and left colon that cause distinct efficacy of molecularly targeted agent in patients with left and right colon [13]. In recent years, molecular biology techniques and high-throughput sequencing technology further promoted the study of the alterations between left- and right-sided colon cancers [14, 15]. However, specific molecules that predict the prognosis of left- and right-sided colon cancers are currently lacking.

In this study, sequencing data from The Cancer Genome Atlas (TCGA) database were used to analyze differentially expressed molecules in left- and right-sided colon cancers and their predictive power for prognosis. We aimed to identify effective prognostic factors for left- and right-sided colon cancers and preliminary investigate their mechanisms, thereby contributing to the search for new clinical therapeutic targets for left- and right-sided colon cancers. The present study was summarized in Fig. 1.

Materials and Methods

Colon cancer samples and clinical parameter downloading

SEER. The SEER database (<https://seer.cancer.gov/>) was used to download the clinical information and survival status of patients with colon cancer from 1988 to 2010 for univariate analysis. Patients were disqualified if the information of tumor location and the T stage was missing. Cases with unclear tumor location, survival time and survival status were excluded.

TCGA. The colon adenocarcinoma sample information, the RNA-seq of transcriptome profiling count data, and the clinical data of the samples in TCGA were downloaded through the GDC data portal. The miRNAs data were downloaded from TCGA project, which provide the expression of precursor miRNAs. Cases with incomplete prognostic information were excluded.

Fig. 1. The flow chart of present study.

Differently transcriptional level of left- and right-sided colon cancers

According to the primary site of the colon tumor, this study included 258 patients of right-sided colon cancer (ileocecum, ascending colon, hepatic flexure of the colon, and transverse colon) and 180 patients of left-sided colon cancer (splenic flexure of the colon, descending colon, and sigmoid colon) (Table 1). Differential expressions various molecules of mRNA, lncRNA and miRNA of left- and right-sided colon cancers were computed using the DESeq method. Genes with an expression value of 0 for greater than 10% of the expression were excluded; $|\log FC| > 1$ and $\text{adjust-P value} < 0.05$ served as the screening conditions for significant difference. The $\log_2$ of the standard expression was used for subsequent analysis.

Prognostic factors of left- and right-sided colon cancers and construction of Cox regression models

To explore the different prognostic molecules of left- and right-sided colon cancers, the impact of the different molecular expression levels on the prognosis of patients with left- and right-sided colon cancers was investigated by univariate Cox regression analysis. The molecules that affect the survival of the patients with left- and right-sided colon cancers were preliminarily screened. Multiple stepwise Cox regression analysis was performed for potentially relevant genes in the preliminary screening, and the impact of these molecules on the survival time and the survival outcome was also analyzed. The screened molecules were used to construct the prognostic index (PI) model: $(\beta_1 \text{Exp}_1 + \beta_2 \text{Exp}_2 + \dots + \beta_x \text{Exp}_x)$ [16-21]. The larger the PI was, the greater the patient's risk and the worse the prognosis. The median of PI was used to divide the PI into a high-risk group and a low-risk group for KM univariate analysis to determine the prognostic power in the high-risk group and the low-risk group. $P < 0.05$ served as a standard condition for the above screening.

Functional analysis of left- and right-sided colon cancers

To explore the variances in biological functions between the left-sided colon and the right-sided colon, the Gene Set Enrichment Assay (GSEA) method was used for the analysis in this study [22]. All genes with different expression levels were divided into the left-sided colon group and the right-sided colon group for comparison. GSEA does not focus on a few individual genes with a significant change in expression. Instead, GSEA focuses on the consistent expression of the entire hybridization data in a specific set of functional genes to interpret the biological information contained in the data. $\text{FDR} < 0.05$ was used as the inclusion criterium in the screening. In addition, the interaction of the differential molecules between the left-sided and right-sided colon was investigated using the PPI interaction analysis of these differential genes.

Verification of the expression of prognostic molecules

The differential molecules were validated using the colon cancer microarray data set in the Gene Expression Omnibus (GEO) database. The microarray data that contained tumor site information and can distinguish between left- and right-sided colon cancers were included in the analysis. The microarray expression profile data were downloaded and normalized. Relevant gene expression data were obtained by re-annotating the probe. T-tests were performed for difference analysis, and $P < 0.05$ was used as the screening standard. We also researched the genetic alterations of prognosis-related genes in colon cancer by cBioPortal web software (<http://www.cbioportal.org/>).

Table 1. Clinical characteristic of left- and right-sided colon cancer

ID	Left colon(n=180)	Right colon(n=258)	χ^2	P
Age			6.699	0.010
>= 65	98(54.4%)	172(66.7%)		
< 65	82(45.6%)	86(33.3%)		
Gender			0.043	0.835
Male	96(53.3%)	135(52.3%)		
Female	84(46.7%)	123(47.7%)		
Stage			6.900	0.075
I	30(16.9%)	44(17.7%)		
II	60(33.9%)	109(43.8%)		
III	54(30.5%)	68(27.3%)		
IV	33(18.6%)	28(11.2%)		
Pathologic M			4.448	0.035
M0	131(80.4%)	194(88.2%)		
M1	32(19.6%)	26(11.8%)		
Pathologic N			8.832	0.012
N0	96(53.3%)	163(63.2%)		
N1	53(29.4%)	45(17.4%)		
N2	31(17.2%)	50(19.4%)		
Pathologic T			2.995	0.392
T1	4(2.2%)	5(1.9%)		
T2	35(19.4%)	42(16.3%)		
T3	125(69.4%)	174(67.7%)		
T4	16(8.9%)	36(14.0%)		
Venous invasion			0.253	0.615
Yes	40(25.3%)	51(23.1%)		
No	118(74.7%)	170(76.9%)		
Lymphatic invasion			2.720	0.099
Yes	72(44.2%)	83(35.9%)		
No	91(55.8%)	148(64.1%)		
Histological			12.599	<0.001
Adenocarcinoma	166(93.3%)	209(81.3%)		
Mucinous adenocarcinoma	12(6.7%)	48(18.7%)		
Colon polyps			1.956	0.162
Yes	22(28.9%)	52(38.5%)		
No	54(71.1%)	83(61.5%)		
Chemotherapy			1.989	0.158
Yes	66(36.7%)	78(30.2%)		
No	114(63.3%)	180(69.8%)		
Treatment outcome			0.159	0.690
SD + PD	19(20.0%)	24(17.9%)		
CR + PR	76(80.0%)	110(82.1%)		
Residual tumor			7.016	0.020
R0	132(89.2%)	199(93.4%)		
R1	0(0.0%)	4(1.9%)		
R2	16(10.8%)	10(4.7%)		
Cancer status			6.548	0.011
With tumor	98(60.5%)	109(47.4%)		
Tumor free	64(39.5%)	121(52.6%)		
Microsatellite instability			3.424	0.064
Yes	1(2.9%)	10(18.9%)		
No	33(97.1%)	43(81.1%)		
Kras mutation			0.123	0.726
Yes	8(42.1%)	10(47.6%)		
No	11(57.9%)	11(52.4%)		
BRAF mutation			1.795	0.529
Yes	0(0.0%)	3(17.6%)		
No	9(100.0%)	14(82.4%)		

Meta-analyses of the expression levels of the hub genes from PPI analysis

We performed meta-analyses of the expression levels of PPI hub genes (CCL5, GNG4, GNLY, GZMH, DRD2, and FASLG) based on data extracted from microarray and RNA-seq databases. All data were converted by corresponding standardization and logarithm 2. Continuous variable expression levels were sent for the calculation of Standard Mean Difference (SMD) by STATA 12. Because of the heterogeneity of different studies, the random effect model was chosen. When the pooled SMD <0 , the gene expression in left colon cancer was higher than that in right colon cancer; when SMD was >0 , the gene expression was higher in right colon cancer than that in left colon cancer.

Immunohistochemistry staining and evaluation of a hub gene

To validate the protein level of the hub genes, one gene was selected as an example. We examined the expression of GNG4 protein levels by immunohistochemistry. The study was approved by the Ethical Committee of the first affiliated hospital of Guangxi Medical University and written informed consent was obtained from all patients. Forty cases of colon cancers (left side, $n=20$, right side, $n=20$) were collected in the first affiliated hospital of Guangxi Medical University from 2016 to 2017. The samples were fixed by formalin. The first antibody (bs-13245R, Anti-G gamma4, Bioss, Beijing, China) was diluted to a concentration (1:500) and incubated at 37°C for one hour. The secondary antibody was incubated at room temperature for half an hour. The immunohistochemistry results were reviewed by two independent pathologists (Jiang-hui Zeng and Gang Chen). We quantified the results using the immunoreactivity score system. The staining intensity ranged from 0 to 3, indicating no staining, weak staining, moderate and strong staining, respectively. The percentage of staining consists of 0-4 points, 0 was $<5\%$; 1 was $<5-25\%$; 2 was $25-50\%$; 3 was $51-75\%$; 4 was $>75\%$. The final result of each case was obtained by a score of multiplying the above two elements. The paired sample T-test was used to assess the difference of GNG4 expression between two groups. $P<0.05$ was considered statistically significant.

Results

Prognosis of left- and right-sided colon cancers in SEER and TCGA

We divided SEER colon cancer cases into left- and right-sided colon cancers. A total of 572, 265 colon cancer patients were collected from 1988 to 2010, including 324, 827 right-sided colon cancer and 247, 438 left-sided colon cancer cases. The K-M univariate analysis results indicated that the overall survival rate of left-sided colon cancer was more favorable than that of right-sided colon cancer (log rank, $P<0.001$, Fig. 2A). The median survival of left-sided colon cancer was 75 months (74.289-75.711 months), and the median survival of right-sided colon cancer was 60 months (59.493-60.507 months). In addition, the prognosis of left-sided colon cancer was enhanced compared with that of right-sided colon cancer at different tumor stages (log rank, $P<0.001$, Fig. 2B, C).

Furthermore, K-M prognostic analysis was performed on 170 cases of left-sided colon cancer and 251 cases of right-sided colon cancer using TCGA. No remarkable alteration in overall survival was noted between the left- and right-sided colon cancers, as shown in Fig. 3A (log rank, $P = 0.064$). However, our analysis of patients at different tumor stages revealed that among patients with high-stage colon cancer, better prognosis was associated with left-sided colon cancer compared with right-sided colon cancer (Log Rank, $P = 0.030$, Fig. 3B). For those patients who were in the early stage, the prognoses of the left-sided and right-sided colon cancers did not change noticeably (Fig. 3C).

Differential molecules in left- and right-sided colon cancers

For the TCGA data, differences in gene expression were analyzed using the DESeq method by dividing colon cancer cases into left- and right-sided colon cancers. A total of 167 differential genes met the criteria of fold change >2 and adj-P value <0.05 , including 109 genes (containing 8 lncRNAs) highly expressed in right-sided colon cancer and 58 genes (containing 6 lncRNAs) highly expressed in left-sided colon cancer, with the most evidential alteration noted in GNG4 (Fig. 4, Table 2). A total of 325 miRNAs could be calculated between

left- and right-sided colon cancers. Only miR-592 was differentially expressed between left- and right-sided colon cancers (data not shown).

Fig. 2. The survival of patients with left- or right-sided colon cancer based on SEER database. Kaplan-Meier (K-M) survival analysis was showed the correlation between survival and tumor location. (A) Left colon cancer had a better survival than right colon cancer (log-rank, $P < 0.001$). (B) In tumor stage I+II, patients with left colon cancer showed longer survival than right colon cancer (log-rank, $P < 0.001$). (C) In advanced tumor stage, survival time of patients with left colon cancer is prolonged than right colon cancer (log-rank, $P < 0.001$)

Fig. 3. The survival of patients with left- or right-sided colon cancer based on TCGA database. Kaplan-Meier (K-M) survival analysis was showed the correlation between survival and tumor location. (A) Survival time of patients with left- and right-sided colon cancers was not different. (log-rank, $P = 0.064$). (B) Survival time of patients with left- and right-sided colon cancer was not different in early stage of tumor. (log-rank, $P = 0.395$) (C) In advanced tumor stage, survival time of patients with left colon cancer is prolonged than right colon cancer (log-rank, $P = 0.030$)

Fig. 4. Differentially expressed genes between left- and right-sided colon cancer. (A) The volcano plot showed 167 differentially genes calculated by DESeq method with criteria ($\log_2FC > 1$, adj- $P < 0.05$). The red dots represented the up expressed genes, as well as green were down expressed genes. (B) Hierarchical clustering of differentially expressed genes expression levels.

Prognosis models of left-sided and right-sided colon cancers

In TCGA, univariate Cox regression analysis was performed for 167 DEGs in left- and right-sided colon cancers to identify five prognostic genes for left-sided colon cancer, including PHACTR3, CKMT2, CYP2W1, ERFE and HOXC4, and eight prognostic genes for the right-sided colon cancer, including EREG, ERFE, HOXC6, SLC22A31,

TFF1, GF11, ZG16, and RASL10B (Table 3). Multiple stepwise Cox regression was used to analyze the effect of five left-sided colon cancer RNAs on the survival time and the patient's outcome. PHACTR3 and CKMT2 genes affected the survival time and the patient's outcome (Fig. 5). A prognostic index model for left-sided colon cancer was constructed using these two genes. The expression data of these two genes were introduced into the PI equation, $PI = 0.299 \times PHACTR3_{EXP} - 0.174 \times CKMT2_{EXP}$ obtaining PI for the 170 cases (Fig. 6). Similarly, multiple stepwise Cox regression was performed for the eight genes for right-sided colon cancer, including EREG, ERFE, HOXC6, SLC22A31, TFF1, GF11, ZG16 and RASL10B. The results revealed that EREG, ERFE, GF11, and RASL10B were

Table 2. 167 differentially expressed genes between left- and right-sided colon cancer

	Colon base mean	Left colon base mean	Right colon base mean	Fold Change	logFC	P-value	Padj
IGF1	1427.813	2322.732	817.2321	0.3318	-1.5879	1.08E-15	1.61E-11
HOXC11	7046.905	12614.665	4261.430	0.3330	-1.5863	9.54E-15	1.07E-11
HOXC6	767.3262	15.4505	167.6839	6.9606	2.8811	4.01E-14	1.61E-10
TNFR1	282.9993	113.3698	394.8779	3.5148	1.8135	8.44E-13	3.12E-09
FEZF1-AS1	164.7138	85.9092	218.8207	2.5395	1.3446	2.21E-12	3.84E-09
RAB27B	508.7979	266.3117	673.7551	2.5304	1.3394	2.47E-12	3.84E-09
FOXP2	103.9465	33.2045	420.29	4.0259	2.0097	2.46E-12	3.84E-09
TPF2A2	256.7811	129.4536	347.5936	3.2814	1.6787	4.18E-11	4.57E-08
AMT	492.4048	702.7515	349.0915	0.4966	-1.0088	1.88E-10	1.72E-07
ARGS6	891.2721	1139.6465	526.4385	0.4567	-1.1143	1.91E-10	1.72E-07
IRS4	189.0195	960.9842	252.75205	2.6301	1.3951	1.04E-09	8.79E-07
IRS1-IB122.5	296.9859	462.9810	201.1261	0.4975	-1.0755	3.01E-09	2.82E-06
IRX5	246.8691	138.7886	276.9684	1.7533	0.9913	4.37E-09	3.75E-06
SLC1A3	331.4128	475.4224	213.3867	0.4949	-1.0266	4.89E-09	2.97E-06
UGALR	364.3388	218.2166	664.8205	1.2099	0.6772	6.23E-09	4.74E-06
PLAZA4	374.6651	231.8638	472.4451	2.0446	1.0118	6.42E-09	1.48E-06
CAI	361.4721	172.4632	498.8074	2.8379	1.5448	8.82E-09	4.42E-06
MATP2	188.1407	539.1714	142.2124	0.3421	-1.2448	9.12E-09	4.48E-06
ULP2	76.5747	33.7279	189.1056	3.2550	1.6938	1.71E-08	7.12E-06
MUC12	5762.1792	8772.6198	3762.1279	0.4290	-1.2211	1.85E-08	7.31E-06
CKMT2	557.0472	865.1826	337.8320	0.3897	-1.3634	1.98E-08	7.88E-06
SLC12A3	318.5410	521.8778	166.6694	0.3194	-1.4647	3.44E-08	1.38E-05
TUBB1	187.7256	584.0633	102.12484	0.2459	-1.0327	3.79E-08	1.41E-05
SLC4A1	113.2283	204.9825	84.3803	0.4116	-1.2885	4.15E-08	1.52E-05
STAG3	128.4345	217.7626	67.5887	0.3301	-1.0885	4.43E-08	1.58E-05
SLC22A3	296.9790	184.7815	373.3595	1.2405	0.6147	6.08E-08	2.06E-05
RT2	288.1616	174.8385	364.4854	2.0957	1.0674	6.95E-08	2.16E-05
SLC16A7	126.6755	274.6187	126.6755	0.4664	-1.0881	6.95E-08	2.16E-05
PRRX3	58.4862	26.5258	66.6483	2.5100	1.3277	7.94E-08	2.45E-05
GAD1	128.8859	73.2581	164.7540	2.2763	1.1867	8.93E-08	2.67E-05
ZNF113	145.8614	218.2492	94.8621	0.4425	-1.1791	9.52E-08	2.81E-05
SERPINB5	246.3498	1597.7992	312.9439	2.0719	1.0509	1.02E-07	2.94E-05
USP1	276.0550	378.6555	176.6555	0.4666	-1.1869	1.06E-07	2.94E-05
TNRC2	513.9686	799.9630	339.6730	0.4411	-1.1807	1.08E-07	3.02E-05
GNAI3	37.8230	18.8263	49.9554	2.7713	1.4705	1.13E-07	3.16E-05
NOT1	168.8277	18.7179	7.0307	0.3748	-1.3748	1.39E-07	3.16E-05
ARL11	142.5625	240.6077	109.4368	0.4548	-1.3366	1.72E-07	4.39E-05
CD38P16.69	327.89169	2.0319	1.0228	0.2019	-1.0228	1.77E-07	4.39E-05
DNAI1	732.3143	455.8667	925.5133	2.0193	1.0138	2.73E-07	4.69E-05
ZIC2	232.8136	126.3449	303.9487	2.4857	1.2465	2.81E-07	4.69E-05
CCL2	648.7888	391.9122	1.0891	0.6829	0.3412	2.89E-07	4.69E-05
CCL1	94.7251	142.5317	62.1798	0.4363	-1.1968	3.57E-07	7.31E-05
ZNF108	114.3322	167.5382	77.8389	0.4635	-1.1094	4.58E-07	8.97E-05
PCOL	112.3237	184.3211	62.8421	0.3421	-1.5418	5.02E-07	9.47E-05
HOXA	33.8175	13.2386	46.4878	3.5137	1.8130	5.28E-07	9.78E-05
MOP1	152.2453	14.2453	1.0453	1.0453	1.0453	5.28E-07	9.78E-05
YXN1	98.5824	54.7423	12.6424	2.3460	1.2362	6.08E-07	1.04E-04
SYN3	96.2798	155.5330	55.9240	0.3597	-1.4752	6.26E-07	1.09E-04
NCN40B	147.0434	59.4788	4.5808	0.2975	-1.2975	1.04E-06	1.24E-04
HOXB2	185.6588	114.2171	214.2492	2.0513	1.0305	1.15E-06	1.77E-04
HOXA1	128.1875	14.2453	1.0453	1.0453	1.0453	1.36E-06	1.77E-04
CITF	767.8700	444.2072	988.8650	2.2216	1.1516	1.47E-06	2.11E-04
HEX2	153.6236	219.5099	108.8390	0.4957	-1.0124	1.51E-06	2.13E-04
TRAF3	53.6483	85.4633	1.8111	0.1844	-1.8111	1.82E-06	2.13E-04
TBX10	117.3425	172.8278	80.1144	0.4637	-1.1025	1.86E-06	2.56E-04
PCP4	117.5749	197.4126	63.2339	0.3203	-1.4447	1.99E-06	2.72E-04
SPNPF2	595.2761	756.5544	344.5154	0.4411	-1.1785	2.38E-06	2.72E-04
HOXA1	85.4290	51.3225	108.6477	2.1170	1.0820	2.58E-06	2.72E-04
CPY42	395.2626	457.4793	208.2626	0.4447	-1.1562	3.58E-06	3.15E-04
CCL13	211.8342	114.3543	278.1955	2.4327	1.2826	3.74E-06	3.45E-04
TNFRSF9	331.1429	191.7382	426.6519	2.2450	1.1667	3.98E-06	4.54E-04
PCOLCE	365.8315	218.2211	2.0281	0.2028	-1.0203	4.42E-06	4.96E-04
SREBF3	567.4581	352.2950	713.9345	2.0265	1.0190	4.61E-06	5.03E-04
ONLY	145.5720	88.8995	1.0453	1.0453	1.0453	4.62E-06	5.03E-04
GF11	145.5720	88.8995	1.0453	1.0453	1.0453	4.62E-06	5.03E-04
ERFE	56.7217	88.7004	38.3131	0.4319	-1.2111	5.38E-06	5.98E-04
PCOL	117.8899	47.8399	1.1889	0.2295	-1.2295	5.58E-06	5.98E-04
RBM27	227.8001	329.3667	158.6459	0.4817	-1.0538	5.64E-06	5.98E-04
BP1-147018.6	145.5720	88.8995	1.0453	1.0453	1.0453	5.64E-06	5.98E-04
ACN4	24.5294	1.4706	3.1455	2.4882	1.2482	6.18E-06	6.44E-04
CAMK2G	18.3938	9.7387	2.4671	2.4671	2.4671	6.18E-06	6.44E-04
GNA1	262.9488	124.4811	1.0203	0.2028	-1.1255	7.38E-06	7.94E-04
CTD1	57.7131	34.4430	21.193	0.1836	-1.8366	8.38E-06	7.57E-04
ARCA12	78.4266	34.2822	10.8788	1.1663	1.6639	9.83E-06	8.12E-04
MYR1	12.4548	16.1148	1.2966	0.1297	-1.4111	9.37E-06	8.29E-04
DISP15	108.6411	57.0964	62.2415	0.3962	-1.3357	1.38E-05	1.06E-03
RAB18	949.4777	580.1941	119.9840	0.2019	-1.0228	1.42E-05	1.27E-03
ACN4	95.7208	48.1107	2.6426	1.4019	1.4019	1.49E-05	1.31E-03
HOXA1	78.4143	124.448	125.4711	0.9258	0.3112	1.71E-05	1.32E-03
TPF2A2-AS1	48.2258	17.1682	2.3430	1.2283	1.2283	1.75E-05	1.34E-03
CTC4	20.4352	11.9962	2.1882	1.1259	1.1259	1.85E-05	1.39E-03
QRS	21.1689	1.1689	2.7289	1.4473	1.4473	2.36E-05	1.47E-03
CTSE	245.5838	345.5152	3.2462	1.6987	1.6987	2.65E-05	1.84E-03
HOXA1	24.8240	34.9080	16.7575	0.4880	-1.0500	2.68E-05	1.84E-03
CEK2	72.4348	107.4378	1.4448	0.1448	-1.1255	2.84E-05	1.92E-03
EMES	34.1176	20.2912	43.5302	2.1453	1.1012	2.88E-05	1.92E-03
HOXA1	185.4418	104.2403	24.8571	2.3227	1.2096	2.89E-05	1.92E-03
CBN1	50.8816	30.1140	1.9918	0.1918	-2.0255	3.05E-05	2.12E-03
BP1-147320.9	94.2623	137.7396	4.6464	0.4646	-1.0909	3.38E-05	2.15E-03
MUC17	21.2962	159.6213	2.6794	1.2092	1.2092	3.41E-05	2.15E-03
LAC1	147.1741	88.8558	188.8990	2.1038	1.0730	3.51E-05	2.20E-03
RPY13	44.5158	64.8022	31.4182	0.4909	-1.0265	3.73E-05	2.29E-03
RASL10B	17.8336	148.9797	1.7871	0.1871	-1.8646	3.74E-05	2.29E-03
PTEN	145.8991	83.7947	188.1136	2.2449	1.1667	3.97E-05	2.38E-03
WSP1	17.7944	1.1790	1.1790	1.1790	1.1790	4.18E-05	2.41E-03
TRDC	38.9262	17.9965	1.1424	1.1424	1.1424	4.18E-05	2.41E-03
RAMP1	446.9699	357.5944	83.8862	2.3319	1.2215	4.25E-05	2.47E-03
RASG	23.9758	16.2923	2.2176	1.1499	1.1499	5.08E-05	2.84E-03
SP140	90.8096	54.3118	115.6425	1.1205	1.0898	5.07E-05	2.88E-03
PRKRA2	146.9876	14.6267	2.1787	1.1235	1.1235	5.08E-05	2.88E-03
HOXA1	17.7944	1.1790	1.1790	1.1790	1.1790	5.08E-05	2.88E-03
AMH	146.5086	81.3858	190.8288	2.3447	1.2294	5.73E-05	3.14E-03
SLC22A4B	21.2962	44.6966	4.6966	0.2090	-1.0904	6.18E-05	3.38E-03
PRRX3	226.6175	372.8079	138.4571	0.3499	-1.1549	6.58E-05	3.45E-03
PCOLN	181.3981	301.2677	99.7672	0.3313	-1.0599	6.98E-05	3.56E-03
SLC12A2	151.4709	52.4135	1.2414	1.0886	1.0886	7.17E-05	3.62E-03
SLC22A2	123.9132	188.8077	82.4583	0.4462	-1.1643	7.24E-05	3.63E-03
SEIPIN	761.5325	336.4643	1.1713	1.1713	1.1713	7.27E-05	3.63E-03
TNFR1	344.6803	206.9122	442.6500	2.2009	1.1381	7.32E-05	3.64E-03
ERFE	94.4903	46.2570	127.6623	2.7598	1.4446	8.12E-05	3.98E-03
ORL1	45.2479	24.1958	1.2854	1.1912	1.1912	8.74E-05	4.23E-03
GZM1	43.1091	36.6572	76.1636	2.1678	1.1162	8.85E-05	4.27E-03
CHN2	29.7418	14.7299	5.9615	0.4958	-1.0120	8.91E-05	4.27E-03
DMR1	29.4845	17.1738	37.7388	2.1970	1.1355	1.04E-04	4.74E-03
RLK10	210.9212	125.9212	310.9212	1.4714	1.3065	1.17E-04	5.18E-03
VNN2	98.2135	52.2139	125.2266	2.4807	1.3108	1.37E-04	5.78E-03
ARZ	94.8992	51.1161	123.3888	2.4133	1.2710	1.41E-04	5.84E-03
DMR2	151.4709	254.8059	76.5654	0.3447	-1.3364	1.54E-04	6.38E-03
TRK1	21.7	1.7	1.7	2.682	1.2529	1.77E-04	6.38E-03
TRK2	192.9212	119.0844	241.3388	2.0178	1.0228	1.89E-04	7.15E-03
VNN1	17.9	205.785	39.8289				

prognostic factors (Fig. 5). The expression data of these four genes were introduced into the prognostic index model for right-sided colon cancer, $PI = -0.125 \times EREG_{EXP} + 0.155 \times ERFE_{EXP} - 0.172 \times GFI1_{EXP} + 0.149 \times RASL10B_{EXP}$ (Fig. 7). The included cases were assigned into a low-risk group and a high-risk group according to the median cutoff of the PI. The PI could be determined as independent prognostic factor according to univariate and multivariate cox regression (Table 4). The K-M prognostic analysis revealed that the prognosis of the low-risk group was better than the high-risk group for both left- and right-sided colon cancers. The diagnostic rate of the 5-year survival rate by the prognostic model constructed with the risk index was $AUC = 0.692$ for left-sided colon cancer and $AUC = 0.730$ for right-sided colon cancer, as shown in Fig. 8. However, the expression level of miR-592 was not associated with OS neither in left-sided, nor right-sided colon cancer by cox regression analyses.

Gene set enrichment analysis for left-sided and right-sided colon cancers

To better understand the differences in biological function between the left- and right-sided colons, gene set enrichment analysis (GSEA) was performed. Four pathways, including *antigen processing and presentation*, *natural killer cell mediated cytotoxicity*, *intestinal immune network for IgA production*, and *type I diabetes mellitus*, were significantly enriched in the right-sided colon, whereas no pathway was significantly enriched in the left-sided colon (Fig. 9, Table 5). In addition, the interaction between the DEGs of the left- and right-sided colon cancers was analyzed by the PPI interaction network. Fig. 10 indicated that the genes CCL5, GNG4, GNLY, GZMH, DRD2, and FASLG genes were more closely related to other genes, which may indicate an important role in regulation.

GEO validation for the expression and genetic alterations of six prognostic genes

A total of 20 microarray datasets were identified by screening the GEO database. In some microarrays, the expression levels of PHACTR3 and CKMT2 for the left-sided colon prognosis model and EREG, ERFE, GFI1 and RASL10B for the right-sided colon prognosis model were verified. PHACTR3, CKMT2, EREG, GFI1 and RASL10B expression levels significantly differed between left- and the right-sided colon cancers, as shown in Fig. 11, which is consistent with the trend of expression noted in TCGA. As for genetic alterations, EREG showed the highest genetic alterations in these genes. ERFE and GFI1 did not show any alterations in colon cancer of TCGA (Fig. 12).

Fig. 6. Prognostic index (PI) model of left-sided colon cancer. The top section showed the survival status of patients with left-sided colon cancer. Y-axis represented survival time of each patient. The middle section of Fig. showed the PI. X-axis represented the patient IDs, which were ranking by PI from low to high. The heatmap of bottom showed PHACTR3 and CKMT2 expression in each left-sided colon cancer patients.

Table 3. Prognostic value of 167 genes of left- and right-sided colon cancer in univariate cox regression

ID	Univariate analysis			
	Estimate	Wald	Hazard ratio (95% CI)	P value
Left-sided colon				
PHACTR3	0.299	6.662	1.348 (1.075 - 1.692)	0.010
CKMT2	-0.174	6.263	0.840 (0.733 - 0.963)	0.012
CYP2W1	0.159	4.133	1.173 (1.006 - 1.368)	0.042
ERFE	0.313	3.911	1.367 (1.003 - 1.864)	0.048
HOXC4	0.199	3.923	1.221 (1.002 - 1.487)	0.048
Right-sided colon				
EREG	-0.125	6.759	0.883 (0.804 - 0.971)	0.009
ERFE	0.155	4.576	1.167 (1.012 - 1.343)	0.034
HOXC6	0.142	6.307	1.153 (1.032 - 1.288)	0.012
SLC22A31	0.152	5.350	1.164 (1.023 - 1.324)	0.021
TFF1	-0.087	4.946	0.917 (0.849 - 0.990)	0.026
GFI1	-0.172	4.566	0.842 (0.719 - 0.986)	0.033
ZG16	-0.073	4.406	0.929 (0.868 - 0.995)	0.036
RASL10B	0.149	3.987	1.161 (1.003 - 1.344)	0.046

Fig. 7. Prognostic index (PI) model of right-sided colon cancer. The top section showed the survival status of patients with right-sided colon cancer. Y-axis represented survival time of each patient. The middle section of Fig. showed the PI curve. X-axis represented the patient IDs, which were ranking by PI from low to high. The heatmap of bottom showed EREG, ERFE, GFI1 and RASL10B expression in each right-sided colon cancer patients.

Fig. 8. The prognostic values of prognostic index (PI) of left- and right-sided colon cancer patients. (A) Kaplan-Meier (K-M) curve showed that low PI group had a better survival than high level PI group in right-sided colon cancer ($P=0.004$). (B) Time-dependent ROC curve represented 5-year survival prediction by the right-sided prognostic model ($AUC=0.692$). (C) Kaplan-Meier (K-M) curve showed that low PI group had a better survival than high level PI group in left-sided colon cancer ($P<0.001$). (D) Time-dependent ROC curve represented 5-year survival prediction by the left-sided prognostic model ($AUC=0.730$).

Table 4. Univariate and multivariate Cox analysis of risk score

ID	Univariate analysis				Multivariate analysis			
	Estimate	Wald	Hazard ratio (95%CI)	P value	Estimate	Wald	Hazard ratio (95% CI)	P value
Left-sided colon								
Age(≥ 65 / <65)	0.131	0.127	1.141 (0.553 - 2.354)	0.722				
Gender(Male/Female)	0.294	0.622	1.342 (0.646 - 2.787)	0.430				
Stage(III+IV/I+II)	1.234	8.750	3.437 (1.517 - 7.787)	0.003	1.929	8.686	6.881 (1.908 - 24.814)	0.003
Cancer status (With tumor/tumor free)	1.261	5.138	3.527 (1.186 - 10.491)	0.023	1.789	6.987	5.985 (1.588 - 22.552)	0.008
prognostic index	0.918	11.332	2.053 (1.467 - 4.271)	<0.001	0.713	3.887	2.040 (1.004 - 4.145)	0.049
Right-sided colon								
Age(≥ 65 / <65)	0.422	2.019	1.525 (0.852 - 2.729)	0.155				
Gender(Male/Female)	0.106	0.171	1.112 (0.673 - 1.838)	0.679				
Stage(III+IV/I+II)	1.257	20.428	3.516 (2.038 - 6.066)	<0.001	1.403	18.251	4.066 (2.136 - 7.738)	<0.001
Cancer status (With tumor/tumor free)	1.236	15.442	3.443 (1.859 - 6.380)	<0.001	0.946	8.151	2.574 (1.345 - 4.927)	0.004
prognostic index	1.321	25.856	3.749 (2.253 - 6.239)	<0.001	1.261	16.873	3.530 (1.934 - 6.444)	<0.001

Table 5. KEGG pathways enrichment in GSEA analysis

NAME	SIZE	ES	NES	P-value	FDR
Kegg_Natural_Killer_Cell_Mediated_Cytotoxicity	103	0.562	1.933	0.006	0.033
Kegg_Intestinal_Immune_Network_For_Iga_Production	38	0.772	1.969	0.002	0.035
Kegg_Antigen_Processing_And_Presentation	56	0.718	1.997	0.006	0.037
Kegg_Type_1_Diabetes_Mellitus	36	0.755	1.938	0.000	0.040
Kegg_Autoimmune_Thyroid_Disease	31	0.805	1.892	0.000	0.051
Kegg_Viral_Myocarditis	60	0.662	2.006	0.002	0.062
Kegg_Graft_Versus_Host_Disease	30	0.800	1.837	0.006	0.085
Kegg_Allograft_Rejection	30	0.797	1.822	0.006	0.088
Kegg_Hematopoietic_Cell_Lineage	72	0.623	1.781	0.028	0.117
Kegg_Primary_Immunodeficiency	30	0.660	1.745	0.022	0.151
Kegg_Sphingolipid_Metabolism	33	0.529	1.667	0.032	0.187
Kegg_Asthma	21	0.750	1.674	0.033	0.190
Kegg_Chemokine_Signaling_Pathway	168	0.468	1.691	0.031	0.195
Kegg_Cytosolic_Dna_Sensing_Pathway	40	0.502	1.651	0.038	0.198
Kegg_Leishmania_Infection	67	0.573	1.698	0.036	0.203
Kegg_Prion_Diseases	30	0.542	1.674	0.030	0.205
Kegg_Colorectal_Cancer	61	0.398	1.529	0.040	0.209
Kegg_Long_Term_Potentialization	59	0.437	1.608	0.020	0.211
Kegg_Pancreatic_Cancer	69	0.415	1.550	0.042	0.211
Kegg_Apoptosis	83	0.411	1.542	0.046	0.214
Kegg_Toll_Like_Receptor_Signaling_Pathway	83	0.469	1.613	0.046	0.215
Kegg_T_Cell_Receptor_Signaling_Pathway	101	0.440	1.618	0.040	0.220
Kegg_B_Cell_Receptor_Signaling_Pathway	73	0.453	1.622	0.049	0.226
Kegg_Rig_I_Like_Receptor_Signaling_Pathway	50	0.443	1.555	0.044	0.228
Kegg_Fc_Epsilon_R1_Signaling_Pathway	69	0.415	1.562	0.044	0.235
Kegg_Vegf_Signaling_Pathway	68	0.406	1.569	0.016	0.236
Kegg_Starch_And_Sucrose_Metabolism	34	0.496	1.573	0.046	0.242

Meta-analyses of hub genes based on microarray and RNA-seq data

According to 13 GEO data series and TCGA dataset, the results showed that GNG4, CCL5, GNLY, GZMH, DRD2 had the same dysregulated trends to our present study except FASLG. GNG4 (SMD=-0.56, 95%CI: -0.87, -0.25, $P<0.001$) and DRD2 (SMD=-0.241, 95%CI: -0.432, -0.05, $P=0.013$) were over-expressed in left-sided colon cancer than right one. CCL5 (SMD=0.36, 95%CI: 0.21, 0.51, $P<0.001$), GNLY (SMD=0.294, 95%CI: 0.112, 0.475, $P=0.002$), and GZMH (SMD=0.308, 95%CI: 0.199, 0.417, $P<0.001$) expression in right-sided were higher than in left-sided one (Fig. 13).

GNG4 expression of immunohistochemistry staining

GNG4 expression levels with 40 paired colon cancers were assessed by immunoreactive score as an example for

Fig. 9. KEGG functional enrichment analysis of left- and right-sided colon cancer based on GSEA. (A) Four KEGG pathways (antigen processing and presentation, natural killer cell mediated cytotoxicity, intestinal immune network for IgA production, and type I diabetes mellitus) were significantly enriched in right-sided colon cancer (FDR<0.05). (B) Heatmap of differentially expressed genes of left- and right-sided colon cancer.

Fig. 10. Protein-protein interaction (PPI) network of 167 differentially expressed genes of left- and right-sided colon cancer. PPI network showed the links between proteins. CCL5, GNG4, GNL, GZMH, DRD2, and FASLG genes were identified as hub genes, which mostly connected with other genes. Medium confidence interaction score was set as cutoff. Disconnected nodes in the network were hidden.

Fig. 11. Six genes expression of prognostic index model in GEO database. Six genes expression were validated in 14 selected data series. CKMT2 was over expressed in left colon cancer in 10 data series (GSE39084, GSE14333, GSE75316, GSE75315, GSE35834, GSE41258, GSE8671, GSE37892, GSE89076, and GSE50592). EREG was higher expression in left-sided than right-sided colon cancer (GSE39084, GSE14333, GSE75316, GSE75315, GSE35834, GSE41258, GSE77953, GSE44076, GSE72970, GSE103512). RASL10B, GF11, PHACTR3 were showed differentially in other data series. Unpaired T-test was performed in analysis. P<0.05 was set as significant.

the validation of the hub genes. The expression level of GNG4 was prominently higher in colon cancer tissues (10.538 ± 2.537) compared with adjacent colon tissues (2.589 ± 2.152 , $P < 0.001$). Furthermore, GNG4 expression in left colon cancer tissues (11.600 ± 1.231) were obviously higher than that in right colon cancer tissues (9.850 ± 2.815 , $P = 0.017$, Fig. 14).

Discussion

In the current study, a differential expression signature of various molecules was performed between the left-sided colon and the right-sided colon based on TCGA sequencing data including mRNA, lncRNA and miRNA, and the predictive power of these differential molecules on the survival of the patients with left- and right-sided colon cancers were further investigated. Molecules that could predict the prognosis of left- and right-sided colon cancers were separately identified, and the corresponding models of prognosis were constructed.

Fig. 12. Genetic alterations of prognostic models. Based on TCGA, we used cBioPortal to detect Genetic alterations in 120 (30% of total 394) colon cancers.

Fig. 13. Forrest plots of hub genes from PPI analysis. A: Forrest plot showed GNG4 overexpression in left-sided colon cancers; B: Forrest plot showed DRD2 overexpression in left-sided colon cancers; C: Forrest plot showed CCL5 overexpression in right-sided colon cancers; D: Forrest plot showed GNLV overexpression in right-sided colon cancers; E: Forrest plot showed GZMH overexpression in right-sided colon cancers; F: Forrest plot showed no statistical significance of FASLG expression in colon cancers.

Prognostic index model of left-sided colon cancer contained PHACTR3 and CKMT2, while another four genes (EREG, ERFE, GFI1 and RASL10B) were included in prognostic model of right-side colon cancer. An enrichment analysis of gene function was also carried out for the left- and the right-sided colon using GSEA. The results showed that right-sided colon cancer was related to the pathways of *antigen processing and presentation*, *natural killer cell mediated cytotoxicity*, *intestinal immune network for IgA production*, and *type I diabetes mellitus*. But the differentially expressed miRNAs and lncRNAs had no significant prognostic value between left- and right-sided colon cancers.

Some studies have focused on factors affecting the prognosis of the left and right colon cancer. Bauer et al. compared recurrence specimens of the left- and right-sided colon cancers and found that NOX4, CDX2, and ITGA3 expression levels differed. The reduced recurrence-free survival in left-sided colon cancer was related to high NOX4 expression. The reduced recurrence-free survival in right-sided colon cancer was related to high CDX2 expression [23, 24]. Although they found some factors related to the prognosis of the left

Fig. 14. GNG4 protein expression in left-/right sided colon cancer. (A) GNG4 showed higher expression level in tumor tissue than normal tissue by IHC. (B) GNG4 showed higher expression level in left-sided colon cancer than right-sided colon cancer by IHC. Magnification: x100 and x400.

and right colon cancer, the predictive ability of a single gene may not be accurate, and the sample size was relatively small compared with the TCGA database. In the current study, a prognostic model to independently predict the prognosis of left-sided colon cancer was constructed using the differential genes PHACTR3 and CKMT2, and a prognostic model to independently predict the prognosis of right-sided colon cancer was constructed using four differential genes, including EREG, ERFE, GF11 and RASL10B. Our GEO verification results revealed that PHACTR3, CKMT2, EREG, ERFE and RASL10B were highly expressed in the left-sided colon, and GF11 was highly expressed in the right-sided colon, which is consistent with the aforementioned findings from TCGA dataset. Among the abovementioned genes, the PHACTR protein family is closely related to cell motivity and morphogenesis by regulating the actin cytoskeleton [25]. PHACTR3 is a new hypermethylated gene in CRC exhibiting good performance in stool DNA testing and has complementary value to the fecal immunochemical test in colorectal cancer [26]. Hypermethylation of the PHACTR3 promoter measured in stool samples could be used as a colorectal cancer biomarker and provide prognostic information [27]. CKMT2 was not studied in colon cancer. Wang H et al. validated that CKMT2 might serve as a key regulating factor participating in osteosarcomagenesis [28]. Saridaki Z et al. demonstrated that OS was significantly reduced in CRC patients with down-regulated EREG (HR: 1.7, $P = 0.021$), which was revealed as an independent prognostic factor for decreased OS [29]. Qu X et al. found that in those patients treated with cetuximab, low EREG methylation was correlated with increased expression of the ligand of EREG, also was related to a better response to the cetuximab treatment. On the contrary, high EREG methylation was correlated with decreased expression of the ligand of EREG, as well as was related to the worse response of therapy [30]. Lee MS et al. found that there were noteworthy inverse correlations between EREG expression and methylation, as well as tumor position, BRAF mutation, and CIMP-high status [31]. Inoue A et al. found that methylated RASL10B could be involved in the development from sessile serrated adenomas/polyps (SSA/Ps) to colon cancer [32]. Zou H et al. demonstrated that profound down-regulation of RASL10B mRNA level could be observed in all breast cancer cell lines and suggested that RASL10B could act as a novel member of the Ras superfamily, which owns the potential to be a tumor suppressor [33]. Xing W et al. explored that GF11 could act as a gating modulator, which shoulders the responsibility for an essentially activated signaling circuit that leads to the metastasis of CRC [34]. Xing W et al. investigated that GF11 could be a prospective therapeutic goal to hinder the progress and metastasis of inflammation-related CRC. Relapse-free survival (RFS) in cases with increased GF11 expression was improved compared with patients with low GF11 expression [34]. The trend in the results of the studies above is similar to our findings for GF11 in right-sided colon cancer. Here, our main purpose is to identify prognostic factors to separately predict left- and right-sided colon cancers. Although these genes have been studied in colon cancer, little has been reported regarding their ability to predict prognosis, no relevant researches have pointed out that these prognosis-related genes were associated with drugs treatment; thus, the results of this study still need to be verified with data from more samples.

In addition, CCL5, GNG4, GNLY, GZMH, DRD2, and FASLG play a central role among all the DEGs through PPI analysis. Accumulating evidence has clearly demonstrated that CCL5 plays a pivotal part in the progress of CRC [35]. CCL5, a crucial issue being involved in immune escape, it can raise the buildup of myeloid-derived suppressor cells and T-regulatory cells in the course of CRC. Hence, CCL5 is essential for intermediating regulative impacts in the process of CRC [36]. Tumor necrosis factor (TNF)- α -preactivated hMSCs exude high levels of CCL5 and then aid the progress of CRC by activation of the epithelial-mesenchymal transition (EMT) process [37]. GNG4 is one of the most hypermethylated and down-regulated genes in glioblastoma. Overexpression of GNG4 inhibits cell growth of glioblastoma cell lines and *in vitro* transformation of immortalized human astrocytes, hence indicating a probable tumor suppressor character of GNG4 in glioblastoma [38]. Palma P et al. identified increased GNG4 expression level in treatment-responsive rectal cancer samples compared with non-responsive rectal tumor patients [30]. Our results revealed that GNG4 is highly expressed in

the left-side colon. The tumor microenvironment comprises a multiplex network of immune T-cell subpopulations (relationship between tumor microenvironment and immunity). Camus M et al. demonstrated that the coexpression of GNLY and IRF1 could precisely forecast patient survival independent of the situation of metastasis [39]. GZMH, a functional cytotoxic serine protease of NK cell granules, could expand the cell death-inducing repertoire of the innate immune system. GZMH expression was reduced in breast cancer patients compared with that in healthy women [40]. Gemignani F et al. pointed to a possible role of the dopamine receptor DRD2 in modulating the risk of colorectal cancer [41]. Fas ligand (FasL, CD95L, or TNFSF6) primarily activated T cells, NKT cells, and NK cells [42]. CD95L is a critical survival factor for cancer cells and protects and promotes cancer stem cells [43]. Raats DA et al. concluded that FASLG prompts senescence in mismatch repair-deficient human colon cancer through chronic caspase-mediated induction of DNA damage [44]. Therefore, these core genes play different roles in the occurrence and development of colon cancer through different mechanisms.

Using GSEA, we found that the right colon is closely related to the pathways of *antigen processing and presentation*, *natural killer cell mediated cytotoxicity*, and *intestinal immune network for IgA production*; therefore, there may be differences in the immune mechanisms between left- and right-sided colon cancers. Hypoxia-induced autophagy boosts antigen presentation in the inflammatory response [45]. Lai MC et al. demonstrated that hypoxia up-regulated the translation of genes involved in antigen processing and presentation in colon cancer cells [46]. *Natural killer cell-mediated cytotoxicity* is related to the process of early-onset CRC and involved in immunosuppression and the epithelial-mesenchymal transition (EMT) in CRC patients [47]. However, differences in the immunological mechanisms of left- and right-sided colon cancers still need to be further explored.

With the development of high-throughput technologies and the improvement of various databases [14, 48-53], some studies have demonstrated that the molecular biological characteristics of left- and right-sided colon cancers impact prognosis. Chromosomal instability (CIN) is one of the manifestations that may cause inactivation of the tumor suppressor gene p53 and is considered an independent prognostic factor for colon cancer [54]. Another study also found that the incidence of p53 mutations in left-sided colon cancer was increased compared with that in right-sided colon cancer. Cases with p53 mutations are at increased risk of death [55]. Microsatellite instability (MSI) is highly associated with right-sided colon cancer [56]. Popat S et al. confirmed that MSI is one of the independent factors that can accurately predict prognosis [57]. The CpG island hypermethylation phenotype (CIMP) often leads to silencing of tumor suppressor genes and tumorigenesis. Iacopetta B found that CIMP often occurred in the right-sided colon [58]. Barault et al. reported that the 5-year survival rate of the patients with a MSI stable genotype and positive for CIMP was poor. Juo et al. found that CIMP is an independent risk factor for poor prognosis in colorectal cancer [59]. This finding indicates that low expression of the genes in right-sided colon cancer is possibly related to hypermethylation. The RAS family is a family of key effector molecules which is downstream of EGFR, and the incidence of EGFR activation via RAS mutations in right-sided colon cancer is significantly increased as compared to the left-sided colon [60]. K-RAS mutations are associated with poor prognosis because patients with these mutations cannot benefit from chemotherapy [61]. BRAF is also a pivotal molecule in the MARK signaling pathway. Studies found that the incidence of BRAF mutations in right-sided colon cancer was increase compared with that in left-sided colon cancer [62, 63]. Correlation meta-analysis revealed that BRAF mutation is a high risk factor for the prognosis of colon cancer [64]. The difference in genetic alterations between left- and the right-sided colon cancers should be further studied.

Our study still has some limitations. We tried to examine the prognostic value of the current model with several genes by the microarray data from GEO database (GSE14333, GSE37892 and GSE39084). But unfortunately those prognostic genes identified by our study showed no prognostic value in GEO data. We consider that the inconsistency may be due to the following reasons. First, sample heterogeneities such as countries, race, and accurate

tumor position could cause altered results. Second, these independent GSE studies only contained limited number of samples to generate precise results. We will utilize clinical samples of our hospital or other independent cohorts for verification in the future.

In conclusion, given differences in clinical practice, molecular biological characteristics, survival, and prognosis between left- and right-sided colon cancers, these cancers can be interpreted as two different diseases. In this study, left- and right-sided colon cancer cases were investigated based on gene expression in the TCGA database to clarify their differences and construct separate prognostic models, providing the basis for the individualized treatment and prognosis assessment of left- and right-sided colon cancers.

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Disclosure Statement

The authors declare to have no competing interests.

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