Original Article

Cyclophilin B overexpression predicts a poor prognosis and activates metastatic pathways in colon cancer

Xiaojing Zhang[1], Jinjing Tan[2], Lei Yang[3], Guangyu An[1]

[1]Oncology Department, Beijing Chao-Yang Hospital, Capital Medical University, Beijing 100020, China; [2]Department of Cellular and Molecular Biology, Beijing Chest Hospital, Capital Medical University & Beijing Tuberculosis and Thoracic Tumor Research Institute, Beijing 101149, China; [3]Medical Research Center, Beijing Chao-Yang Hospital, Capital Medical University, Beijing 100020, China

Contributions: (I) Conception and design: G An; (II) Administrative support: G An, L Yang; (III) Provision of study materials or patients: G An, L Yang; (IV) Collection and assembly of data: X Zhang, J Tan; (V) Data analysis and interpretation: X Zhang, J Tan, L Yang; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Guangyu An, MD. Oncology Department, Beijing Chao-Yang Hospital, No. 8, Gongti South Road, Chaoyang District, Beijing 100020, China. Email: agybjcy@163.com; Lei Yang, MD. Medical Research Center, Beijing Chao-Yang Hospital, No. 8, Gongti South Road, Chaoyang District, Beijing 100020, China. Email: yl6649084@163.com.

Background: Cyclophilin B (CypB) has been found overexpressed in various malignant tumors. To date, there are few studies on CypB in colon cancer. In this study, we aimed to analyze the CypB expression pattern and to further evaluate its clinical significance, especially its prognostic value for colon cancer.

Methods: CypB expression was investigated in colon cancer tissue microarrays (TMA) by RNAscope in situ hybridization and immunohistochemical (IHC) staining. The correlation between CypB and clinicopathological characteristics was analyzed. The Cancer Genome Atlas (TCGA) RNA-seq dataset of colon adenocarcinoma (COAD) was further analyzed to validate our main findings. Gene Set Enrichment Analysis (GSEA) and Search Tool for the Retrieval of Interacting Genes (STRING) analysis were performed to enrich CypB related biological pathways. *In vitro* experiments by knockdown of CypB in colon cancer cell HCT116 were performed to verify the bioinformatics results and analyze its role in the metastatic pathways in colon cancer.

Results: We found that CypB expression was highly upregulated in colon cancer tissues (P<0.05). Importantly, the overall survival (OS) time of patients with high CypB expression was significantly shorter than those with low CypB expression, and overexpressed CypB was identified as an independent prognostic indicator for poor survival (P=0.015). Subgroup analysis indicated that a high level of CypB was associated with a shorter OS time, especially for advanced cancer patients, such as later T stage, lymph node metastasis, larger tumor size (P<0.05). Analysis of TCGA RNA-seq dataset of COAD provided us with a larger clinical sample verification. Bioinformatics analysis and the following *in vitro* study revealed that CypB was involved in tumor metastatic associated signaling pathways.

Conclusions: CypB overexpression predicts a poor prognosis and may activate metastatic pathways in colon cancer.

Keywords: Colon cancer; cyclophilin B (CypB); metastasis; prognosis; RNAscope

Submitted Dec 25, 2019. Accepted for publication Apr 16, 2020.
doi: 10.21037/tcr-19-2960
View this article at: http://dx.doi.org/10.21037/tcr-19-2960

Introduction

Colorectal cancer is a leading cause of cancer-related death. The incidence of colorectal cancer ranks fourth among all malignant tumors, with approximately 140,000 new cases and 50,000 cases of death each year in the United States (1). In China, due to the huge population base and dramatic changes in the environment, the number of deaths per year is approximately 190,000 (2).

Colonoscopy has been used in the clinic for the early diagnosis of colorectal cancer and has promoted a 5-year survival rate of almost 90%. Unfortunately, many patients lose the chance for early diagnosis and effective treatment and often develop distant metastases, and the 5-year survival rate for those patients is only 12.5% (3). For these distant-stage patients, we more urgently need to find effective biomarkers closely related to prognosis and their pathological mechanisms in order for more precise targeted treatments.

Cyclophilins (Cyps) have been reported to exhibit peptidyl-prolyl isomerase enzymatic activity and are involved in a variety of cell functions (4,5). CypB (cyclophilin B) is a member of the Cyps family, which is predominantly located in the endoplasmic reticulum (ER) and was indicated to act as the target of cyclosporin A (an immunosuppressive drug). CypB has also been shown to be involved in many biological processes, including protein folding (6), virus replication (7), immunosuppression (8) and osteogenesis (9). Recently, a high level of CypB was found in pancreatic, breast, gastric and liver cancer (10-13). CypB was found to promote cancer by accelerating cell proliferation, decreasing cell apoptosis, and facilitating cell migration and invasion (10,14-16). However, the clinical significance of CypB overexpression remains to be investigated in colorectal cancer.

In this study, we analyzed the expression of CypB by RNAscope *in situ* hybridization and immunohistochemical (IHC) staining in colon cancer. Furthermore, we analyzed the correlation between CypB expression and clinicopathological characteristics. Then, we focused on the prognostic significance and signaling pathways of CypB in colon cancer. Our study demonstrates that CypB was overexpressed in colon cancer tissues and that the upregulation of CypB was associated with poor survival. Bioinformatics analysis and the *in vitro* study revealed that CypB was involved in tumor metastatic signaling pathways. Hence, we propose that CypB serves as a promising prognostic biomarker and may promote metastasis in colon cancer.

Methods

Patients and tumor tissue microarray (TMA)

The colonic TMA (HCol-Ade180Sur-07, Shanghai Outdo Biotech Co., Shanghai, China) used in RNAscope analysis contained 90 cases of colonic adenocarcinoma and paired adjacent noncancerous tissues. All tissues were retrospectively collected from patients after surgery from January 2009 to October 2009. Before surgery the patients did not receive any chemotherapy or radiotherapy. And the follow-up data of patients were acquired from February 2009 to May 2014. The included patients were followed-up routinely either till their expiry or at least 5 years from their surgery date. Detailed clinicopathological characteristics are listed in supplementary *Table S1*. The HCol-Ade030PG-01 TMA (Shanghai OUTDO Biotech Co., Shanghai, China) used in IHC analysis consisted of 15 paired colorectal adenocarcinoma tissues and matched normal mucosa; All tissues were retrospectively collected from patients underwent surgery from January 2009 to October 2009. Before surgery the patients did not receive any chemotherapy or radiotherapy. The TMAs were stored in 4 ℃ before use. This TMA has no clinicopathological or follow-up data. Tumor T staging, N staging and TNM staging were performed based on the 7th Edition of American Joint Committee on Cancer (AJCC) staging system. Histological grading was performed according to the World Health Organization (WHO) classification of tumors of the digestive system of 2010. According to the location of the tumor, tumors located before the splenic flexure of the transverse colon were defined as right colon tumors, and tumors located at or after the splenic flexure of the transverse colon were defined as left colon tumors. Our study design, tissue sample, and data collection were accomplished according to our institutional protocols, which approved by Institutional Ethics Committee, Beijing Chao-Yang Hospital of Capital Medical University (No. 2018-Research-61) and informed consent was taken from all the patients. Our primary endpoint of the study was overall survival (OS) that is stated as the time from the date of surgery to death or the last follow-up date.

RNAscope in situ hybridization and image analysis

RNAscope *in situ* hybridization analysis was performed on colon cancer TMAs using a probe that targeted human CypB (Cat. No. 476701; Advanced Cell Diagnostics, Hayward, CA, USA) based on the manufacturer's instruction, and a standard pretreatment protocol was

used. RNAscope 2.5 High Definition (HD) Reagent Kit-brown (Cat. No. 322310; Advanced Cell Diagnostics, Hayward, CA, USA) was adopted to amplify and visualize the hybridization signals. Then, the slide image was taken with an Aperio scanner and viewed with AperioImageScope software (v12.3.1.6002, Leica Biosystems). CypB mRNA molecules are shown as brown spots and were counted manually. According to the manufacturer's guidelines, a 5-tier scoring system was developed for semiquantitative microscopic evaluations: score 0 (–), no staining or less than 1 dot in each of ten cells; score 1 (+), 1–3 dots per cell; score 2 (++), 4–10 dots per cell, very few dot clusters; score 3 (+++), >10 dots per cell and the cells with dot clusters were <10% of all cells; and score 4 (++++), >10 dots per cell and the cells with dot clusters were >10% of all cells. Scores of 0–2 were considered low CypB mRNA expression, and scores of 3–4 were considered high CypB mRNA expression. Bacillus subtilis DapB mRNA (Cat. No. 310043; Advanced Cell Diagnostics, Hayward, CA, USA) was probed as a negative control. All the staining scores were reviewed by two pathologists through blinded-reading.

IHC staining analysis

The TMA slide was deparaffinized and rehydrated and rinsed in water. To quench endogenous peroxidase activity, the TMA slide was treated with 0.3% H_2O_2 for 10 minutes at room temperature. Antigen retrieval was performed in 0.01 M sodium citrate (pH =6.0) with heating in a pressure cooker. The sections were then blocked in 2% goat serum and were incubated with the primary antibody for 1 hour at room temperature. This study used rabbit polyclonal anti-CypB antibody (ab16045, Abcam Inc., Cambridge, MA, USA) as the primary antibody with 1:500 dilution. Then the second antibody from SP reagent kit (Zhongshan Goldenbridge Biotechnology Co., Beijing, China) was exerted to incubate the TMA sections for 20 minutes at room temperature, followed by further incubation with streptavidin-horseradish peroxidase complex. Staining with 3,3'-diaminobenzidine kit (DAB; Zhongshan Goldenbridge Biotechnology Co.), TMA sections were counter-stained with hematoxylin and evaluated. Score is the combination of staining intensity (0= negative, 1= mild staining, 2= moderate staining and 3= strong staining) and percentage of positive cells (0: <5%, 1: 6% to 25%, 2: 26% to 50%, 3: 51% to 75% and 4: >76%) (17). Finally the CypB staining was assigned to one of 4 levels as follows: negative (–) (score of 0), weak (+) (score of 1–4), moderate (++) (score of 5–8) to strong (+++) (score of 9–12). Negative (–) and weak (+) were considered as low expression, and moderate (++) and strong (+++) were considered as high expression.

Colon Adenocarcinoma (COAD) RNA-seq data from the Cancer Genome Atlas (TCGA)

The COAD RNA-seq datasets of TCGA, which enrolled 286 COAD tissues and 41 adjacent noncancerous tissues, were downloaded through the UCSC cancer genome browser (https://xenabrowser.net). The Illumina HiSeq 2000 RNA Sequencing platform was used to experimentally measure gene expression at the University of North Carolina TCGA genome characterization center. Level 3 data was downloaded from TCGA data coordination center. This dataset shows the gene-level transcription estimates, as in log2(x+1) transformed RSEM normalized count.

Gene set enrichment analysis (GSEA) and network construction

Gene Set Enrichment Analysis (GSEA, http://software.broadinstitute.org/gsea/) was applied for enriching CypB related pathways. At first, the top 50 up-regulated and 50 down-regulated differential genes between normal and cancer tissues from COAD datasets of TCGA were selected using Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/) (18). Finally, 87 genes were selected after deleting non-coding RNA. Then CypB related signaling pathways were enriched using GSEA by dividing those differential genes into two sets according to the median value of CypB. The gene set permutations analysis was repeated 1,000 times, according to the default weighted enrichment statistical method. Nominal P value, enrichment score (ES) and false discovery rate (FDR) were calculated to verify the significant difference for GSEA. After gene enrichment, the Search Tool for the Retrieval of Interacting Genes (STRING, https://string-db.org/) was used to construct protein-protein interactions (PPI) and screen the CypB related signaling pathways.

Cell lines, cell culture and cell transfection

The human colon cancer cell line HCT116 purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA) was used for our experiment. Cells were cultured in RPMI-1640 (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 10%

fetal bovine serum (FBS; HyClone; Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 37 ℃ in a humidified atmosphere containing 5% CO_2. HCT116 cells were seeded in six-well plates and allowed to attach overnight. With the application of lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA), CypB small interfering RNA (siRNA) and control siRNA were transfected into the cells respectively according to the manufacturer's recommendations. Then the cells were further cultured at 37 ℃ in a 5% CO_2 atmosphere. CypB siRNA-1 sequence was 5'-GCAUGGAGGUGGUGCGG-3', CypB siRNA-2 sequence was 5'-CUUAGCUACAGGAGAGAA-3', and the negative control siRNA sequence was 5'-TTCTCCGAACGTGTCACGT-3'. Both of them were designed and synthesized by the Beijing Hesheng Gene Technology Co., Ltd. (Beijing, China).

RNA extraction and real-time quantitative PCR

Total cellular RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Total RNA was then reverse transcribed to cDNA using the EasyScript® First-Strand cDNA Synthesis kit (Transgene, Beijing, China). Gene expression analysis was performed by qRT-PCR using a SYBR Premix Ex Taq Kit (Takara, Dalian, China). Relative gene expression was quantified using the comparative threshold cycle ($2^{-\Delta\Delta Ct}$) method. The PCR program was as follows: pre-denaturation at 95 ℃ for 2 min, 40 cycles of denaturation at 95 ℃ for 5 s, and annealing and elongation at 60 ℃ for 30 s. The primers used in the experiment were as follows:

CypB: Forward, AAGTCACCGTCAAGGTGTATTTT; Reverse, TGCTGTTTTTGTAGCCAAATCCT.

CNN1: Forward, AGGTTAAGAACAAGCTGGCCC; Reverse, ATGAAGTTGTTGCCGATGCG.

MYL9: Forward, CTCGCTGGGGAAGAACCCC; Reverse, CGTTGCGAATCACATCCTCG.

MYH11: Forward, AGACACAAGTATCACGGGAGAG; Reverse, TTGCCGAATCGTGAGGAGTT.

E-cadherin: Forward, GTCACTGACACCAACGATAATCCT; Reverse, TTTCAGTGTGGTGATTACGACGTTA.

Snail: Forward, GCCATGTCCGGACCCACACTG; Reverse, GGCAGGGGCAGGTATGGAGA.

TWIST: Forward, GTCCGCAGTCTTACGAGGAG; Reverse, GCTTGAGGGTCTGAATCTTGCT.

Vimentin: Forward, CCTGAACCTGAGGGAAACTAA; Reverse, GCAGAAAGGCACTTGAAAGC.

18s: Forward, AAACGGCTACCACATCCA; Reverse, CACCAGACTTGCCCTCCA.

Statistical analysis

Statistical analyses were conducted using SPSS software for Windows, version 17.0 (SPSS, Chicago, IL, USA). GraphPad Prism for Windows, version 5.0 (GraphPad Software, San Diego, CA, USA) was used to create the artwork. Quantitative variables were compared by means of the student *t*-test. Categorical variables were compared using the χ^2 test. The Cox proportional hazards regression model and the Kaplan-Meier test were used to assess the OS rates. The survival curves were plotted by the log-rank test. P<0.05 was considered statistically significant.

Results

RNAscope in situ hybridization and IHC staining present the overexpression of CypB in colon cancer

TMA that contained 90 paired cancer and adjacent normal tissues was used to determine the expression of CypB. Finally, 80 cancer tissues and 84 adjacent normal tissues were successfully stained to show the mRNA levels of CypB by RNAscope. According to the expression level of CypB mRNA (representative images were provided in *Figure 1*), staining intensities of score 0 (–), score 1 (+) and score 2 (++) were classified as the low expression group, and score 3 (+++) and score 4 (++++) were classified as the high expression group. CypB mRNA was found to be significantly overexpressed in colon cancer tissues compared with adjacent normal tissues (P<0.001; *Table 1*).

We also used TMA with a small sample size to detect the expression of CypB protein (*Figure 2*). As in mRNA level, the expression of CypB protein in colon cancer is significantly higher than that in adjacent tissues (P<0.05; *Table 2*).

Clinicopathological analysis reveals that CypB is associated with advanced T stage

The correlation between CypB levels and the clinicopathological parameters of 80 colon cancer patients was analyzed. The clinicopathological data of the patients were summarized in the supplementary *Table S1*. Our analysis indicated that the levels of CypB were significantly higher in patients with T4 stage than in those with T1–3 stage (P=0.043; *Table 3*). However, there were no significant correlations between the levels of CypB and other parameters, including age, sex, tumor size, N stage, histological grade, TNM stage and tumor position (P>0.05; *Table 3*).

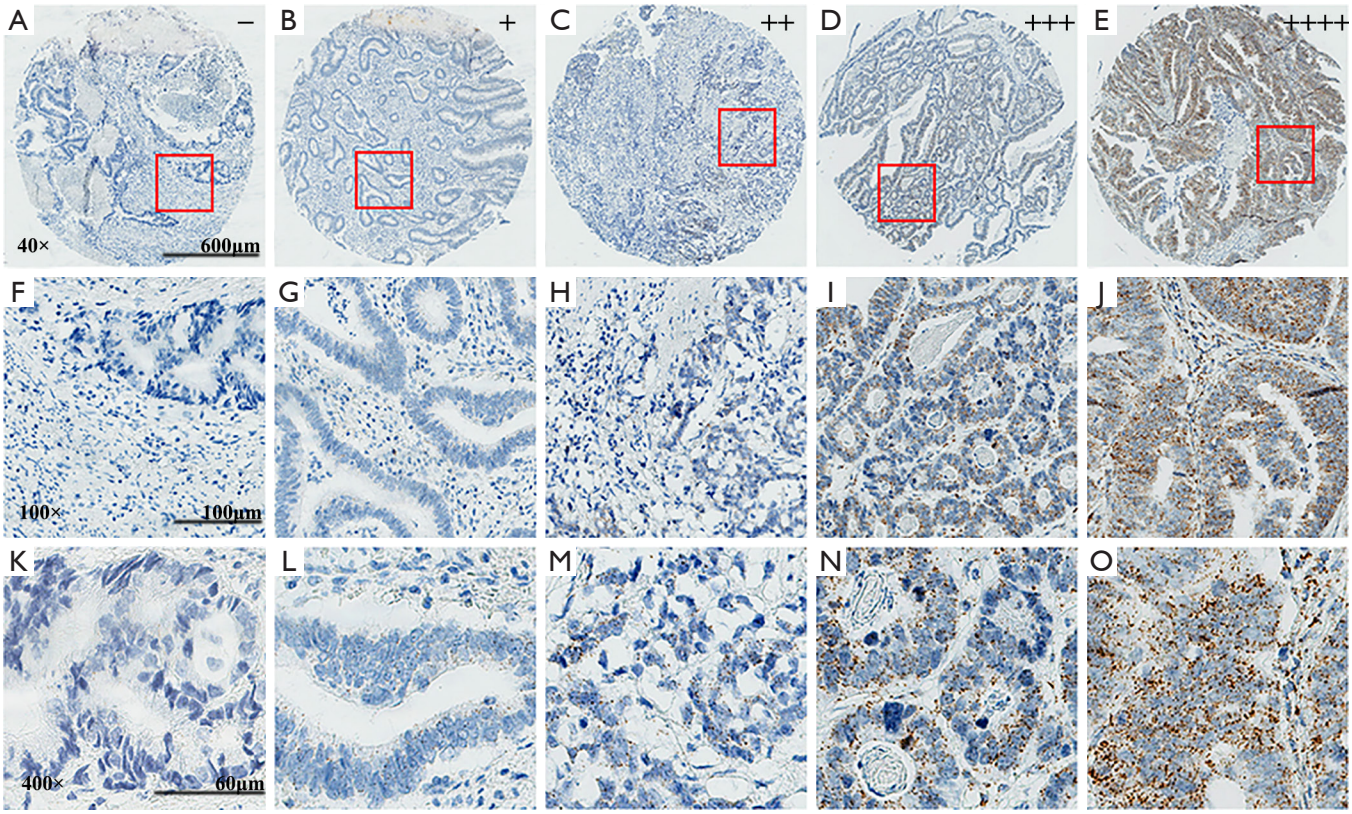

Figure 1 Different expression levels of CypB mRNA in a colon cancer tissue microarray performed by RNAscope *in situ* hybridization. Score 0 (–), no staining or less than 1 dot in each of ten cells; score 1 (+), 1–3 dots per cell; score 2 (++), 4–10 dots per cell, very few dot clusters; score 3 (+++), >10 dots per cell and the cells with dot clusters were <10% of all cells; score 4 (++++), >10 dots per cell and the cells with dot clusters were >10% of all cells. CypB, cyclophilin B. Red boxes indicate the amplified part of the entire image. Staining method RNAscope *in situ* hybridization.

Table 1 Expression of CypB mRNA in colon cancer and adjacent noncancerous tissues

Histological type	Case numbers	CypB expression		P value
		Low	High	
Tumor tissues	80	39	41	<0.001*
Nontumor tissues	84	70	14	

*, P value less than 0.05. CypB, cyclophilin B.

CypB mRNA overexpression predicts a poor prognosis of colon cancer patients

The prognostic significance of CypB mRNA expression was further investigated in colon cancer patients. In total, 80 patients were followed up for 0.4–64 months (mean ± SD, 47.02±19.48 months). At the end of follow up, 27 patients had died. Kaplan-Meier analysis revealed that the high expression of CypB was associated with a shorter OS (*Figure 3A*, P=0.0139). Univariate and multivariate Cox regression analyses indicated that TNM stage (P=0.000) and CypB expression (P=0.015) were independent prognostic indicators for poor survival (*Table 4*). Furthermore, subgroup analysis indicated that high levels of CypB were associated with poor survival for patients with stage T3–4, lymph node metastasis, tumor size ≥5 cm or right colonic cancer (*Figure 3B,C,D,E*, P<0.05). In addition, our analysis also indicated that patients in TNM stages III–IV with high CypB expression had a shorter survival time, although the difference was not significant (*Figure 3F*, P=0.0616).

Validation of CypB overexpression and its prognostic significance in COAD RNA-seq dataset of TCGA

To further validate our findings, we analyzed the CypB

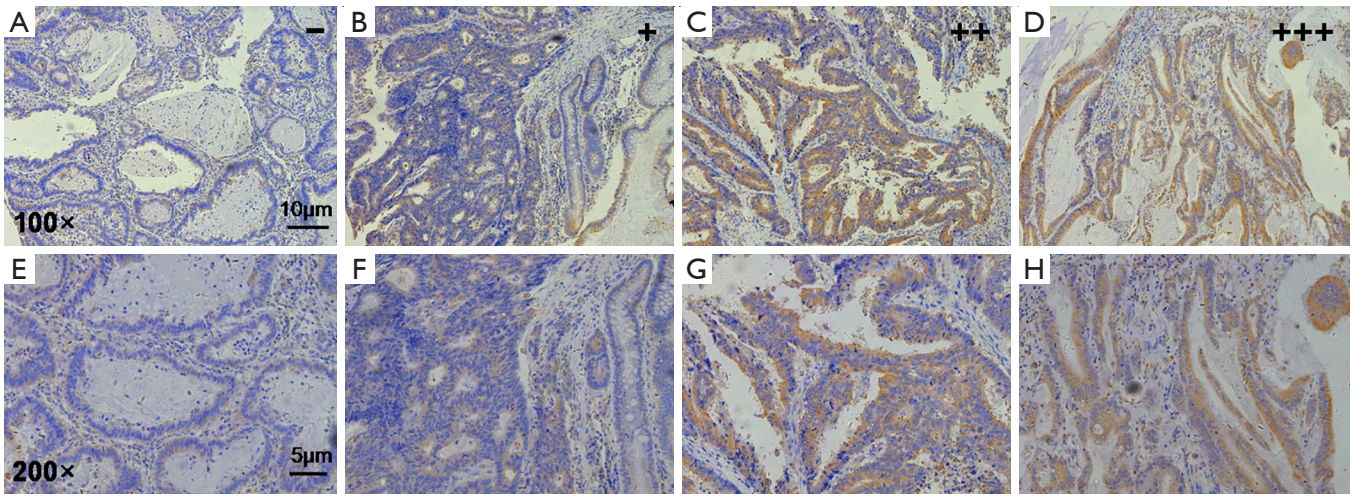

Figure 2 Different expression levels of CypB protein in a colon cancer tissue microarray performed by immunohistochemical (IHC) staining. The levels of CypB range from negative (–) (score of 0), weak (+) (score of 1–4), moderate (++) (score of 5–8) to strong (+++) (score of 9–12). CypB, cyclophilin B.

Table 2 Expression of CypB protein in colon cancer and adjacent noncancerous tissues

Histological type	Case numbers	CypB expression		P value
		Low	High	
Tumor tissues	15	10	5	0.042*
Nontumor tissues	15	15	0	

*, P value less than 0.05. CypB, cyclophilin B.

mRNA expressions in COAD RNA-seq dataset of TCGA. First, we compared the CypB mRNA levels between 286 cancerous tissues and 41 normal tissues (*Figure 4A*). As expected, the CypB levels were significantly higher in cancer tissues than in normal tissues (P<0.0001). Furthermore, in the TCGA 26 paired cancer and corresponding normal tissues, the CypB mRNA levels were also markedly increased in cancer tissues compared to normal tissues (*Figure 4B*, P=0.0146).

Next, we determined the prognostic significance of CypB mRNA in 286 COAD patients. The CypB mRNA expression levels and clinicopathological parameters are summarized in the supplementary *Table S2*. OS differences between patients with high or low CypB expression were analyzed by Cox regression models and log-rank tests. As shown by Kaplan-Meier plots, a high level of CypB mRNA was associated with a reduced OS time (P=0.048, *Figure 5A*). In subgroup analysis, we found that a higher level of CypB mRNA was associated with a shorter OS time for patients with advanced tumors, such as in patients with stage T3–4, lymph node metastasis and TNM stage III-IV (*Figure 5B,C,D*, P<0.05). Furthermore, Cox multivariate analyses confirmed that CypB mRNA was associated with the OS time of COAD patients (*Table 5*, P=0.007).

GSEA and STRING analyses indicate that CypB is enriched in the metastatic pathways

To identify potential function of CypB, we performed GSEA using TCGA data. The cut-off criterion is set to nominal P value <0.05 and |enrichment score (ES)| >0.55. As shown in *Figure 5*, the gene set "FGFR1_TARGETS_IN_PROSTATE_CANCER_MODEL_DN" was enriched with CypB lowly expressed (*Figure 5E*, P<0.05). This gene set is involved in the regulation of epithelial-to-mesenchymal transition (EMT) and Wnt signaling pathway (19). Furthermore, the enriched genes were analyzed by STRING to generate visual images of PPIs and the potential biological processes (*Figure 5F*). The results uncovered that CypB was closely involved in tumor metastatic pathways, including cell adhesion, tight junction, cell-cell junction organization, extracellular matrix organization and adherens junctions interactions (*Table S3*).

CypB is associated with myosin related genes and may involve in Snail-mediated EMT in colon cancer

Based on the enriched gene set with GSEA analysis,

Table 3 The correlation between CypB expression and clinicopathological characteristics in patients with colon cancer

Parameters	Group	Case numbers	CypB expression		P value
			Low expression	High expression	
Age	<65	33	18	15	0.385
	≥65	47	21	26	
Sex	Male	43	21	22	0.987
	Female	37	18	19	
Tumor size	<5 cm	33	14	19	0.296
	≥5 cm	46	25	21	
	NA	1	0	1	
T stage	T1–3	55	31	24	0.043*
	T4	25	8	17	
N stage	N0	53	28	25	0.306
	N1–2	27	11	16	
Histological grade	I–II	69	34	35	0.814
	III–V	11	5	6	
TNM stage	I–II	51	26	25	0.597
	III–V	29	13	16	
Tumor position	Left colon	37	15	22	0.17
	Right colon	41	23	18	
	NA	2	1	1	

*, P value less than 0.05. CypB, cyclophilin B; TNM, tumor-node-metastasis.

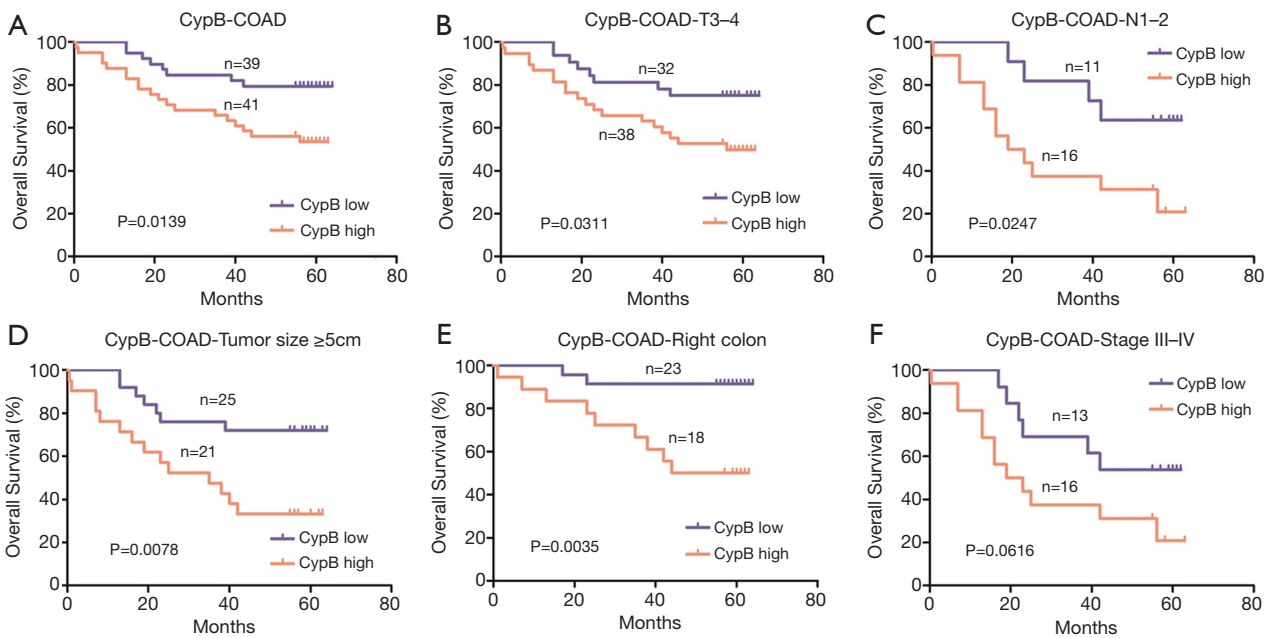

Figure 3 High CypB mRNA expression was correlated with poor prognosis in colon cancer. Kaplan-Meier curves showed that patients with high CypB mRNA expression had significantly shorter OS than those with low CypB mRNA expression in all colon cancer patients (A), T3–4 patients (B), N1–2 patients (C), patients with a tumor size ≥5 cm (D) and patients with right colon cancer (E) (P=0.0139, 0.0311, 0.0247, 0.0078 and 0.0035, respectively; log-rank test). The OS of patients in TNM stage III–IV (F) did not differ significantly according to CypB expression (P=0.0616; log-rank test). CypB, cyclophilin B; OS, overall survival; TNM, tumor-node-metastasis.

Table 4 Univariate and multivariate analysis of prognostic parameters in patients with colon cancer

Parameters	OS					
	Univariate analysis			Multivariate analysis		
	HR	95% CI	P value	HR	95% CI	P value
Age (<65 *vs.* ≥65)	1.067	0.495–2.301	0.868			
Sex (male *vs.* female)	1.832	0.822–4.080	0.138			
Tumor size (<5 *vs.* ≥5 cm)	3.183	1.282–7.902	0.013*			
T stage (T1–3 *vs.* T4)	3.432	1.599–7.364	0.002*			
N stage (N0 *vs.* N1–2)	3.702	1.712–8.006	0.001*			
Histological grade (I–II *vs.* III–IV)	3.534	1.485–8.411	0.004			
TNM stage (I–II *vs.* III–IV)	4.805	2.146–10.755	0.000*	4.918	2.193–11.03	0.000*
Tumor position (left *vs.* right colon)	0.586	0.269–1.277	0.179			
CypB mRNA expression (low *vs.* high)	2.693	1.178–6.155	0.019*	0.36	0.157–0.823	0.015*

*, P value less than 0.05. CI, confidence interval; CypB, cyclophilin B; HR, hazard ratio; OS, overall survival; TNM, tumor-node-metastasis.

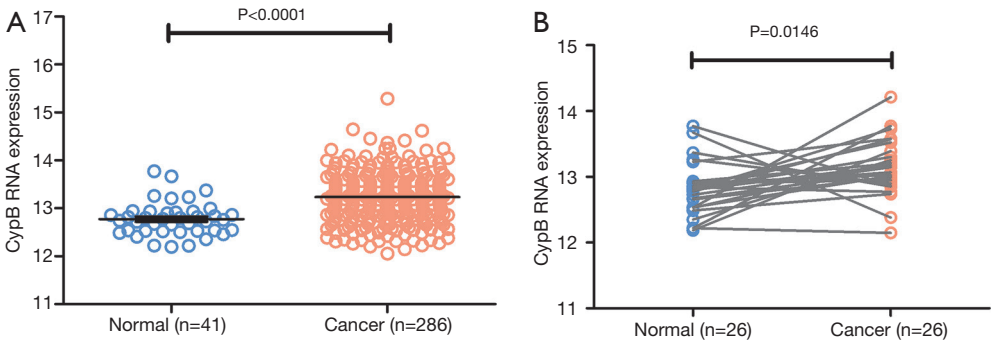

Figure 4 Expression of CypB mRNA was upregulated in COAD patients from TCGA. The analysis of COAD RNA-seq data showed that CypB mRNA was highly upregulated in cancer tissues compared with unpaired (A) and paired (B) adjacent normal tissues (P<0.0001 and P=0.0146, respectively; *t*-test). COAD, colon adenocarcinoma; CypB, cyclophilin B; TCGA, the Cancer Genome Atlas.

we found that the expressions of calponin 1 (CNN1), myosin light chain 9 (MYL9) and myosin heavy chain 11 (MYH11) were positively correlated with the expression of CypB. These three genes are all necessary in cell movement, cytokinesis and spindle formation, which are related to tumor invasion and metastasis. Therefore, *in vitro* experiments by knockdown of CypB in colon cancer cell HCT116 were performed to verify the bioinformatics results. We found that compared with the NC-siRNA group, CypB silencing significantly reduced the expressions of MYL9, MYH11 and CNN1 (*Figure 6A,B,C,D*, P<0.05).

Subsequently, GSEA and STRING analyses revealed that CypB may closely involved in tumor metastatic pathways, such as EMT. During EMT, epithelial cells lose epithelial characteristics and acquire a mesenchymal, highly invasive phenotype. In this process, many transcriptional regulators, such as TWIST, ZEB, Snail and Slug are activated, leading to the downregulation of E-cadherin expression. In HCT116 cells, CypB silencing significantly reduced Snail expression (*Figure 6E*, P=0.0048). Although there was no statistical significance, the expression of E-cadherin increased (*Figure 6F*, P>0.05) after CypB decreased. But there were no significant changes in Vimentin and TWIST expressions (*Figure 6G,H*, P>0.05). These data suggest that Snail-mediated EMT may be associated with CypB in colon cancer.

Discussion

Previous studies have found that CypB was involved in many pathophysiological processes, including osteogenesis,

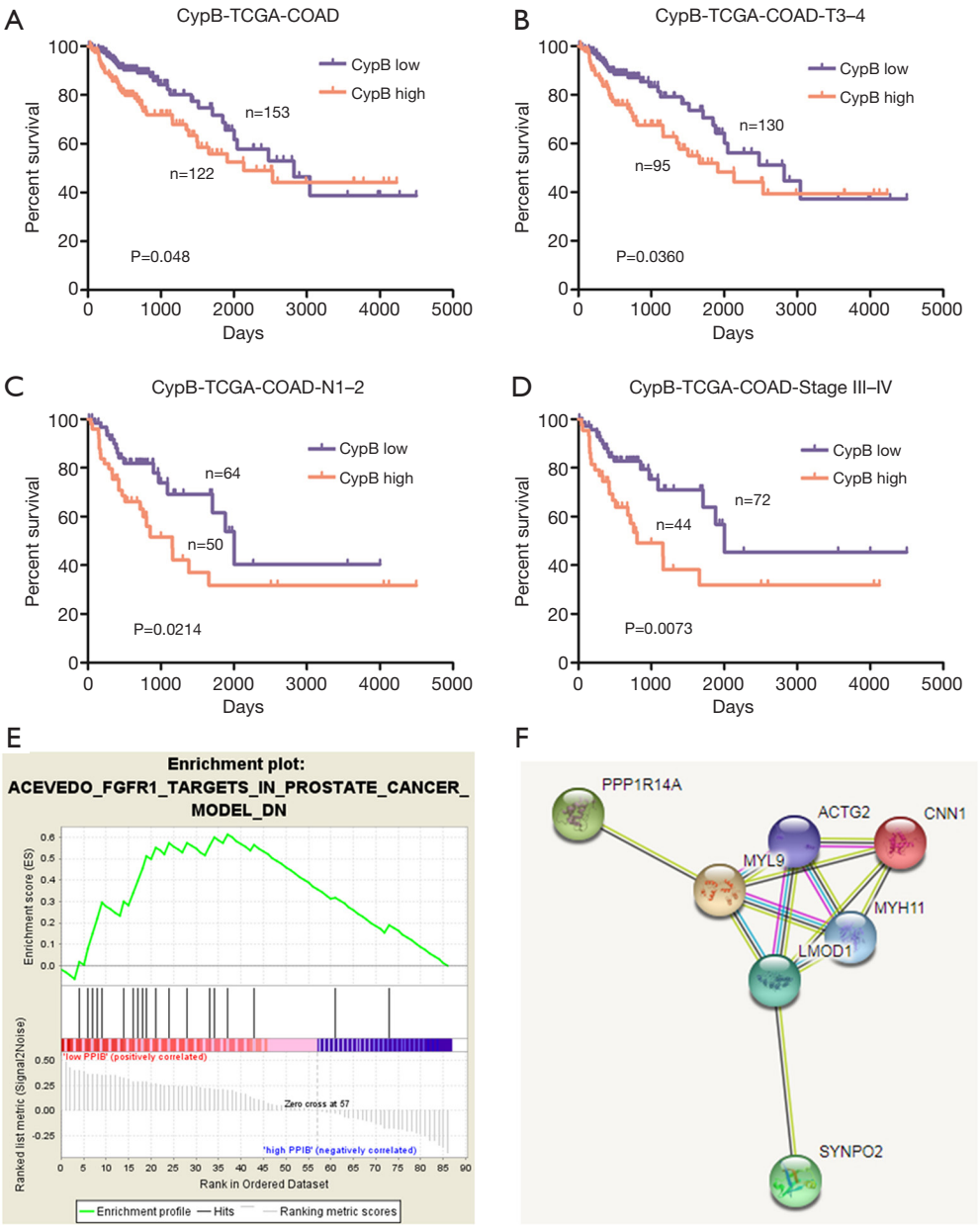

Figure 5 Association between CypB expression and the prognosis of patients with COAD from TCGA and CypB related biological pathways. Kaplan-Meier curves showed that patients with high CypB mRNA expression had significantly shorter OS than those with low CypB mRNA expression in all COAD patients (A), T3–4 patients (B), N1–2 patients (C) and patients in TNM stage III–IV (D) (P=0.048, 0.0360, 0.0214 and 0.0073, respectively; log-rank test). GSEA and STRING analysis of CypB related biological pathways. The gene set of "FGFR1_TARGETS_IN_PROSTATE_CANCER_MODEL_DN" was enriched with CypB lowly expressed by GSEA (E). STRING analysis was employed to generate a visual image of protein-protein interactions using the enriched genes (F). COAD, colon adenocarcinoma; CypB, cyclophilin B; DN, down; FGFR1, fibroblast growth factor receptor 1; GSEA, Gene set enrichment analysis; OS, overall survival; STRING, the Search Tool for the Retrieval of Interacting Genes; TCGA, the Cancer Genome Atlas; TNM, tumor-node-metastasis.

Table 5 Univariate and multivariate analysis of prognostic parameters in patients with colon cancer in TCGA

Parameters	OS					
	Univariate analysis			Multivariate analysis		
	HR	95% CI	P value	HR	95% CI	P value
Age (<65 *vs.* ≥65)	1.521	0.914–2.531	0.106			
Sex (male *vs.* female)	0.686	0.420–1.121	0.133			
T stage (Tis–2 *vs.* T3–4)	2.516	1.009–6.271	0.048*			
N stage (N0 *vs.* N1–2)	2.432	1.499–3.947	0.000*			
TNM stage (I–II *vs.* III–IV)	2.548	1.538–4.219	0.000*	2.808	1.688–4.671	0.000*
Tumor position (left *vs.* right colon)	1.325	0.779–2.254	0.299			
CypB mRNA expression (low *vs.* high)	1.614	0.998–2.612	0.049*	2.007	1.212–3.323	0.007*

*, P value less than 0.05. CI, confidence interval; CypB, cyclophilin B; HR, hazard ratio; OS, overall survival; TNM, tumor-node-metastasis.

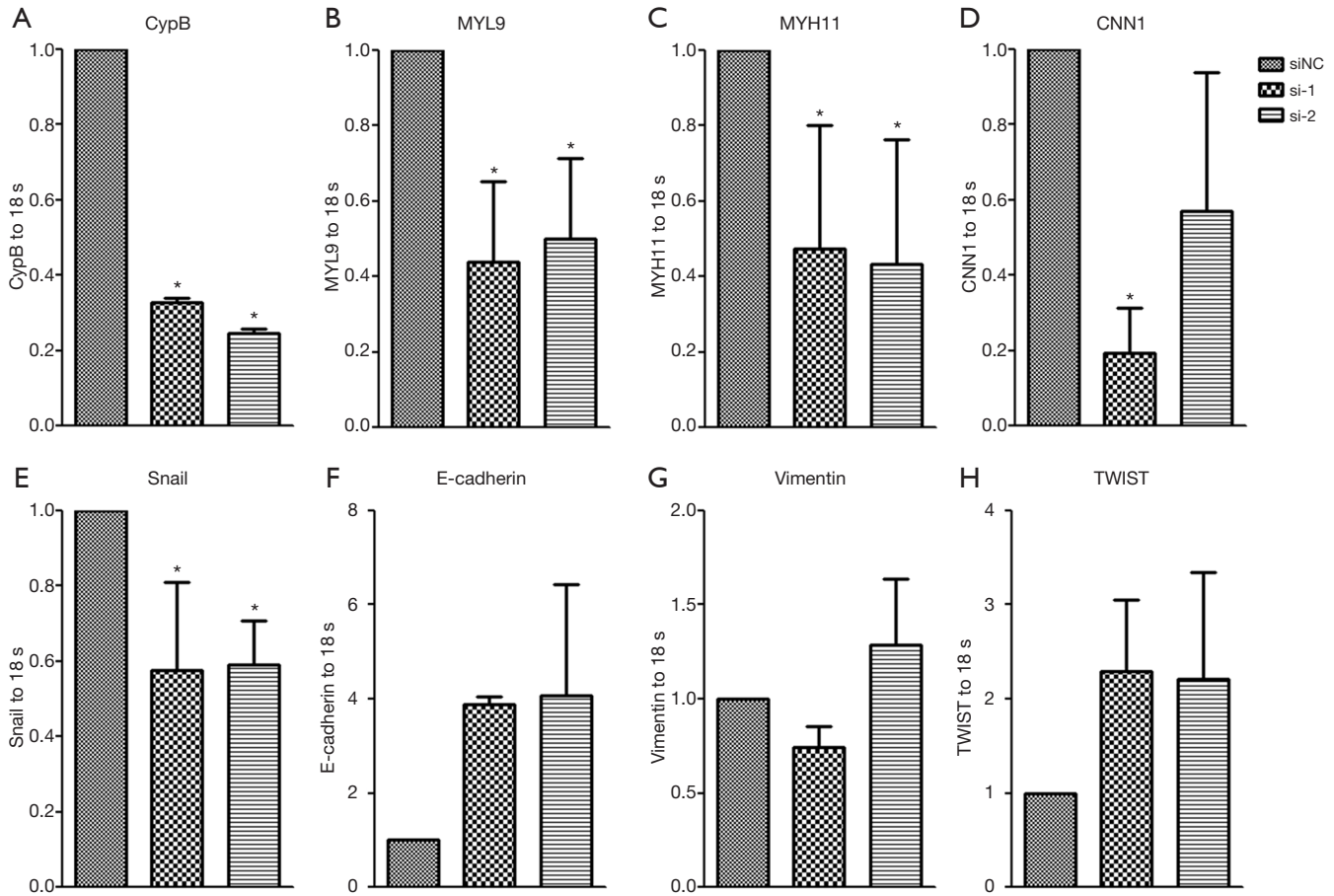

Figure 6 CypB is associated with myosin related genes and may involve in Snail-mediated EMT in colon cancer. After transfection of CypB siRNAs, the transcriptional level of CypB (A), MYL9 (B), MYH11 (C), CNN1 (D), Snail (E), E-cadherin (F), Vimentin (G) and TWIST (H) in HCT116 cells were detected by qRT-PCR. *, P<0.05, versus siNC. CNN1, calponin 1; CypB, cyclophilin B; EMT, epithelial-to-mesenchymal transition; MYH11, myosin heavy chain 11; MYL9, myosin light chain 9.

hepatitis virus replication, and immunosuppression. In recent years, CypB overexpression has been observed in stomach, liver, pancreatic, breast and several other types of cancers (10,11,13,20,21). Some *in vitro* studies have shown that CypB could promote tumor cell proliferation, protect tumor cells against oxidative stress, and stimulate neovascularization (22-24). So far, only one research team has analyzed the relationship between CypB expression and prognosis in colon cancer. Their research was only at the protein level, and the correlation between CypB and clinicopathological parameters was not further explored (12). Our study applied a new technique of RNA *in situ* hybridization-RNAscope, and explored the expression pattern and clinical significance of CypB in colon cancer at the RNA level. We also detected the CypB protein expression using IHC. Bioinformatics analysis was applied to find out the CypB involved signaling pathways, which provides a new clue to reveal the function of CypB in colon cancer.

For formalin-fixed, paraffin-embedded tissue sections, immunohistochemistry remains the overwhelming technique of choice. However, validations can be complex, with significant specificity, sensitivity and reproducibility issues. Commercial antibodies from many available vendors may also lead to nonstandard approaches. The RNAscope *in situ* hybridization method enabled a realistic alternative with fewer validation steps and more stringent and reproducible assessment criteria (25,26). In our analyses, we used this method to stain CypB mRNA in single colon cancer cells and adjacent normal cells. We also analyzed the CypB protein expression using IHC. We found that CypB mRNA and protein were distributed in the cytoplasm and nucleus. Furthermore, we observed that CypB was apparently overexpressed in colon cancer tissues compared with adjacent normal tissues. The high expression of CypB mRNA was significantly higher in patients with T4 stage than in those with T1–3 stage. However, there were no significant correlations between CypB mRNA expression and other parameters. To the best of our knowledge, this is the first study to demonstrate the relation between CypB and clinicopathological parameters in colon cancer.

Additionally, the patients who had relatively high levels of CypB showed poorer prognoses than their low-level counterparts, and further Cox regression analyses indicated that CypB mRNA expression was an independent prognostic indicator. The expression of CypB is not significantly correlated with clinicopathological parameters, such as T and N stages, but its high expression is related to a poor prognosis, suggesting that CypB may not directly promote the tumor proliferation but may affect the prognosis in other ways. For example, Choi's study found that the overexpression of CypB could promote oxaliplatin resistance and inhibit oxaliplatin-induced apoptosis in colon cancer cells (27), therefore, further research is needed on this perspective. In addition, in subgroup analysis, we found that CypB had prognostic significance in more advanced tumors, such as in patients with T3–4, lymph node metastasis and clinical stage III-IV, suggesting that CypB may play a vital role in late stage of colon cancer, such as promoting cancer migration.

With the wide application of sequencing technology, TCGA datasets contain differentially expressed transcripts of many cancers (28,29). Here, the COAD RNA-seq dataset in the TCGA was downloaded and analyzed. We confirmed that CypB mRNA was highly upregulated and served as a prognostic biomarker in colon cancer, especially in more advanced tumors. These results further validate our main findings from the TMA.

The mechanism and signaling pathways which CypB is involved in several cancers are studied in depth (10,15,21,30,31). However, the detailed mechanism for CypB in colon cancer progression still needs to be elucidated. In our study, bioinformatic analysis showed that the CypB may be closely associated with metastatic related processes, such as EMT and Wnt signaling pathway. Further cell experiments revealed that compared with the NC-siRNA group, CypB silencing significantly reduced the expressions of MYL9, MYH11 and CNN1. These genes all belong to the myosin family and more and more evidences show that this family may play important roles in tumor invasion and metastasis development, including EMT process (32,33). During EMT, epithelial cells lose epithelial characteristics and acquire a mesenchymal, highly invasive phenotype (34,35). Therefore we next tested several EMT related genes after knockdown of CypB. And results showed that CypB may be associated with Snail-mediated EMT in colon cancer. But further *in vivo* experiments should be designed to verify our findings *in vitro*.

Conclusions

Collectively, we report here that CypB is remarkedly overexpressed in human colon cancer. Overexpressed CypB is an independent prognostic indicator for poor survival, especially for advanced tumors. Bioinformatic and *in vitro* study analysis revealed that CypB is associated with some myosin related genes and may involve in Snail-mediated

EMT process in colon cancer. CypB may have an important role in the regulation of tumor metastasis. In this regard, we suggest that CypB could serve as a promising poor prognostic biomarker for colon cancer.

Acknowledgments

Funding: This research was supported by the National Natural Science Foundation of China (No. 81802349).

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr-19-2960). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted under the approval of the Institutional Ethics Committee, Beijing Chao-Yang Hospital of Capital Medical University (No. 2018-Research-61). Written informed consent was obtained from the patient for publication of this study and any accompanying images. The study outcomes will not affect the future management of the patients.

References

1. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA Cancer J Clin 2017;67:7-30.
2. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. CA Cancer J Clin 2016;66:115-32.
3. Siegel R, Desantis C, Jemal A. Colorectal cancer statistics, 2014. CA Cancer J Clin 2014;64:104-17.
4. Hoffmann H, Schiene-Fischer C. Functional aspects of extracellular cyclophilins. Biol Chem 2014;395:721-35.
5. Yao Q, Li M, Yang H, et al. Roles of cyclophilins in cancers and other organ systems. World J Surg 2005;29:276-80.
6. Skagia A, Zografou C, Vezyri E, et al. Cyclophilin PpiB is involved in motility and biofilm formation via its functional association with certain proteins. Genes Cells 2016;21:833-51.
7. DeBoer J, Madson CJ, Belshan M. Cyclophilin B enhances HIV-1 infection. Virology 2016;489:282-91.
8. Lee J, Choi TG, Ha J, et al. Cyclosporine A suppresses immunoglobulin G biosynthesis via inhibition of cyclophilin B in murine hybridomas and B cells. Int Immunopharmacol 2012;12:42-9.
9. Terajima M, Taga Y, Chen Y, et al. Cyclophilin-B Modulates Collagen Cross-linking by Differentially Affecting Lysine Hydroxylation in the Helical and Telopeptidyl Domains of Tendon Type I Collagen. J Biol Chem 2016;291:9501-12.
10. Li T, Guo H, Zhao X, et al. Gastric Cancer Cell Proliferation and Survival Is Enabled by a Cyclophilin B/STAT3/miR-520d-5p Signaling Feedback Loop. Cancer Res 2017;77:1227-40.
11. Ray P, Rialon-Guevara KL, Veras E, et al. Comparing human pancreatic cell secretomes by in vitro aptamer selection identifies cyclophilin B as a candidate pancreatic cancer biomarker. J Clin Invest 2012;122:1734-41.
12. Kim Y, Jang M, Lim S, et al. Role of cyclophilin B in tumorigenesis and cisplatin resistance in hepatocellular carcinoma in humans. Hepatology 2011;54:1661-78.
13. Fang F, Flegler AJ, Du P, et al. Expression of cyclophilin B is associated with malignant progression and regulation of genes implicated in the pathogenesis of breast cancer. Am J Pathol 2009;174:297-308.
14. Ray P, Sullenger BA, White RR. Further characterization of the target of a potential aptamer biomarker for pancreatic cancer: cyclophilin B and its posttranslational modifications. Nucleic Acid Ther 2013;23:435-42.
15. Kim K, Kim H, Jeong K, et al. Release of overexpressed CypB activates ERK signaling through CD147 binding for hepatoma cell resistance to oxidative stress. Apoptosis 2012;17:784-96.
16. Xiong L, Ding L, Ning H, et al. CD147 knockdown improves the antitumor efficacy of trastuzumab in HER2-positive breast cancer cells. Oncotarget 2016;7:57737-51.
17. Li X, Lv L, Zheng J, et al. The significance of LRPPRC overexpression in gastric cancer. Med Oncol 2014;31:818.
18. Tang Z, Li C, Kang B, et al. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 2017;45:W98-W102.

19. Acevedo VD, Gangula RD, Freeman KW, et al. Inducible FGFR-1 activation leads to irreversible prostate adenocarcinoma and an epithelial-to-mesenchymal transition. Cancer Cell 2007;12:559-71.
20. Guo L, Han Y. Surgery combined with local 5-aminolevulinic acid-photodynamic therapy on skin cancer and its effect on the expression of cyclophilin A, cyclophilin B and CD147. Oncol Lett 2017;14:1449-54.
21. Choi JW, Schroeder MA, Sarkaria JN, et al. Cyclophilin B supports Myc and mutant p53-dependent survival of glioblastoma multiforme cells. Cancer Res 2014;74:484-96.
22. Meng DQ, Li PL, Xie M. Expression and role of cyclophilin B in stomach cancer. Genet Mol Res 2015;14:5346-54.
23. Jeong K, Kim K, Kim H, et al. Hypoxia induces cyclophilin B through the activation of transcription factor 6 in gastric adenocarcinoma cells. Oncol Lett 2015;9:2854-8.
24. Jeong K, Kim H, Kim K, et al. Cyclophilin B is involved in p300-mediated degradation of CHOP in tumor cell adaptation to hypoxia. Cell Death Differ 2014;21:438-50.
25. Bingham V, McIlreavey L, Greene C, et al. RNAscope in situ hybridization confirms mRNA integrity in formalin-fixed, paraffin-embedded cancer tissue samples. Oncotarget 2017;8:93392-403.
26. Wang F, Flanagan J, Su N, et al. RNAscope: a novel in situ RNA analysis platform for formalin-fixed, paraffin-embedded tissues. J Mol Diagn 2012;14:22-9.
27. Choi TG, Nguyen MN, Kim J, et al. Cyclophilin B induces chemoresistance by degrading wild-type p53 via interaction with MDM2 in colorectal cancer. J Pathol 2018;246:115-26.
28. Tomczak K, Czerwinska P, Wiznerowicz M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. Contemp Oncol (Pozn) 2015;19:A68-77.
29. Cancer Genome Atlas Research N, Weinstein JN, Collisson EA, et al. The Cancer Genome Atlas Pan-Cancer analysis project. Nat Genet 2013;45:1113-20.
30. Fang F, Zheng J, Galbaugh TL, et al. Cyclophilin B as a co-regulator of prolactin-induced gene expression and function in breast cancer cells. J Mol Endocrinol 2010;44:319-29.
31. Oh Y, Jeong K, Kim K, et al. Cyclophilin B protects SH-SY5Y human neuroblastoma cells against MPP(+)-induced neurotoxicity via JNK pathway. Biochem Biophys Res Commun 2016;478:1396-402.
32. Arabzadeh A, Quail DF. Myosin II in Cancer Cells Shapes the Immune Microenvironment. Trends Mol Med 2019;25:257-9.
33. Ouderkirk JL, Krendel M. Non-muscle myosins in tumor progression, cancer cell invasion, and metastasis. Cytoskeleton (Hoboken) 2014;71:447-63.
34. Derynck R, Weinberg RA. EMT and Cancer: More Than Meets the Eye. Dev Cell 2019;49:313-6.
35. Saitoh M. Involvement of partial EMT in cancer progression. J Biochem 2018;164:257-64.

Cite this article as: Zhang X, Tan J, Yang L, An G. Cyclophilin B overexpression predicts a poor prognosis and activates metastatic pathways in colon cancer. Transl Cancer Res 2020;9(5):3573-3585. doi: 10.21037/tcr-19-2960

Table S1 CypB mRNA expression levels and clinicopathological parameters of the patients in the colonic tumor tissue microarray (TMA)

Tissue code	CypB expression*	OS (m)	OS#	Age (y)	Sex&	T stage	N stage	M stage	TNM stage	Histological grade	Tumor position	Tumor size (cm^3)
D15A1454-B30-C1	1	64	0	72	1	T3	N0	M0	2A	II	Right colon	6.5×5×1.7
D15A1455-B30-C1		1	1	57	0	T2	N1b	M0	3A	II	Right colon	6.5×5.5×2
D15A1456-B30-C1	1	13	1	76	1	T3	N0	M0	2A	II	Descending colon	5.5×4.5×2
D15A1458-B30-C1	1	64	0	63	0	T3	N0	M0	2A	II	Sigmoid colon	4.5×3.5×1.5
D15A1516-B30-C1		76	0	53	1	T4b	N0	M0	2C	I–II	Sigmoid colon	5×3×1.5
D15A1461-B30-C1	2	63	0	78	0	T3	N2b	M0	3C	II	Ascending colon	7×5×1
D15A1464-B30-C1	2	7	1	63	1	T4a	N2b	M0	3C	III	Sigmoid colon	5.5×4.5×1.5
D15A1462-B30-C1	1	22	1	78	1	T3	N0	M1b	4B	I–II	Sigmoid colon	7.5×3×1.5
D15A1502-B30-C1	1	63	0	68	0	T3	N0	M0	2A	II	Hepatic flexure	6×3×2
D15A1503-B30-C1	1	63	0	39	1	T4a	N0	M0	2B	II	Transverse colon	6×4×3
D15A1504-B30-C1		23	1	68	1	T2	N0	M0	1	I–II	Hepatic flexure	5.5×4×2
D15A1505-B30-C1	1	63	0	62	1	T3	N0	M0	2A	I–II	Ascending colon	2.5×2×0.5
D15A1508-B30-C1	1	63	0	78	1	T3	N0	M0	2A	II	Ascending colon	5×4×2
D15A1510-B30-C1	2	44	1	50	0	T4a	N0	M0	2B	II	Hepatic flexure	4×3.5×1
D15A1556-B30-C1	2	62	0	73	1	T3	N0	M0	2A	I–II	Hepatic flexure	11×6×2
D15A1557-B30-C1	2	38	1	68	1	T4a	N0	M0	2B	II	Hepatic flexure	6×4×1
D15A1558-B30-C1	2	13	1	87	0	T4b	N1b	M0	3C	I–II	Right colon	6×4×1
D15A1559-B30-C1	2	8	1	52	0	T4a	N0	M0	2B	II–III	Sigmoid colon	7×5×2.5
D15A1560-B30-C1	2	62	0	51	0	T1	N0	M0	1	II	Sigmoid colon	2.7×1.7×1.3
D15A1561-B30-C1	2	56	1	55	1	T4a	N2a	M0	3C	II	Splenic flexure	3.5×3.5×1
D15A1562-B30-C1	1	17	1	73	1	T4a	N0	M1b	4B	III–IV	Ascending colon	6.5×5×1.5
D15A1563-B30-C1	1	62	0	61	0	T3	N0	M0	2A	II	Right colon	3×3×2
D15A1564-B30-C1		12	1	48	0	T3	N0	M0	2A	II–III	Transverse colon	1.5×1×1
D15A1565-B30-C1	1	62	0	59	0	T2	N0	M0	1	II	Sigmoid colon	3×2.5×1
D15A1566-B30-C1		40	1	77	0	T2	N0	M0	1	II	Sigmoid colon	4×4×3
D15A1567-B30-C1	2	42	1	78	1	T3	N1a	M0	3B	II	Ascending colon	5×5×1.5
D15A1570-B30-C1	1	62	0	31	1	T3	N1b	M0	3B	I–III	Ascending colon	4×3×1
D15A1571-B30-C1		33	1	79	0	T3	N0	M0	2A	II	Sigmoid colon	7×5×2
D15A1572-B30-P1	1	61	0	81	1	T3	N1b	M0	3B	II	Sigmoid colon	4×3×1
D15A1573-B30-C1	2	61	0	85	1	T3	N0	M0	2A	I–II	Ascending colon	4.3×2×0.5
D15A1574-B30-C1	2	40	1	90	1	T4a	N0	M0	2B	II	Sigmoid colon	7×5×5
D15A1576-B30-C1	1	61	0	70	0	T2	N0	M0	1	II	Sigmoid colon	4.5×2×1
D15A1577-B30-C1	2	23	1	66	0	T4b	N1b	M0	3C	II–III	Ascending colon	5×4×1.5
D15A1579-B30-P1	2	61	0	73	1	T3	N0	M0	2A	II	Descending colon	3.5×3×1
D15A1614-B30-C1	2	61	0	54	0	T3	N0	M0	2A	II	Descending colon	3.5×3×2
D15A1628-B30-C1	2	25	1	76	0	T3	N1a	M0	3B	II	Ascending colon	8×8×4
D15A1615-B30-C1	1	61	0	50	1	T1	N0	M0	1	I	Ascending colon	4×3×3
D15A1616-B30-C1	1	61	0	74	0	T3	N0	M0	2A	I	Ascending colon	5×2.5×1
D15A1617-B30-C1	1	61	0	80	1	T3	N0	M0	2A	II	Right colon	8×7×1
D15A1619-B30-C1	2	61	0	65	0	T3	N0	M0	2A	I–II	Ascending colon	4×3.5×1
D15A1620-B30-C1	1	61	0	59	0	T3	N0	M0	2A	II	Sigmoid colon	4.5×3.5×1.2
D15A1622-B30-C1	2	61	0	79	1	T3	N0	M0	2A	I–II	Descending colon	4×4×1
D15A1629-B30-C1	2	61	0	56	1	T3	N0	M0	2A	I–II	Ascending colon	4×3×1
D15A1624-B30-C1	2	13	1	76	0	T4a	N1b	M0	3B	II	Sigmoid colon	3.5×3.5×1
D15A1625-B30-C1	2	60	0	76	0	T3	N0	M0	2A	I–II	Ascending colon	8×6×1
D15A1626-B30-C1	1	39	1	63	1	T4a	N1b	M0	3B	I–II	Sigmoid colon	5×3×1.5
D15A1630-B30-C1	1	60	0	44	0	T2	N0	M0	1	II	Ascending colon	8×8×4
D15A1663-B30-C1	1	13	1	73	1	T3	N0	M0	2A	II	Sigmoid colon	5.5×3.5×2
D15A1668-B30-C1	1	60	0	66	0	T1	N1a	M0	3A	II	Transverse colon	7.5×6.5×0.5
D15A1669-B30-C1	2	1	1	48	0	T3	N0	M0	2A	II	Right colon	8×7×2
D15A1732-B30-C1	1	59	0	79	1	T3	N0	M0	2A	I–II	Ascending colon	6×3×1
D15A1733-B30-C1	1	59	0	55	1	T4a	N1b	M0	3B	II–III	Hepatic flexure	5×4×1
D15A1735-B30-C1	2	59	0	65	0	T3	N0	M0	2A	II	Sigmoid colon	4×3×1
D15A1740-B30-C1	2	7	1	73	0	T3	N1a	M0	3B	II	Right colon	8×5×2.5
D15A1741-B30-C1	1	59	0	81	1	T3	N1a	M0	3B	II	Left colon	8×7×1.5
D15A1742-B30-C1	2	59	0	61	1	T2	N0	M0	1	I–II	Descending colon	4.5×3.5×1.5
D15A1745-B30-C1	2	59	0	80	1	T3	N0	M0	2A	II	Ascending colon	4×3×2
D15A1743-B30-C1	2	16	1	65	1	T4b	N1b	M0	3C	III	Colon	6×5×1.3
D15A1744-B30-C1	2	16	1	61	1	T4a	N2a	M1a	4A	II–III	Sigmoid colon	4×4×3
D15A1756-B30-C1	2	58	0	71	0	T3	N1a	M0	3B	II	Sigmoid colon	3×1.5×1
D15A1758-B30-C1	1	58	0	55	0	T3	N0	M0	2A	II–III	Ascending colon	11×6×3
D15A1765-B30-C1	2	21	1	55	1	T4a	N0	M0	2B	II	Sigmoid colon	4×2.5×1
D15A1767-B30-C1	1	58	0	83	1	T3	N0	M0	2A	I	Ascending colon	5×3×2
D15A1762-B30-C1	1	58	0	69	0	T4a	N0	M0	2B	II	Transverse colon	8×5×4
D15A1764-B30-C1		58	0	80	0	T4a	N1a	M0	3B	II–III	Ascending colon	6×5.5×1
D15A1990-B30-C1	1	57	0	43	1	T3	N0	M0	2A	II	Right colon	4×3×1.5
D15A1811-B30-C1	2	57	0	73	1	T4a	N0	M0	2B	I	Ascending colon	7×4×1
D15A1813-B30-C1	1	57	0	82	0	T3	N1a	M0	3B	II–III	Transverse colon	4×4×1
D15A1814-B30-C1	2	57	0	69	0	T2	N0	M0	1	I–II	Ascending colon	2×2×1.5
D15A1991-B30-C1	2	57	0	83	1	T4a	N0	M0	2B	II	Descending colon	4×2×1
D15A1815-B30-C1	2	57	0	46	0	T3	N0	M0	2A	II	Sigmoid colon	6×6×0.7
D15A1819-B30-C1	2	57	0	56	0	T3	N0	M0	2A	II	Descending colon	4.5×3.5×1.5
D15A1992-B30-C1		56	0	66	1	T3	N0	M0	2A	II	Ascending colon	4×2.5×0.6
D15A1993-B30-C1	2	0.4	1	82	1	T3	N2b	M0	3C	III	Splenic flexure	7×6×1
D15A1820-B30-P1	1	56	0	78	0	T3	N0	M0	2A	II	Sigmoid colon	4.5×4×1.5
D15A1836-B30-C1	1	56	0	81	0	T4a	N0	M0	2B	II	Colon	6×5×3.5
D15A1839-B30-C1	2	56	0	73	1	T4a	N0	M0	2B	II	Sigmoid colon	5×5×1.5
D15A1841-B30-C1	1	56	0	50	0	T3	N0	M0	2A	II	Right colon	6×4×1
D15A1904-B30-C1		19	1	27	0	T4a	N2a	M0	3C	III	Descending colon	4×4×1.5
D15A1907-B30-C1	2	35	1	54	1	T3	N0	M0	2A	II	Right colon	5×5×2
D15A1914-B30-C1	2	55	0	77	0	T4a	N1a	M0	3B	I–II	Splenic flexure	
D15A1915-B30-C1	1	55	0	55	0	T4a	N1b	M0	3B	II	Sigmoid colon	9×6×2
D15A1917-B30-C1	1	55	0	66	1	T2	N0	M0	1	II	Sigmoid colon	2.7×2.2×1.3
D15A1918-B30-C1	1	42	1	60	1	T3	N2b	M0	3C	II	Sigmoid colon	3.5×2×1.5
D15A1919-B30-C1	1	19	1	65	1	T3	N2a	M0	3B	II	Sigmoid colon	5×5×1.8
D15A1921-B30-C1		15	1	56	1	T3	N1b	M0	3B	II	Sigmoid colon	6×6×2.5
D15A1923-B30-C1	2	55	0	54	1	T4a	N1b	M0	3B	II	Sigmoid colon	6.5×5×2.5
D15A1928-B30-C1	1	55	0	52	0	T2	N0	M0	1	II	Transverse colon	5.5×4.5×1.5
D15A1929-B30-C1	1	23	1	62	0	T4a	N1b	M0	3B	I–II	Ascending colon	5×4×3
D15A1927-B30-C1	2	19	1	67	1	T3	N1a	M0	3B	II	Sigmoid colon	6×5×1

*, CypB (score 0–2 =1, score 3–4 =2); #, OS (event =1); &, Sex (male =1, female =2).

Table S2 CypB mRNA expression levels and clinicopathological parameters of the colon adenocarcinoma (COAD) patients from the Cancer Genome Atlas (TCGA)

Sample	CypB mRNA expression	OS time (days)	OS status (event =1)	M stage	N stage	T stage	TNM stage	Gender	Age (year)	Neoplasm_subdivision
TCGA-3L-AA1B-01	13.3798	475	0	M0	N0	T2	I	Female	61	Cecum
TCGA-4N-A93T-01	12.6538	146	0	M0	N1b	T4a	IIIB	Male	67	Ascending colon
TCGA-4T-AA8H-01	12.83	385	0	MX	N0	T3	IIA	Female	42	Descending colon
TCGA-5M-AAT4-01	12.5424	49	1	M1b	N0	T3	IV	Male	74	Ascending colon
TCGA-5M-AAT5-01	13.5081									
TCGA-5M-AAT6-01	13.9539	290	1	M1a	N2b	T4a	IV	Female	40	Transverse colon
TCGA-5M-AATA-01	13.2919									
TCGA-5M-AATE-01	12.5337	1,200	0	M0	N0	T3	IIA	Male	76	Ascending colon
TCGA-A6-2675-01	12.1484	1,321	0	MX	N0	T3	IIA	Male	78	Sigmoid colon
TCGA-A6-2682-01	13.725	424	1	M1	N1	T4b	IV	Male	70	[Discrepancy]
TCGA-A6-2684-01	13.1669	1,127	0	M0	N0	T2	I	Female	75	Cecum
TCGA-A6-2685-01	12.9514	1,133	0	M0	N0	T3	IIA	Female	48	Sigmoid colon
TCGA-A6-2686-01	13.1465	1,126	1	M0	N0	T3	IIA	Female	81	Cecum
TCGA-A6-4105-01	13.7982	442	1	M0	N0	T3	IIA	Male	79	Ascending colon
TCGA-A6-5656-01	13.305	1,001	0	M0	N0	T2	I	Male	74	Sigmoid colon
TCGA-A6-5657-01	12.9149	962	0	M0	N1	T3	IIIB	Male	65	[Discrepancy]
TCGA-A6-5659-01	13.0106	926	0	M0	N0	T2	I	Male	82	Cecum
TCGA-A6-5660-01	12.8449	888	0	M0	N2b	T3	IIIC	Male	73	Cecum
TCGA-A6-5661-01	13.3289	1,020	0	M0	N0	T3	IIA	Female	80	Ascending colon
TCGA-A6-5662-01	13.254	718	0	M1	N2	T3	IVA	Male	46	Splenic flexure
TCGA-A6-5664-01	13.7724	672	0	MX	N2a	T4a	IIIC	Male	80	Cecum
TCGA-A6-5665-01	13.7718	671	0	M0	N0	T3	IIA	Female	84	Ascending colon
TCGA-A6-5666-01	13.8249	995	0	M0	N0	T4b	IIC	Male	78	Sigmoid colon
TCGA-A6-5667-01	12.7441	887	0	MX	N1a	T3	IIIB	Female	40	Sigmoid colon
TCGA-A6-6137-01	12.8026	824	0	M0	N1c	T3	IIIB	Male	55	Hepatic flexure
TCGA-A6-6138-01	12.2254	685	0	M0	N0	T2	I	Male	61	Cecum
TCGA-A6-6140-01	13.035	734	0	M0	N0	T3	IIA	Male	62	Descending colon
TCGA-A6-6141-01	13.3598	130	0	M0	N0	T3	IIA	Male	31	Cecum
TCGA-A6-6142-01	13.4184	763	0	M1a	N1a	T3	IVA	Female	56	Sigmoid colon
TCGA-A6-6648-01	12.4873	766	0	M1a	N0	T3	IVA	Male	56	[Discrepancy]
TCGA-A6-6649-01	12.9726	735	0	M0	N1b	T3	IIIB	Male	66	Hepatic flexure
TCGA-A6-6650-01	12.5784	627	0	M0	N0	T3	IIA	Female	69	Cecum
TCGA-A6-6651-01	13.1779	662	0	MX	N1b	T3	IIIB	Female	55	Transverse colon
TCGA-A6-6652-01	12.7351	751	0	M1	N0	T3	IVA	Male	59	Sigmoid colon
TCGA-A6-6653-01	13.8395	742	0	M0	N0	T2	I	Male	82	Ascending colon
TCGA-A6-6654-01	13.3981	726	0	M0	N1	T3	IIIB	Female	65	Descending colon
TCGA-A6-6780-01	13.771	612	0	MX	N0	T3	IIA	Male	74	[Discrepancy]
TCGA-A6-6781-01	14.0498	598	0	MX	N1b	T4b	IIIC	Male	43	Transverse colon
TCGA-A6-6782-01	13.0313	617	0	MX	N0	T4a	IIB	Male	82	Transverse colon
TCGA-A6-A565-01	13.0484	494	1	MX	N2	T3	IIIC	Female	34	Transverse colon
TCGA-A6-A566-01	13.5206	758	1	M0	N1	T4	IIIB	Female	55	Descending colon
TCGA-A6-A567-01	12.2014	1,881	1	M1	N1	T3	IV	Male	56	Sigmoid colon
TCGA-A6-A56B-01	12.4469	1,711	1	M0	N1	T3	IIIB	Male	57	Sigmoid colon
TCGA-A6-A5ZU-01	13.256	293	0	M0	N1	T3	IIIB	Male	59	Transverse colon
TCGA-AA-3489-01	12.8667	214	1	M0	N0	T3	II	Male	75	Sigmoid colon
TCGA-AA-3492-01	13.3061	92	1	M0	N0	T3	II	Female	90	Ascending colon
TCGA-AA-3495-01	13.2578	1,127	0	M0	N0	T2	I	Male	79	Hepatic flexure
TCGA-AA-3496-01	13.0737	31	0	M0	N0	T3	II	Female	83	Ascending colon
TCGA-AA-3502-01	12.9701	1,065	0	M0	N0	T2	I	Male	73	Transverse colon
TCGA-AA-3506-01	13.5302	1,765	0	M0	N0	T2	I	Male	77	Hepatic flexure
TCGA-AA-3509-01	13.2673	1,915	0	M0	N0	T3	II	Female	54	Sigmoid colon
TCGA-AA-3511-01	12.7691	212	0	M0	N0	T4	II	Male	64	Sigmoid colon
TCGA-AA-3526-01	14.0539	580	0	M0	N0	T2	I	Male	57	Sigmoid colon
TCGA-AA-3655-01	13.1989	1,856	0	M0	N0	T3	II	Male	68	Sigmoid colon
TCGA-AA-3660-01	13.0301	2,375	0	M0	N0	T3	II	Female	51	Sigmoid colon
TCGA-AA-3662-01	12.9868	184	0	M1	N2	T4	IV	Female	80	Sigmoid colon
TCGA-AA-3663-01	14.2051	212	0	M0	N0	T3	II	Male	42	Cecum
TCGA-AA-3675-01	13.3136	1,431	0	M0	N0	T3	II	Male	84	Hepatic flexure
TCGA-AA-3685-01	13.8864	1,127	0	M0	N0	T3	II	Male	69	Sigmoid colon
TCGA-AA-3697-01	12.3795	2,587	0	M0	N0	T3	II	Male	77	Sigmoid colon
TCGA-AA-3712-01	13.2138			M0	N2	T3	III	Male	65	Descending colon
TCGA-AA-3713-01	12.8525	579	0	M1	N0	T3	IV	Male	68	Ascending colon
TCGA-AA-A01P-01	13.7176	1,158	1	M0	N1	T3	III	Female	80	Ascending colon
TCGA-AA-A01X-01	12.6789	791	0	M0	N1	T2	III	Female	80	Sigmoid colon
TCGA-AA-A01Z-01	13.3704	1,126	0	M0	N0	T3	II	Male	68	Ascending colon
TCGA-AA-A02K-01	12.5992	426	1	M1	N2	T4	IV	Male	50	Ascending colon
TCGA-AA-A02Y-01	13.3555	1,216	0	M0	N0	T2	I	Male	73	Cecum
TCGA-AD-5900-01	13.3362	370	0	MX	N0	T2	I	Male	67	Ascending colon
TCGA-AD-6548-01	13.4931	650	0	M0	N0	T2	I	Female	81	Splenic flexure
TCGA-AD-6888-01	13.8273	472	1	M0	N1b	T3	IIIB	Male	73	Hepatic flexure
TCGA-AD-6889-01	15.2878	2,532	1	M0	N0	T3	IIA	Male	76	Ascending colon
TCGA-AD-6890-01	13.9953	746	0	MX	N0	T1		Male	65	Ascending colon
TCGA-AD-6895-01	13.6519	763	0	M0	N1a	T3	IIIB	Male	84	Cecum
TCGA-AD-6899-01	12.7928	176	1	MX	N2b	T4a	IIIC	Male	84	Cecum
TCGA-AD-6901-01	13.1607	682	1	MX	N0	T3		Male	78	Cecum
TCGA-AD-6963-01	12.9269	834	0	MX	N0	T3		Male	58	Ascending colon
TCGA-AD-6964-01	13.844	331	1		N2b	T4a		Male	58	Cecum
TCGA-AD-6965-01	13.391	805	0	M0	N2b	T4a	IIIC	Male	62	Cecum
TCGA-AD-A5EJ-01	14.104			MX	N0	T3	IIA	Female	74	Cecum
TCGA-AD-A5EK-01	12.9109	500	0	MX	N0	T2	I	Male	51	Ascending colon
TCGA-AM-5820-01	13.1072	14	0	M1	N2	T4a	IVA	Female	59	Sigmoid colon
TCGA-AM-5821-01	13.9842	28	0	M0	N0	T3	IIA	Female	68	Sigmoid colon
TCGA-AU-3779-01	12.9971			M0	N0	T3	IIA	Female	80	Rectosigmoid junction
TCGA-AU-6004-01	12.4943	824	0	M0	N0	T2	I	Female	69	Cecum
TCGA-AY-5543-01	12.6835	1,004	0	M1	N1a	T3	IVA	Female	65	Ascending colon
TCGA-AY-6196-01	12.8902				N2b	T3	IIIC	Male	47	Cecum
TCGA-AY-6197-01	13.1902	652	0		N0	T3	IIA	Male	60	Cecum
TCGA-AY-6386-01	13.7194	542	0	M0	N1a	T3	IIIB	Female	66	Cecum
TCGA-AY-A54L-01	13.4659	525	0	M0	N0	T2	I	Female	74	Transverse colon
TCGA-AY-A69D-01	12.8051	543	0	M0	N0	T3	IIA	Female	55	Transverse colon
TCGA-AY-A71X-01	13.3791	588	0	M0	N0	T2	I	Female	54	Cecum
TCGA-AY-A8YK-01	12.3245	573	0	M1	N2a	T3	IVA	Male	44	Sigmoid colon
TCGA-AZ-4313-01	14.2427	2,310	0	M0	N0	T1	I	Female	51	Descending colon
TCGA-AZ-4315-01	13.733	1,776	0	M0	N0	T3	IIA	Male	61	Cecum
TCGA-AZ-4323-01	13.2983	43	1	M1	N2	T4	IV	Male	37	Cecum
TCGA-AZ-4614-01	13.5968	172	1	M1	N1	T4a	IVA	Female	71	
TCGA-AZ-4615-01	13.3781	1,002	0	M0	N1	T3	IIIB	Male	84	
TCGA-AZ-4616-01	14.3653	156	1	M1	N2	T3	IV	Female	82	Cecum
TCGA-AZ-4682-01	13.873	680	1	M1	N0	T3	IVA	Male	61	Sigmoid colon
TCGA-AZ-4684-01	12.7842	1,977	0	M1	N2	T3	IVA	Male	49	
TCGA-AZ-5403-01	13.307	1,910	1	MX	N0	T3	II	Male	43	Sigmoid colon
TCGA-AZ-5407-01	12.756	2,683	0	M0	N0	T1	I	Female	51	Cecum
TCGA-AZ-6598-01	13.516	1,503	1	MX	N0	T3	II	Female	77	[Discrepancy]
TCGA-AZ-6599-01	13.5732	206	1	MX	N0	T2	I	Male	72	Cecum
TCGA-AZ-6600-01	13.2994			M1	N1	T4	IV	Male	64	Hepatic flexure
TCGA-AZ-6601-01	13.0715	3,042	1	M0	N0	T3	II	Male	68	Sigmoid colon
TCGA-AZ-6603-01	12.8988	899	1	MX	N1	T2		Female	77	Sigmoid colon
TCGA-AZ-6605-01	13.3891	159	1	M0	N1	T4	IIIB	Male	77	Ascending colon
TCGA-AZ-6606-01	12.7094	357	1	M1	N2	T4	IV	Male	81	Cecum
TCGA-AZ-6607-01	12.8187	97	1	M1	N2	T4	IV	Male	69	Sigmoid colon
TCGA-AZ-6608-01	13.6897	59	1	M0	N1	T2	IIIA	Female	55	Sigmoid colon
TCGA-CA-5254-01	14.2203	386	0	M0	N0	T3	IIA	Female	42	Transverse colon
TCGA-CA-5255-01	13.548	376	0	M0	N0	T3	IIA	Male	45	Ascending colon
TCGA-CA-5256-01	13.6084	379	0	M0	N0	T3	IIA	Female	54	Hepatic flexure
TCGA-CA-5796-01	12.7443	377	0	M0	N0	T3	IIA	Female	52	Ascending colon
TCGA-CA-5797-01	13.4717	383	0	M0	N0	T3	IIA	Male	56	Sigmoid colon
TCGA-CA-6715-01	13.6661	383	0	M0	N1	T3	IIIB	Male	63	Sigmoid colon
TCGA-CA-6716-01	13.0409	371	0	M0	N0	T3	IIA	Male	65	Ascending colon
TCGA-CA-6717-01	12.8855	388	0	M0	N0	T3	IIA	Male	57	Ascending colon
TCGA-CA-6718-01	13.3607	306	1	M0	N0	T3	IIA	Male	46	Ascending colon
TCGA-CA-6719-01	12.9851	435	0	M0	N0	T3	IIA	Male	77	Descending colon
TCGA-CK-4947-01	13.2784	534	0	M0	N1	T4	IIIB	Female	46	Sigmoid colon
TCGA-CK-4948-01	13.296	4,502	0	M0	N1	T3	III	Female	45	Sigmoid colon
TCGA-CK-4950-01	13.4453	2,599	0	M0	N1	T3	IIIB	Female	68	Cecum
TCGA-CK-4951-01	13.3511	2,134	1	M0	N0	T3	IIA	Female	79	Ascending colon
TCGA-CK-4952-01	13.4197	475	0	M0	N2	T4	IIIC	Female	48	Ascending colon
TCGA-CK-5912-01	13.2997	1,493	1	MX	N0	T2	I	Male	81	Cecum
TCGA-CK-5913-01	13.4065	1,561	0	MX	N0	T3	IIA	Female	58	Cecum
TCGA-CK-5914-01	13.1163	304	0	MX	N1	T3	IIIB	Male	81	Sigmoid colon
TCGA-CK-5915-01	12.3319			MX	N0	T2	I	Male	63	Sigmoid colon
TCGA-CK-5916-01	13.7034	643	1	M0	N0	T1	I	Female	71	Cecum
TCGA-CK-6746-01	14.0824			MX	N0	T4b	IIB	Female	84	Cecum
TCGA-CK-6747-01	13.402	2,523	0	MX	N0	T3	IIA	Female	87	Cecum
TCGA-CK-6748-01	13.2504	58	0	M1	N1	T3	IV	Female	45	Sigmoid colon
TCGA-CK-6751-01	13.8717	3,780	0	MX	N0	T2	I	Female	88	Ascending colon
TCGA-CM-4743-01	14.6461	701	0	M0	N0	T3	IIA	Male	69	Hepatic flexure
TCGA-CM-4744-01	14.4513	609	0	M0	N0	T2	I	Male	69	Cecum
TCGA-CM-4747-01	12.6867	761	0	M1a	N1b	T4a	IVA	Male	47	Cecum
TCGA-CM-4751-01	12.6197	822	0	M0	N1b	T3	IIIB	Male	62	Cecum
TCGA-CM-5344-01	13.9646	670	0	M0	N1b	T3	IIIB	Female	39	Sigmoid colon
TCGA-CM-5348-01	12.6828	699	0	M0	N1a	T3	IIIB	Male	72	Cecum
TCGA-CM-5349-01	13.4487	915	0	M0	N0	T3	IIA	Female	68	Cecum
TCGA-CM-5860-01	13.2636	974	0	M0	N0	T3	IIA	Male	44	Ascending colon
TCGA-CM-5861-01	13.9718	457	0	M0	N0	T3	IIA	Female	63	Cecum
TCGA-CM-5862-01	13.4638	153	1	M1a	N1a	T3	IVA	Male	80	Ascending colon
TCGA-CM-5863-01	13.1017	457	0	M0	N1b	T3	IIIB	Female	60	Ascending colon
TCGA-CM-5864-01	13.0053	457	0	M0	N0	T2	I	Male	60	Cecum
TCGA-CM-5868-01	13.1335	518	0	M1a	N1a	T4a	IVA	Female	59	Sigmoid colon
TCGA-CM-6161-01	13.1885	457	0	M0	N0	T2	I	Female	36	Sigmoid colon
TCGA-CM-6162-01	13.1823	365	0	M0	N1a	T3	IIIB	Female	48	Ascending colon
TCGA-CM-6163-01	12.274	427	0	M0	N0	T1	I	Male	74	Sigmoid colon
TCGA-CM-6164-01	13.0871	883	0	M0	N0	T3	IIA	Female	46	Sigmoid colon
TCGA-CM-6165-01	12.0513	488	0	M0	N0	T3	IIA	Male	74	Sigmoid colon
TCGA-CM-6166-01	13.4988	669	0	M0	N0	T2	I	Female	48	Ascending colon
TCGA-CM-6167-01	12.9749	456	0	M0	N2b	T3	IIIC	Female	57	Cecum
TCGA-CM-6168-01	13.2209	395	0	M0	N0	T3	IIA	Female	84	Ascending colon
TCGA-CM-6169-01	12.6992	396	0	M0	N0	T3	IIA	Male	67	Cecum
TCGA-CM-6170-01	12.3852	457	0	M0	N0	T2	I	Female	73	Descending colon
TCGA-CM-6171-01	14.4106	427	0	M0	N0	T2	I	Female	77	Ascending colon
TCGA-CM-6172-01	12.7955	335	0	M0	N1a	T3	IIIB	Female	70	Sigmoid colon
TCGA-CM-6674-01	13.8921	394	0	M0	N0	T3	IIA	Male	39	Hepatic flexure
TCGA-CM-6675-01	13.0063	397	0	M1b	N2b	T3	IVB	Male	35	Cecum
TCGA-CM-6676-01	12.8475	337	0	M0	N0	T2	I	Male	82	Sigmoid colon
TCGA-CM-6677-01	12.6611	337	0	M0	N0	T3	IIA	Female	75	Hepatic flexure
TCGA-CM-6678-01	13.5735	335	0	M1a	N1c	T4a	IVA	Female	63	Sigmoid colon
TCGA-CM-6679-01	13.2632	306	0	M0	N0	T3	IIA	Male	58	Sigmoid colon
TCGA-CM-6680-01	12.8609	366	0	M0	N2a	T3	IIIB	Female	78	Cecum
TCGA-D5-5537-01	13.707	1,381	1	MX	N2	T3	IIA	Male	83	Ascending colon
TCGA-D5-5538-01	13.5236	1,661	1	M0	N1b	T3	IIIB	Female	60	Cecum
TCGA-D5-5539-01	13.0102	596	0	M0	N1	T3	IIIA	Male	60	Ascending colon
TCGA-D5-5540-01	13.899	1,706	0	M0	N0	T3	IIA	Male	73	Cecum
TCGA-D5-5541-01	13.097	1,701	0	M0	N1a	T3	IIIB	Male	63	Sigmoid colon
TCGA-D5-6529-01	12.8576	614	0	M0	N0	T3	IIA	Male	69	[Discrepancy]
TCGA-D5-6530-01	13.5343	621	0	M0	N0	T2	I	Male	53	[Discrepancy]
TCGA-D5-6531-01	13.5619	540	0	M0	N0	T3	IIA	Male	75	Hepatic flexure
TCGA-D5-6532-01	13.2976	555	0	M0	N0	T3	IIA	Male	61	Sigmoid colon
TCGA-D5-6533-01	12.4948	775	0	M0	N0	T4b	[Discrepancy]	Female	68	Transverse colon
TCGA-D5-6534-01	13.1514	1,316	0	M0	N0	T3	IIA	Female	62	Ascending colon
TCGA-D5-6535-01	12.3147	460	0	MX	N1	T3	IIIB	Female	80	Ascending colon
TCGA-D5-6536-01	13.7486	543	0	M0	N0	T3	IIA	Male	73	Sigmoid colon
TCGA-D5-6537-01	13.3376	146	1	MX	N1a	T3	IIIB	Male	64	Transverse colon
TCGA-D5-6538-01	13.2516	521	0	M0	N2	T3	IIIB	Female	79	Hepatic flexure
TCGA-D5-6539-01	12.3064	380	0	M0	N0	T3	[Discrepancy]	Female	45	Transverse colon
TCGA-D5-6540-01	13.827	491	0	M0	N0	T2	I	Male	66	Cecum
TCGA-D5-6541-01	13.2981	474	0	M0	N0	T3	IIA	Male	49	Splenic flexure
TCGA-D5-6898-01	12.4218	229	0	M0	N0	T2	I	Female	51	Sigmoid colon
TCGA-D5-6920-01	13.3308	377	0	M0	N0	T3	IIA	Female	77	Sigmoid colon
TCGA-D5-6922-01	12.3456	308	0	M0	N1	T3	IIIA	Male	76	Sigmoid colon
TCGA-D5-6923-01	12.909	378	0	M0	N0	T2	I	Male	57	Sigmoid colon
TCGA-D5-6924-01	13.2702	435	0	M0	N0	T3	IIA	Male	68	Sigmoid colon
TCGA-D5-6926-01	12.9835	275	0	M0	N1	T4a	IIIB	Male	65	Sigmoid colon
TCGA-D5-6927-01	13.9284			M0	N0	T3	IIA	Male	34	Transverse colon
TCGA-D5-6928-01	13.1599	354	0	M0	N0	T3	IIA	Male	80	Ascending colon
TCGA-D5-6929-01	13.5511	408	0	M1	N1	T3	IV	Female	49	Sigmoid colon
TCGA-D5-6930-01	13.9947	406	0	M0	N0	T3	IIA	Male	67	Ascending colon
TCGA-D5-6931-01	13.1779	365	0	M0	N2	T4b	IIIC	Male	77	Transverse colon
TCGA-D5-6932-01	13.1223	346	0	M0	N0	T3	IIA	Male	69	Transverse colon
TCGA-D5-7000-01	13.1931	312	0	M0	N0	T2	I	Female	79	Cecum
TCGA-DM-A0X9-01	13.3966	3,641	0	M0	N0	T3	IIA	Female	71	[Discrepancy]
TCGA-DM-A0XD-01	13.9004	743	1	M0	N0	T3	IIA	Male	65	[Discrepancy]
TCGA-DM-A0XF-01	13.5163	1,162	1	M0	N2	T3	IIIC	Female	68	[Discrepancy]
TCGA-DM-A1D0-01	12.3348	3,974	0	M0	N0	T3	IIA	Female	79	Sigmoid colon
TCGA-DM-A1D4-01	12.6627	2,821	1	M0	N0	T3	IIA	Male	80	Cecum
TCGA-DM-A1D6-01	12.2639	1,518	1	M0	N0	T3	IIA	Male	88	Splenic flexure
TCGA-DM-A1D7-01	13.3092	405	1	M0	N0	T3	IIA	Male	82	Sigmoid colon
TCGA-DM-A1D8-01	12.7949	383	1		N1	T3		Female	50	Ascending colon
TCGA-DM-A1D9-01	12.5488	4,270	0	M0	N0	T3	IIA	Female	67	Cecum
TCGA-DM-A1DA-01	13.3777	228	1	M0	N2	T3	IIIC	Female	71	Cecum
TCGA-DM-A1DB-01	13.8398	1,348	1	M0	N0	T3	IIA	Male	68	Sigmoid colon
TCGA-DM-A1HA-01	12.4432	4,000	0	M0	N2	T3	IIIC	Male	82	Ascending colon
TCGA-DM-A1HB-01	14.6197	4,126	0	M0	N1	T3	IIIB	Male	75	Transverse colon
TCGA-DM-A280-01	14.2422	236	1	M0	N0	T3	IIA	Female	70	Ascending colon
TCGA-DM-A282-01	13.9437	4,233	0	M0	N0	T3	IIA	Female	60	Hepatic flexure
TCGA-DM-A285-01	13.4791	179	1	M1	N2	T3	IV	Female	71	Ascending colon
TCGA-DM-A288-01	13.5002	427	1	M0	N2	T3	IIIC	Male	68	Cecum
TCGA-DM-A28A-01	13.7039	805	1	M0	N2	T3	IIIC	Male	78	Cecum
TCGA-DM-A28C-01	12.6539	2,475	1	M0	N0	T3	IIA	Male	74	Sigmoid colon
TCGA-DM-A28E-01	13.4291	3,648	0	M0	N0	T3	IIA	Female	72	Sigmoid colon
TCGA-DM-A28F-01	13.009	1,094	1	M0	N1	T3	IIIB	Male	73	Sigmoid colon
TCGA-DM-A28G-01	13.1773	1,849	1	M0	N0	T3	IIA	Male	75	Ascending colon
TCGA-DM-A28H-01	13.1818	3,561	0	M0	N2	T3	IIIC	Male	50	Cecum
TCGA-DM-A28K-01	13.9535	2,988	0	M0	N0	T3	IIA	Male	75	Hepatic flexure
TCGA-DM-A28M-01	12.7494	2,895	0	M0	N0	T3	IIA	Male	63	Descending colon
TCGA-F4-6459-01	12.7603	262	1	M0	N2a	T3	IIIB	Female	61	Sigmoid colon
TCGA-F4-6460-01	12.8791	972	1	M0	N1	T3	IIIB	Female	51	Sigmoid colon
TCGA-F4-6461-01	13.4939	338	1	M0	N2	T4b	IIIC	Female	41	Hepatic flexure
TCGA-F4-6463-01	13.5646	1,087	0	M0	N0	T3	IIA	Male	51	Transverse colon
TCGA-F4-6569-01	13.2891	1,087	0	M0	N0	T2	I	Male	60	Transverse colon
TCGA-F4-6570-01	13.4011	188	1	M0	N0	T3	IIA	Female	78	Transverse colon
TCGA-F4-6703-01	13.3259	1,456	0	M0	N0	T3	IIA	Male	64	Ascending colon
TCGA-F4-6704-01	13.5814	47	0	MX	N2b	T3	IIIC	Male	60	Sigmoid colon
TCGA-F4-6805-01	13.2868	1,047	0	M0	N0	T3	IIA	Female	58	Descending colon
TCGA-F4-6806-01	13.355	1,260	0	M0	N0	T2	I	Female	59	Sigmoid colon
TCGA-F4-6807-01	12.5661	1,309	0	M0	N2b	T3	IIIC	Female	51	Hepatic flexure
TCGA-F4-6808-01	13.6601	1,024	0	M0	N0	T1	I	Female	54	Sigmoid colon
TCGA-F4-6809-01	12.8493	403	1	M1	N1	T3	IVA	Female	52	Sigmoid colon
TCGA-F4-6854-01	12.4674	16	0	M0	N0	T3	IIA	Female	77	Sigmoid colon
TCGA-F4-6855-01	13.0115	1,442	0	M0	N0	T3	IIA	Female	70	Sigmoid colon
TCGA-F4-6856-01	13.9564	1,074	0	M0	N0	T2	I	Male	45	Cecum
TCGA-F4-6857-01	13.0872									
TCGA-G4-6293-01	13.4778	4,051	0	M0	N1	T3	III	Female	49	Transverse colon
TCGA-G4-6294-01	13.2546	858	1	M1	N1	T3	IV	Male	75	Cecum
TCGA-G4-6295-01	12.6653	254	0	M0	N0	T3	II	Female	70	Cecum
TCGA-G4-6297-01	13.6856	2,506	0	M1	N2	T3	IV	Female	55	Cecum
TCGA-G4-6298-01	13.3551	715	1	MX	N1	T4a	IIIB	Male	90	Cecum
TCGA-G4-6299-01	13.2354	2,268	0	M0	N2	T3	IIIC	Male	69	Descending colon
TCGA-G4-6302-01	13.2234	2,047	1	M0	N0	T3	IIA	Female	90	Cecum
TCGA-G4-6303-01	12.5696	2,003	1	M1	N1	T3	IV	Female	54	Sigmoid colon
TCGA-G4-6304-01	14.0855	1,631	0	M0	N0	T4	IIB	Female	66	Transverse colon
TCGA-G4-6306-01	13.5552	1,359	0	M0	N0	T2	[Discrepancy]	Male	71	Ascending colon
TCGA-G4-6307-01	12.7383	1,674	0	M0	N1	T3	IIIB	Female	37	Sigmoid colon
TCGA-G4-6309-01	14.0657	2,600	0	M0	N1	T3	IIIB	Female	40	Sigmoid colon
TCGA-G4-6310-01	13.0093	1,935	0	M0	N1	T3	IIIB	Male	69	Cecum
TCGA-G4-6311-01	12.8804	1,199	0	MX	N1	T3	III	Male	80	Ascending colon
TCGA-G4-6314-01	13.0451	1,093	0	M1	N2	T3	IV	Female	76	Cecum
TCGA-G4-6315-01	13.0198	1,883	0	M1	N1	T3	IV	Male	66	Descending colon
TCGA-G4-6317-01	12.7918	1,095	0	MX	N2	T3	IIIC	Female	51	Sigmoid colon
TCGA-G4-6320-01	13.278	804	0	MX	N1	T3	III	Male	73	Hepatic flexure
TCGA-G4-6321-01	12.88	672	0	MX	N1	T2	III	Female	60	Cecum
TCGA-G4-6322-01	14.2467	792	0	MX	N1	T3	IIIB	Male	65	Descending colon
TCGA-G4-6323-01	13.1754	419	0	MX	N0	Tis	IA	Male	50	Cecum
TCGA-G4-6586-01	13.683	1,089	0	M0	N0	T3	IIA	Female	73	Ascending colon
TCGA-G4-6588-01	13.4753	796	0	M0	N0	T3	IIA	Female	58	Cecum
TCGA-G4-6625-01	12.8892	2,792	0	M0	N0	T3	IIA	Female	77	Sigmoid colon
TCGA-G4-6626-01	12.547	1,422	1	M0	N0	T3	IIA	Male	90	Ascending colon
TCGA-G4-6627-01	12.8671	2,275	0	M0	N0	T3	IIA	Male	84	Ascending colon
TCGA-G4-6628-01	13.7375	2,424	0	M0	N0	T2	I	Male	78	Cecum
TCGA-NH-A50T-01	13.6842	553	0	MX	N0	T3	IIA	Female	68	Splenic flexure
TCGA-NH-A50U-01	12.8574	334	1	M1a	N0	T4a	IVA	Male	42	Cecum
TCGA-NH-A50V-01	12.4816	588	0	M0	N2a	T3	IIIB	Male	69	Cecum
TCGA-NH-A5IV-01	13.1119	588	0	MX	N0	T3	IIA	Female	90	Transverse colon
TCGA-NH-A6GA-01	12.697	302	1	MX	N2a	T4a	IIIC	Male	58	Ascending colon
TCGA-NH-A6GB-01	13.4996	476	0	MX	N2b	T3	IIIC	Female	71	Transverse colon
TCGA-NH-A6GC-01	12.5818	389	0	M1b	N1b	T4b	IVB	Female	66	Descending colon
TCGA-NH-A8F7-01	12.7693	543	0	MX	N0	T3	IIA	Female	53	Sigmoid colon
TCGA-NH-A8F8-01	13.432	511	1	M1	N2b	T4a	IV	Male	79	Ascending colon
TCGA-QG-A5YV-01	13.4551	1,301	0	MX	N1a	T4b	IIIC	Female	64	Sigmoid colon
TCGA-QG-A5YW-01	13.0881	896	0	MX	N2b	T3	IIIC	Female	55	Cecum
TCGA-QG-A5YX-01	13.1771	1,003	0	MX	N0	T3	IIA	Female	61	Sigmoid colon
TCGA-QG-A5Z1-01	12.6054	256	1	MX	N1b	T3	IIIB	Male	71	Sigmoid colon
TCGA-QG-A5Z2-01	13.0875	952	0	M0	N0	T2	I	Male	61	Cecum
TCGA-QL-A97D-01	12.3149	666	0	MX	N0	T2	I	Female	84	Cecum
TCGA-RU-A8FL-01	12.7671	1,177	0	MX	N2a	T3	IIIB	Male	51	Cecum
TCGA-SS-A7HO-01	13.7814	1,829	0	M0	N0	T4a	IIB	Female	44	Cecum
TCGA-T9-A92H-01	12.5539	362	0	M0	N0	T3	IIA	Male	82	Sigmoid colon
TCGA-WS-AB45-01	13.4247	2,130	0	MX	N0	T3	IIA	Female	52	Cecum

Table S3 The potential CypB related biological processes and signaling pathways generated by the Search Tool for the Retrieval of Interacting Genes (STRING)

#term ID	Term description	False discovery rate	Matching proteins in your network
hsa04514	Cell adhesion molecules (CAMs)	0.00072	CDH1, CDH3, CLDN1, CLDN2, CLDN4
hsa04530	Tight junction	0.00085	CLDN1, CLDN2, CLDN4, MYH11, MYL9
hsa04270	Vascular smooth muscle contraction	0.0019	ACTG2, MYH11, MYL9, PPP1R14A
hsa04670	Leukocyte transendothelial migration	0.0019	CLDN1, CLDN2, CLDN4, MYL9
hsa00480	Glutathione metabolism	0.002	GPX2, GPX3, RRM2
hsa05130	Pathogenic Escherichia coli infection	0.002	CDH1, CLDN1, KRT18
hsa04657	IL-17 signaling pathway	0.0079	CCL20, LCN2, MMP1
hsa05160	Hepatitis C	0.0185	CLDN1, CLDN2, CLDN4
hsa05219	Bladder cancer	0.0209	CDH1, MMP1
hsa00590	Arachidonic acid metabolism	0.0396	GPX2, GPX3
HSA-446728	Cell junction organization	8.90E-06	CDH1, CDH3, CLDN1, CLDN2, CLDN4, LIMS2
HSA-421270	Cell-cell junction organization	2.73E-05	CDH1, CDH3, CLDN1, CLDN2, CLDN4
HSA-445355	Smooth muscle contraction	3.79E-05	ACTG2, LMOD1, MYH11, MYL9
HSA-420029	Tight junction interactions	0.0014	CLDN1, CLDN2, CLDN4
HSA-397014	Muscle contraction	0.0018	ACTG2, DES, LMOD1, MYH11, MYL9
HSA-5625740	RHO GTPases activate PKNs	0.0079	MYH11, MYL9, PPP1R14A
HSA-416572	Sema4D induced cell migration and growth-cone collapse	0.0162	MYH11, MYL9
HSA-5625900	RHO GTPases activate CIT	0.0162	MYH11, MYL9
HSA-5627117	RHO GTPases activate ROCKs	0.0162	MYH11, MYL9
HSA-5627123	RHO GTPases activate PAKs	0.0162	MYH11, MYL9
HSA-2022854	Keratan sulfate biosynthesis	0.0191	B3GNT3, PRELP
HSA-3928663	EPHA-mediated growth cone collapse	0.0191	MYH11, MYL9
HSA-1592389	Activation of matrix metalloproteinases	0.0227	MMP1, MMP7
HSA-195258	RHO GTPase effectors	0.0227	CDH1, MYH11, MYL9, PPP1R14A
HSA-3299685	Detoxification of reactive oxygen species	0.0227	GPX2, GPX3
HSA-418990	Adherens junctions interactions	0.0227	CDH1, CDH3
HSA-202733	Cell surface interactions at the vascular wall	0.0231	CEACAM6, EPCAM, MMP1
HSA-1474228	Degradation of the extracellular matrix	0.0239	CDH1, MMP1, MMP7
HSA-1474244	Extracellular matrix organization	0.026	CDH1, CEACAM6, MMP1, MMP7
HSA-2142753	Arachidonic acid metabolism	0.0465	DPEP1, GPX2