XPF Expression And Its Relationship With The Risk And Prognosis Of Colorectal Cancer

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Abstract

Background: XPF is a key factor contributing to DNA damage excision of nucleotide excision repair pathway. The relationship between XPF expression and the risk and prognosis of colorectal cancer (CRC) is unclear.

Methods: In this experiment, a total of 824 cases of colorectal tissue were collected. XPF protein expression was detected by immunohistochemical staining. In order to explore the differential expression of XPF between CRC and non-cancer controls, and the correlation between XPF expression and CRC clinicopathological parameters, we conducted a non-parametric test. Univariate and multivariate Cox regression analyses were conducted to investigate the relationship between XPF expression and CRC prognosis. The Java based software GSEA as well as STRING, David, GO, KEGG were used to explore the function and regulation network of XPF.

Results: The results demonstrated that the XPF expression in CRC was significantly up-regulated compared with non-tumor controls ($P < 0.001$) and adenoma tissues ($P < 0.001$). XPF protein was highly expressed in the dynamic sequence of anal diseases to adenoma tissues to CRC. Expression of XPF was related to tumor location ($P=0.005$) and with tumor growth pattern ($P=0.009$). The results of prognosis analysis suggested that in individuals with T1-2 invasive extent, XPF low expression may be significantly associated with better overall survival (HR = 7.978, 95% CI: 1.208-52.673, $P = 0.031$). XPF and its interacting genes played a vital role in different processes of nucleotide excision repair pathway. XPF expression was related with Ubiquitin like protein specific protease activity.

Conclusions: XPF might be a promising biomarker for CRC risk, and also showed potential as a prognostic predictor in CRC patients.

Background

DNA damage caused by endogenous or exogenous genetic toxicants can contribute to genomic instability and directly lead to a variety of cancers. Cells have evolved a series of DNA repair pathways to avoid the deleterious result[1]. Nucleotide excision repair (NER) can identify many types of underlying damage and cut damaged DNA strands at precise distances on both sides of the lesion, as well as base-damaged oligonucleotide fragments[2]. NER pathway has four main steps which are damage identification, damage partitioning and unwinding, damage incision and new strand synthesis[3, 4]. XPF, locating on chromosome 16p13.12, has 11 exons with a span of 28.2 kb[4]. The heterodimer of XPF-ERCC1 is involved with the 5 'incision step of the NER pathway. The catalytic area located in XPF can determine the activity of NER[5]. It is an essential human gene in the NER pathway responsible for the removal of UV-C photoproducts and large volume adducts from DNA[6, 7]. Cells or animals that lack XPF cannot perform the NER pathway[4].

Given its important function in the NER pathway, XPF may be involved in diseases associated with imbalance between DNA damage and repair. A number of researches have focused on its role in different cancers. XPF has been reported in the literature that its expression in renal cell carcinoma is significantly higher compared with bladder cancer and testicular cancer, and is related to the clinical features and chemotherapy sensitivity[6]. XPF expression is increased in gastric cancer (GC) tissue, and the prognosis of patients with high expression of XPF is poor[7]. XPF is also highly expressed in oral cancer tissues, while its high expression indicates a low survival rate[8]. CRC is a malignant tumor which is the third cause of cancer death in China[9]. There have been some previous studies on the relationship between XPF polymorphism and the risk of CRC. Previous studies showed that there was an association between XPF polymorphisms and risk of CRC[10–12]. XPF polymorphisms was associated with Chinese population, especially smokers[13]. So far, although there have been small samples to study the relationship between XPF expression and the risk of CRC[14], the pathological process from colorectal benign diseases to precancerous lesions to cancer has not been studied with anyone, and there is no large sample size of studies on the relationship between XPF expression and

CRC. In the study of the relationship between onset and prognosis, we first studied the expression tendency of XPF in the progression of lesions from anal disease to adenoma to CRC, and its relationship with risk and prognosis. Further, we analyzed the association of XPF expression with clinicopathological parameters and survival of CRC patients, thus to investigate the effect of XPF on development, progression, and prognosis of CRC.

Methods

Patients and tissue specimens

The design of this study was approved by the Human Ethics Committee of China Medical University. Each subject participated in the study provided the written informed consent. The patients undergoing surgery were from the First Hospital of China Medical University between November 2012 and June 2016. We enrolled a total of 824 cases of colorectal tissue for risk study, including 276 cases of CRC and 284 adjacent non-tumor tissue (248 cases of CRC had survival time, 230 pairs had cancer tissues and its matched adjacent tissues), 202 cases of adenoma and 62 cases of anal disease; and 248 cases of CRC tissue with survival time were used for prognosis study.

We collected the tissues of CRC, which were derived from the histological results, and the collection was according to the World Health Organization standards. The TNM staging of CRC was evaluated based on the International Union Against Cancer (UICC) / United Joint Cancer Committee (AJCC) (7th edition in 2010)[6]. There were 3 cases of CRC patients that needed to be excluded: (1) patients with XP disease; (2) patients who received chemotherapy or radiation therapy before surgery; (3) patients with hereditary nonpolyposis colorectal cancer (HNPCC).

Follow-up study was conducted until April 2018. We performed prognostic analysis of 248 patients enrolled (the follow-up time was 12 to 63 months, the average survival time was 48.15 months, and there was no death). We excluded 14 patients who lacked visits in the OS analysis. Patients with the habit of smoking at least one cigarette a day for at least one year were considered to have a history of smoking. In the meantime, the study defined the drinking history as an average daily intake of at least 50 grams of alcohol for at least one year. Clinical characteristics of cancer patients included gender, age, whether smoking or drinking, tumor location, TNM stage, invasive extent, lymph infiltrative, distant metastasis, tumor deposit, perineural invasion, vessel carcinoma embolus, growth pattern, differentiation degree, maximum diameter and family history.

Immunohistochemistry

The tissue was fixed in formalin and embedded in paraffin, then cut into 4 μm thick sections, and the sections were mounted on glass slides[15]. Antigen retrieval was performed after routine dewaxing. The tissue sections were washed with phosphate buffered saline (PBS, pH 7.4). Then the sections were blocked with 10% normal goat serum for 10 minutes. The expression of XPF protein was detected with mouse anti-XPF monoclonal antibody (ab-85140, 1: 200 dilution; Abcam, Cambridge, UK), and the primary antibody was used to incubate at room temperature for one hour. We spined off the primary antibody on the slice, and then used a biotinylated secondary antibody (goat anti-rabbit antibody, Fujian Maixin) to incubate the tissue for 10 minutes. The tissue was rinsed with PBS for 10 minutes. After that, we used streptavidin Biotin-biotin peroxide at a temperature of 24-27 ° C for incubating the tissue for 10 minutes, and stained with DAB (DAB-0031, Maixin City, Fujian Province, China) on a glass slide. When the tissue stain become brown (about 30 seconds), we rinsed the DAB with PBS. Finally, the slides were dehydrated, the tissue was fixed with resin and the coverslips were covered to observe the staining.

Evaluation of immunohistochemistry

Two experienced pathologists scored XPF's expression in different tissue independently, and this process followed the double-blind principle. The pathologists scored the staining intensity and staining area of XPF respectively. If there are differences in the scores of the pathologists, two pathologists will discuss and summarize the final scores. Semi-quantitative scoring criteria were used to assess the expression of XPF. Scoring standard: (1) staining intensity was classified into four levels, including 0 (no staining), 1 (light brown), 2 (brown staining), and 3 (heavy brown staining); (2) percentage of stained cells was divided into: 0(0–5); 1(6–25); 2(26–50); 3(51–75); 4(76–100%). We got the final IS (immunoreactivity score) by multiplying staining intensity and percentage of stained scores. Finally, the IS score was classified as: negative (-), score = 0; weak positivity (+), score = 1-4; medium positivity (++), score = 5-8; and strong positivity (+++), score = 9-12.

The function and regulation network of XPF by GO and KEGG analysis

STRING is a database designed to collect, score and integrate all public sources of information on protein–protein interactions[16]. Gene ontology (GO) analysis is a major bioinformatics tool that unifies the characterization of genes and gene products through the three components of biological processes, cell composition and molecular function[17]. Kyoto Encyclopedia of Genes and Genomes (KEGG) is a set of databases whose main purpose is to study genetic pathways, and contains information about biological pathways, genomes, chemicals and diseases[18]. The Database for Annotation, Visualization and Integrated Discovery (DAVID; v.6.8; https://david.ncifcrf.gov/home.jsp; accessed on September 16, 2020) was applied to perform the enrichment analyses of GO and KEGG [19]. DAVID is an online portal that provides comprehensive annotation analysis of large gene lists. GO analysis comprises groups of molecular function, cellular function and biological process[20]. We used STRING to explore the genes closely correlated with XPF. XPF and its interacting genes were enriched and analyzed by David for GO and KEGG pathways, respectively. The ggplot2 package in the R platform (Version 3.6.3) was used to show the obtained results. Gene Set Enrichment Analysis (GSEA) is an analysis method for whole-genome expression profiling chip data, which compares genes with predefined gene sets[21]. By analyzing the gene expression profile data, we can understand the expression status of XPF in a specific functional gene set, and whether there is some statistical significance in this expression status. We searched the expression of XPF in normal and cancerous colorectal tissues in the Oncomine database [22].

Statistical analysis

All the statistical analyses were conducted using SPSS 20.0 software (IL, Chicago). The difference of XPF expression between CRC and adjacent non-tumor tissues was compared by non-parametric tests. We performed nonparametric test to evaluate the relationship between XPF expression and clinicopathological parameters of CRC. Survival analysis was performed by Kaplan-Meier method. When we compared the differences between subgroups, and the log-rank test was used. To evaluate the effect of XPF expression on CRC prognosis, the Cox proportional hazard model was used, and the multivariate Cox proportional hazard model was adjusted by age, gender, TNM stage, and differentiation degree. $P < 0.05$ was considered as statistically significant.

Results

Baseline information of the participants

The baseline information of 248 patients with CRC were shown in Table 1. Of the 143 male and 105 female patients who participated, the MST (median survival time) for men and women was 48.20 and 48.42 months, respectively. A total

of 129 patients with CRC were over 60 years old and 119 were younger than 60 years old. After analysis, the survival time was related to perineural invasion that patients without perineural invasion lived longer ($P < 0.001$). The results also showed that patients with vessel carcinoma embolus had shorter survival time ($P = 0.003$), and individuals without lymph node metastasis had longer survival time.

Table 1
Clinicopathological parameters and survival in CRC

Characteristics	CRC	Cases of events	MST	P
Gender				
Male	143	43	48.2	0.649
Female	105	31	48.42	
Age(years)				
>60	129	31	48.6	0.145
≤60	119	43	47.66	
Smoking				
Yes	59	21	46.41	0.124
No	189	53	48.69	
Drinking				
Yes	40	12	49.53	0.498
No	208	62	47.88	
Tumor location				
Colon	82	28	48.62	0.416
Rectum	166	46	47.91	
TNM stage[1]				
	6	2	50	0.133
	89	29	46.53	
	132	36	50.39	
	18	7	38.5	
Invasive extent[1]				
T1-2	44	13	46.61	0.214
T3-4	155	42	49.3	
Lymph node metastasis				
Positive	148	45	46.6	0.009
Negative	100	29	50.43	
Distant metastasis				
Positive	64	19	46.84	0.173
Negative	184	55	48.6	
Perineural invasion				

Characteristics	CRC	Cases of events	MST	P
Positive	148	39	46.35	< 0.001
Negative	100	35	50.8	
Vessel carcinoma embolus				
Positive	60	16	44.78	0.003
Negative	188	58	49.22	
Growth patten				
Infiltrative	152	43	47.78	0.316
Nested/cloddy	96	31	48.73	
Differentiation degree				
Poor/mucinous	80	26	48.74	0.794
Well/moderate	168	48	47.86	
Family history				
Positive	43	15	47.37	0.653
Negative	205	59	48.31	
CRC, colorectal cancer; MST, median survival time.				
[1]Incomplete information.				

XPF was highly expressed in CRC tissues compared with non-tumor adjacent tissues

Figure 1 showed the immunohistochemical staining of CRC tissues and adjacent non-tumor tissues. We enrolled 276 cases of CRC tissues, of which 230 pairs contained cancer and its matched adjacent tissues. The results demonstrated that XPF was highly expressed in CRC tissues compared with adjacent non-tumor tissues ($P < 0.001$). Subgroup analysis showed that XPF expression continued to be significantly up-regulated in cancer tissues in men ($P < 0.001$), women ($P < 0.001$), age > 60 ($P < 0.001$), age ≤ 60 ($P < 0.001$), colon cancer ($P < 0.001$) and rectal cancer ($P < 0.001$) (Table 2). Besides, in the other stratified analyses were all meaningful, which XPF expression had significant relationship with the risk of CRC cancer. XPF was highly expressed in colon adenocarcinoma tissue than in colon and rectum tissue in Oncomine (Fig. 8).

Table 2
XPF expression in CRC and nontumor adjacent tissues

			(-)	(+)	(++)	(+++)		
Category	Group	Cases	n(%)	n(%)	n(%)	n(%)	PR(%)	P
Overall	CRC	230	115	94	15	6	50.0%	< 0.001
	Adjacent	230	228	1	1	0	0.9%	
Male	CRC	129	59	57	9	4	54.3%	< 0.001
	Adjacent	129	127	1	1	0	1.6%	
Female	CRC	101	56	37	6	2	44.6%	< 0.001
	Adjacent	101	101	0	0	0	0.0%	
≤ 60	CRC	112	58	45	7	2	48.2%	< 0.001
	Adjacent	112	111	1	0	0	0.9%	
60	CRC	118	57	49	8	4	51.7%	< 0.001
	Adjacent	118	117	0	1	0	0.8%	
Smoking	CRC	54	26	22	2	4	51.9%	< 0.001
	Adjacent	54	53	1	0	0	1.9%	
No smoking	CRC	176	89	72	13	2	49.4%	< 0.001
	Adjacent	176	175	0	1	0	0.6%	
Drinking	CRC	36	19	15	1	1	47.2%	< 0.001
	Adjacent	36	36	0	0	0	0.0%	
No drinking	CRC	194	96	79	14	5	50.5%	< 0.001
	Adjacent	194	192	1	1	0	1.0%	
Colon	CRC	74	48	21	3	2	35.1%	< 0.001
	Adjacent	74	74	0	0	0	0.0%	
Rectum	CRC	156	67	73	12	4	57.1%	< 0.001
	Adjacent	156	154	1	1	0	1.3%	
Lymph node metastasis	CRC	134	72	51	8	3	46.3%	< 0.001
	Adjacent	134	133	1	0	0	0.7%	
Without Lymph node metastasis	CRC	96	43	43	7	3	55.2%	< 0.001
	Adjacent	96	95	0	1	0	1.0%	
Distant metastasis	CRC	57	26	25	3	3	54.4%	< 0.001
	Adjacent	57	56	1	0	0	1.8%	
No Distant metastasis	CRC	173	89	69	12	3	48.6%	< 0.001
	Adjacent	173	172	0	1	0	0.6%	

			(-)	(+)	(++)	(+++)		
Perineural invasion	CRC	136	72	52	9	3	47.1%	< 0.001
	Adjacent	136	135	0	1	0	0.7%	
Without Perineural invasion	CRC	94	43	42	6	3	54.3%	< 0.001
	Adjacent	94	93	1	0	0	1.1%	
Vessel carcinoma embolus	CRC	53	31	19	3	0	41.5%	< 0.001
	Adjacent	53	53	0	0	0	0.0%	
Without Vessel carcinoma embolus	CRC	177	84	75	12	6	52.5%	< 0.001
	Adjacent	177	175	1	1	0	1.1%	
Infiltrative	CRC	139	76	54	5	4	45.3%	< 0.001
	Adjacent	139	138	0	1	0	0.7%	
Nested/cloddy	CRC	91	39	40	10	2	57.1%	< 0.001
	Adjacent	91	90	1	0	0	1.1%	
Poor/mucinous	CRC	70	41	24	4	1	41.4%	< 0.001
	Adjacent	70	69	1	0	0	1.4%	
Well/moderate	CRC	160	74	70	11	5	53.8%	< 0.001
	Adjacent	160	159	0	1	0	0.6%	
Family history	CRC	41	21	14	4	2	48.8%	< 0.001
	Adjacent	41	40	1	0	0	2.4%	
Without Family history	CRC	189	94	80	11	4	50.3%	< 0.001
	Adjacent	189	188	0	1	0	0.5%	
T1	CRC	6	2	3	1	0	66.7%	
	Adjacent	6	6	0	0	0	0.0%	0.022
T2	CRC	85	34	42	7	2	60.0%	
	Adjacent	85	84	0	1	0	1.2%	< 0.001
T3	CRC	119	68	43	5	3	42.9%	
	Adjacent	119	118	1	0	0	0.8%	< 0.001
T4	CRC	18	10	5	2	1	44.4%	
	Adjacent	18	18	0	0	0	0.0%	0.002
T1-2	CRC	42	19	18	3	2	54.8%	
	Adjacent	42	41	0	1	0	2.4%	< 0.001
T3-4	CRC	141	78	51	8	4	44.7%	
	Adjacent	141	140	1	0	0	0.7%	< 0.001

	(-)	(+)	(++)	(+++)
PR, positive rate. Negative (−), light positive (+), positive (++), strong positive (+++) staining. Mann–Whitney U- test of nonparametric test to compare the XPF protein expression between CRC and adjacent tissues.				
The bold values: P < 0.05				

XPF was highly expressed in CRC compared with adenoma and anal benign disease

As shown in Fig. 2, XPF expression increased from anal benign disease, adenoma to CRC (P < 0.001). Subgroup analysis demonstrated a similar expression trend of XPF in men (P < 0.001), women (P = 0.001, age ≤ 60 (P = 0.001), age > 60(P < 0.001) and rectal cancer (P < 0.001) (Table 3).

Table 3
XPF expression in CRC, adenoma and anal benign disease tissues.

			(-)	(+)	(++)	(+++)		
Category	Group	Cases	n(%)	n(%)	n(%)	n(%)	PR(%)	P
Overall	CRC	276	131	116	17	12	52.5%	< 0.001
	Adenoma	202	116	67	15	4	42.6%	
	Anal disease	62	55	6	1	0	11.3%	
Male	CRC	143	66	62	9	6	53.8%	< 0.001
	Adenoma	24	19	4	0	1	20.8%	
	Anal disease	19	17	1	1	0	10.5%	
Female	CRC	105	58	39	6	2	44.8%	0.001
	Adenoma	26	16	6	2	2	38.5%	
	Anal disease	42	37	5	0	0	11.9%	
≤ 60	CRC	119	62	47	7	3	47.9%	0.001
	Adenoma	26	18	6	1	1	30.8%	
	Anal disease	38	32	5	1	0	15.8%	
60	CRC	129	62	54	8	5	51.9%	< 0.001
	Adenoma	24	17	4	1	2	29.2%	
	Anal disease	22	21	1	0	0	4.5%	
Colon	CRC	82	54	21	3	4	34.1%	0.275
	Adenoma	10	8	2	0	0	20.0%	
Rectum	CRC	166	70	80	12	4	57.8%	0.109
	Adenoma	33	22	6	2	3	33.3%	
PR, positive rate. Negative (−), light positive (+), positive (++), strong positive (+++) staining. Mann–Whitney U- test of nonparametric test to compare the XPF protein expression between CRC and adjacent tissues.								
The bold values: P < 0.05								

The expression of XPF in CRC was higher than that in benign anal disease (P < 0.001). In the subgroup analysis of the following groups: male (P = 0.001), female (P < 0.001), age ≤ 60 (P < 0.001), age > 60 (P < 0.001), it showed the same result that XPF expression was significantly up-regulated in cancer tissues (Table 4).

Table 4
XPF expression in CRC and anal disease tissues.

			(-)	(+)	(++)	(+++)		
Category	Group	Cases	n(%)	n(%)	n(%)	n(%)	PR(%)	P
Overall	CRC	276	131	116	17	12	52.5%	< 0.001
	Anal disease	62	55	6	1	0	11.3%	
Male	CRC	143	66	62	9	6	53.8%	0.001
	Anal disease	19	17	1	1	0	10.5%	
Female	CRC	105	58	39	6	2	44.8%	< 0.001
	Anal disease	42	37	5	0	0	11.9%	
≤ 60	CRC	119	62	47	7	3	47.9%	< 0.001
	Anal disease	38	32	5	1	0	15.8%	
60	CRC	129	62	54	8	5	51.9%	< 0.001
	Anal disease	22	21	1	0	0	4.5%	
PR, positive rate. Negative (−), light positive (+), positive (++), strong positive (+++) staining. Mann–Whitney U- test of nonparametric test to compare the XPF protein expression between CRC and adjacent tissues.								
The bold values: P < 0.05								

Four different grades of immunoreactivity score (IS) including negative (-), weak positivity (+), moderate positivity (++), and strong positivity (+++) are displayed in Fig. 3.

Relationship between XPF protein expression and clinicopathological parameters in CRC patients

We used the Mann-Whitney U test to study the differences between the XPF groups (Table 5) and stratified the CRC patients based on age, gender, smoking, alcohol consumption, location, TNM stage, invasive extent, lymph node metastasis, distant metastasis, perineural invasion, vessel carcinoma embolus, growth pattern, differentiation degree and family history. The results of stratified analysis showed that XPF protein expression was related to tumor location: XPF expression in rectal cancer patients was higher than that of colon cancer patients (P = 0.005). XPF protein expression was also related to growth patterns: XPF was highly expressed in nested/cloddy CRC compared with infiltrating CRC (P = 0.009). There was a trend that XPF was highly expressed in male than female (P = 0.056). In the meanwhile, XPF was more likely to be expressed in well/moderate CRC than in poor/mucinous CRC. However, except for the above points, there was no statistical difference for other clinical pathological parameters (P > 0.05).

Table 5
Association between XPF expression and clinicopathological parameters in CRC

Variables	Cases	(-) n(%)	(+) n(%)	(++) n(%)	(+++) n(%)	PR(%)	P
Gender							
Male	143	66	62	9	6	53.8%	0.056
Female	105	58	39	6	2	44.8%	
Age(years)							
≥60	119	62	47	7	3	47.9%	0.319
≤60	129	62	54	8	5	51.9%	
Smoking							
Yes	59	27	25	2	5	54.2%	0.304
No	189	97	76	13	3	48.7%	
Drinking							
Yes	40	20	17	1	2	50.0%	0.672
No	208	104	84	14	6	50.0%	
Tumor location							
Colon	82	54	21	3	4	34.1%	0.005
Rectum	166	70	80	12	4	57.8%	
TNM stage[1]							
Ⅰ	6	2	3	1	0	66.7%	0.151
Ⅱ	89	36	44	7	2	59.6%	
Ⅲ	132	74	48	5	5	43.9%	
Ⅳ	18	10	5	2	1	44.4%	
Invasive extent[1]							
T1-2	44	20	19	3	2	54.5%	0.395
T3-4	155	84	57	8	6	45.8%	
Lymph node metastasis							
Positive	148	79	56	8	5	46.6%	0.106
Negative	100	45	45	7	3	55.0%	
Distant metastasis							
Positive	64	30	28	3	3	53.1%	0.341
Negative	184	94	73	12	5	48.9%	
Perineural invasion							

Positive	148	78	57	9	4	47.3%	0.605
Negative	100	46	44	6	4	54.0%	
Vessel carcinoma embolus							
Positive	60	36	20	3	1	40.0%	0.131
Negative	188	88	81	12	7	53.2%	
Growth patten							
Infiltrative	152	85	58	5	4	44.1%	0.009
Nested/cloddy	96	39	43	10	4	59.4%	
Differentiation degree							
Poor/mucinous	80	47	27	4	2	41.3%	0.083
Well/moderate	168	77	74	11	6	54.2%	
Family history							
Positive	43	22	15	4	2	48.8%	0.611
Negative	205	102	86	11	6	50.2%	
PR, positive rate. Negative (−), light positive (+), positive (++), strong positive (+++) staining.							
The association of XPF expression with TNM stage was analyzed by Kruskal–Wallis H- test of nonparametric test. For other clinicopathological parameters, Mann–Whitney U- test of nonparametric test was used.							
1Incomplete information.							
The bold values: P<0.05							

Relationship between XPF expression and CRC prognosis

In this study, the immunohistochemical score 1.8 was used as the cutoff value. To explore whether XPF was a prognostic indicator for patients with CRC, the association of XPF protein expression and CRC overall survival was assessed and summarized in Table 6. As shown in Figure 4, stratified analysis showed that in individuals whose invasive extent were T1-2, low XPF expression was significantly correlated with better survival rate (95% CI: 1.208-52.673, HR = 7.978, P = 0.031). Otherwise, there's a trend that patients who were in II TNM stage with low XPF expression had better survival (P=0.057), though the statistical difference was not significant. Patients with vessel carcinoma embolus had the same tendency (P=0.06).

Table 6
Correlation between XPF expression and survival in CRC.

		Cases of events	Univariate				Multivariate		
	Case		MST	HR	95% CI	P	HR	95% CI	P
XPF expression									
Low	167	54	50						
High	81	20	48	0.884	0.528-1.478	0.638	1.227	0.668-2.255	0.509
Stratification									
Sex									
Male									
Low	90	28	49						
High	53	15	48	0.968	0.516-1.815	0.919	1.288	0.603-2.752	0.513
Female									
Low	77	26	52						
High	28	5	48	0.767	0.291-2.025	0.592	1.035	0.294-3.635	0.958
Age									
≤60									
Low	86	36	49						
High	33	7	47	0.726	0.320-1.647	0.443	0.796	0.233-2.723	0.716
>60									
Low	81	18	51						
High	48	13	51.5	1.263	0.619-2.579	0.521	1.541	0.723-3.285	0.263
Smoking									
Yes									
Low	37	14	49						
High	22	7	41.5	1.016	0.407-2.535	0.972	0.605	0.109-3.346	0.564
No									
Low	130	40	50						
High	59	13	49	0.835	0.445-1.564	0.573	1.264	0.596-2.682	0.541
Drinking									
Yes									
Low	25	10	49						
High	15	2	49	0.333	0.073-1.519	0.155	0	0.000-(3.315E+126)	0.899
No									

Low	142	44	50						
High	66	18	48	1.109	0.637-1.931	0.714	1.46	0.750-2.843	0.265
Location									
Colon									
Low	61	19	51						
High	21	9	51	1.485	0.671-3.286	0.329	2.373	0.849-6.636	0.099
Rectum									
Low	106	35	49						
High	60	11	48	0.652	0.330-1.287	0.218	0.748	0.293-1.911	0.544
TNM stage[1]									
I									
Low	3	1	61						
High	3	1	48	65.289	0.000-628084630.4	0.61	13.593	0.000-(4.626E+22)	0.918
II									
Low	55	21	48						
High	34	8	46.5	0.794	0.349-1.808	0.583	4.1	0.956-17.581	0.057
III									
Low	96	28	52						
High	36	8	51	0.789	0.359-1.731	0.554	0.952	0.411-2.208	0.91
IV									
Low	11	4	39						
High	7	3	42	1.226	0.273-5.509	0.791	3346.862	0.000-(3.231E+188)	0.97
Invasive extent[1]									
T1-2									
Low	28	7	48.5						
High	16	6	47.5	2.483	0.750-8.222	0.137	7.978	1.208-52.673	0.031
T3-4									
Low	107	31	51						
High	48	11	51	0.799	0.401-1.592	0.524	0.958	0.466-1.973	0.908
Lymph node metastasis									
Yes									

Low	108	34	49						
High	40	11	47	1.002	0.507-1.980	0.995	1.462	0.694-3.081	0.318
No									
Low	59	20	54						
High	41	9	51	0.842	0.381-1.861	0.672	0.439	0.096-2.009	0.289
Distant metastasis									
Yes									
Low	39	12	49						
High	25	7	47	1.253	0.487-3.222	0.64	2.592	0.650-10.341	0.177
No									
Low	128	42	50						
High	56	13	48.5	0.773	0.415-1.441	0.418	1.112	0.531-2.332	0.778
Perineural invasion									
Yes									
Low	96	27	49						
High	52	12	48	0.851	0.431-1.681	0.643	1.362	0.608-3.052	0.452
No									
Low	71	27	55						
High	29	8	49	0.907	0.411-2.002	0.81	1.068	0.355-3.209	0.907
Vessel carcinoma embolus									
Yes									
Low	43	9	48						
High	17	7	42	2.241	0.826-6.083	0.113	3.805	0.944-15.333	0.06
No									
Low	124	45	52						
High	64	13	50	0.666	0.359-1.237	0.198	0.82	0.374-1.794	0.619
Growth patten									
Infiltrative									
Low	111	33	49						
High	41	10	48	0.985	0.484-2.005	0.966	1.18	0.523-2.662	0.69
Nested/cloddy									
Low	56	21	52.5						
High	40	10	48	0.767	0.361-1.631	0.49	0.824	0.290-2.340	0.716

Differentiation degree									
Low									
Low	60	22	49						
High	20	4	48.8	0.539	0.183-1.586	0.262	0.939	0.240-3.673	0.928
High									
Low	107	32	50						
High	61	16	48	1.029	0.562-1.882	0.927	1.531	0.701-3.343	0.286
Family history									
Yes									
Low	26	10	54						
High	17	5	47	1.249	0.409-3.820	0.696	8.39E+15	0.000-(3.821E+081)	0.635
No									
Low	141	44	49						
High	64	15	49	0.829	0.461-1.491	0.531	1.218	0.622-2.384	0.565
CI, confidence interval; HR, hazard radio; MST, median survival time. IS, the immunohistochemistry score.									
The bold values: P<0.05									

Functional enrichment analysis of XPF and its interacting genes

We analyzed XPF and its interacting genes in STRING (Figure 5) database. As shown in Figure 6A, B, C, GO analysis showed that XPF and its interacting genes were mainly correlated with nucleoplasm, nucleotide-excision repair factor 1 complex, DNA repair, nucleotide-excision repair, preincision complex stabilization, damaged DNA binding single-stranded DNA binding and so on. As for KEGG analysis, these genes were mainly enriched in nucleotide excision repair pathway (Figure 6D). The results of GSEA for GO analysis (Figure 7A, B) showed that XPF expression was associated with Ubiquitin like protein specific protease activity, microtubule organizing center organization, cytoplasmic stress granule, peptide-n-acetytransferase activity, centriole, ciliary basal body, microtubule organizing center localization, WNT signaling pathway, calcium modulating pathway and so on. The results of GSEA for KEGG analysis

Discussion

Although there have been some studies on the correlation between XPF expression and CRC[10–14], this is the first research on XPF expression that covers dynamic CRC development. In addition, this study explored the relationship between XPF expression and clinical traits of CRC. Our results showed that XPF expression was upregulated in CRC tissues compared with adjacent non-tumor tissues, adenoma and anal benign disease. Overexpression of XPF was related to poor prognosis of CRC patients with T1-2 invasive extent. Therefore, XPF might be a promising biomarker for CRC risk, and also a potential prognostic predictor in metastatic CRC patients.

Firstly, we detected the differential expression of XPF in cancerous and non-cancerous tissues. It found that the XPF protein expression was significantly higher in CRC tissues than that in adjacent colorectal tissues. Moreover, XPF expression showed an obviously trend of increasing with the development from anal disease, adenoma to CRC. Previous

studies of XPF expression in other tumors have yielded similar results: Li.P et al[7] found a significant increase of the XPF expression in GC tissues compared with adjacent tissues. Meanwhile, XPF protein played a vital role in the occurrence and progress of GC[7]; Increased expression of XPF might be associated with drug resistance in non-small cell lung cancer[23]. In summary, the results of other types of cancer can partially confirm our experimental results on the up-regulation of XPF in CRC, at any rate.

Further analysis combined with clinicopathological features of patients brought to light that increased expression of XPF was closely related to clinical features, including rectal cancer and cloddy/nested pattern. XPF expression was related to the invasion of hepatic capsules and microvascular tumor embolus in human hepatocellular carcinoma[24]. The expression of XPF was significantly related to some clinical features such as family history and Laurén classification in GC($P < 0.05$)[7]. XPF abundance was associated with positive ER status in breast cancer, through clinicopathological parameter analysis[25]. Therefore, XPF may have a certain significance for predicting the biologic activities and the progression of CRC, and it also can be considered as a direction of future research.

This study also explored the association between XPF expression and prognosis of CRC patients. We observed that the XPF expression was not significantly associated with the survival time in overall analysis. As for patients with T1-2 invasive extent, those with low XPF expression can survive longer than those with high expression. Previously, low XPF expression was also found to predict better prognosis in other types of cancer: XPF-positive patients had shorter survival time than XPF-negative patients in GC[7]. XPF can be used as an important index to affect the survival time of GC patients because of the gene polymorphisms[26]. Mesquita KA et al. found that low ERCC1/XPF expression was related to better progression-free survival in 331 ovarian cancer patients[27]. With the increase of damage in cancer tissues, damage repair activities increase, so the high expression of XPF in colorectal cancer tissues also indicates a worse prognosis. In summary, the correlation between low expression of XPF and longer survival time may be applicable to CRC as well as other types of cancer, but its molecular mechanism still needs further research to be clarified.

The expression pattern of XPF in CRC and its potential prognostic role inspired our understanding of XPF in development and progression of CRC. Therefore, we further performed PPI and functional enrichment analysis to reveal the interacting network and the biological function of XPF. By STRING database, we queried the genes interacted with XPF, which were ERCC1, XPA, ERCC5, MSH2, XPC, ERCC3, ERCC2 and so on. Firstly, functional analysis of this PPI network showed that positive regulation of DNA secondary structure binding and damaged DNA binding were the most significant. Besides, the network was also correlated with UV protection and DNA repair complex. XPF is an essential human gene in the NER pathway responsible for the removal of UV-C photoproducts and large volume adducts from DNA[4]. KEGG analysis also showed that XPF-related PPI network was mainly enriched in nucleotide excision repair pathway. Studies have mentioned that XPF was in charge of the 5' incision process in the NER pathway[27]. GSEA analysis showed that with the expression of XPF increased, some pathways can be activated, such as ubiquitin like protein specific protease activity, WNT signaling pathway and calcium modulating pathway. There are reports in the literature showing that high expression of ubiquitin-specific protease 6 N-terminal-like protein can regulate proliferation activity of CRC cell via Wnt/β-catenin pathway[21]. Therefore, we suspected that increased XPF expression may be related to CRC risk and progression by activating the above mentioned pathways.

Conclusions

In conclusion, we investigated the expression of XPF in adjacent non-tumor tissues, benign disease, adenoma and CRC by immunohistochemistry. We found that the expression of XPF was gradually increased with the progress of CRC. Besides, it mentioned that XPF protein expression was associated with tumor location and growth patterns of CRC. XPF may be a promising biomarker for CRC risk, and also showed potential as a prognosis predictor in metastatic patients with CRC.

Abbreviations

CRC: colorectal cancer; NER: Nucleotide excision repair; GC: gastric cancer; UICC: International Union Against Cancer; AJCC: United Joint Cancer Committee; HNPCC: hereditary nonpolyposis colorectal cancer; GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; GSEA: Gene Set Enrichment Analysis; MST: median survival time; IS: immunoreactivity score

Declarations

Ethics approval and consent to participate

This study was permitted by the Ethics Committee of the First Hospital of China Medical University. And the written informed consent was obtained from all the participants.

Consent for publication

Not applicable.

Competing interests

All authors declare that there is no conflict of interest.

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Author’s contributions

Conceived and designed the experiments: CX and YY . Performed the experiments: HH. Collected the samples and Analyzed the data: HH. Analyzed the data: XL. Contributed reagents/materials/analysis tools: CX. Wrote and revised the paper: HH JJ and YY. All authors read and approved the final manuscript.

Availability of data and materials

The authors declare that the data supporting the conclusion of this study are included within the article.

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Figures

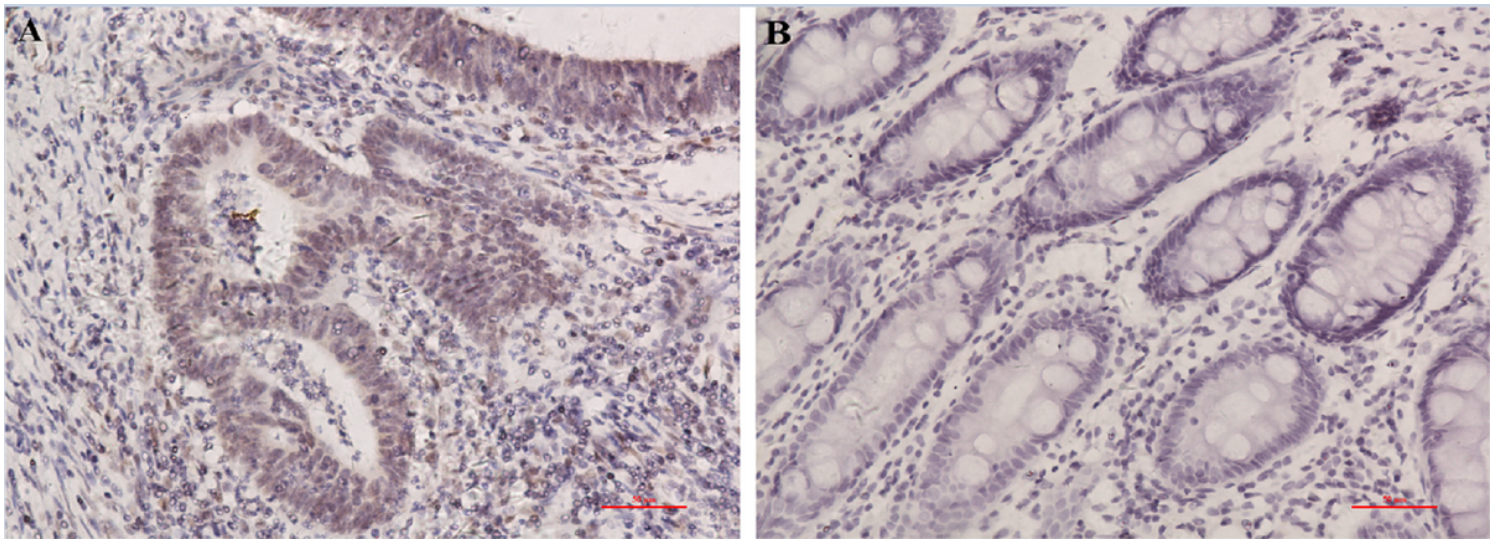

Figure 1

XPF expression in CRC and its matched non-tumor adjacent specimens (A) Colorectal cancer tissues and (B) adjacent nontumor tissues of CRC. Original magnification, ×200.

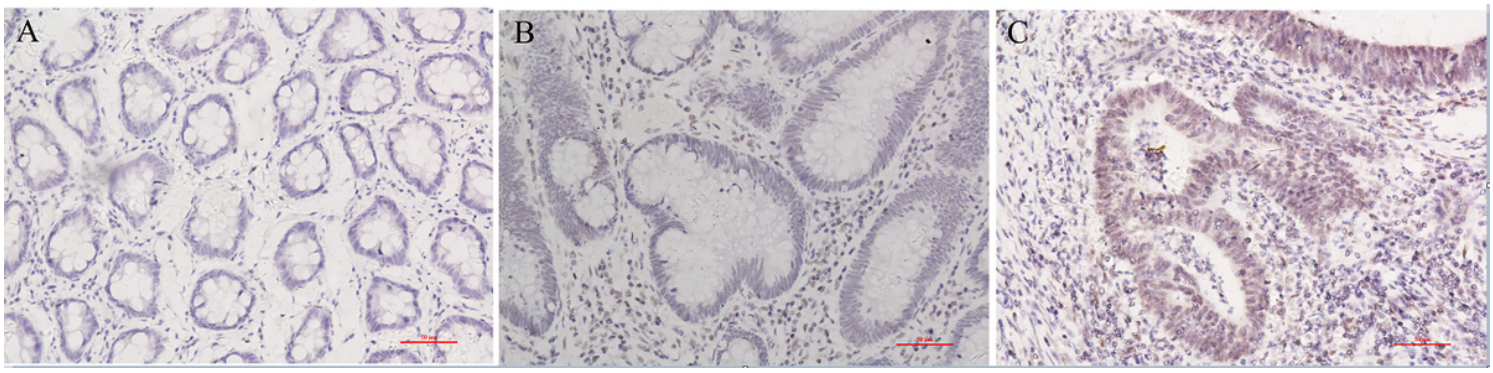

Figure 2

Representative photomicrographs of immunohistochemical staining of XPF in different colorectal specimens. (A)Low nuclear expression of XPF in anal disease. (B)Middle nuclear expression of XPF in adenoma tissues. (C)High nuclear expression in CRC. Showing the dynamic expression in colorectal diseases. Original magnification, ×200.

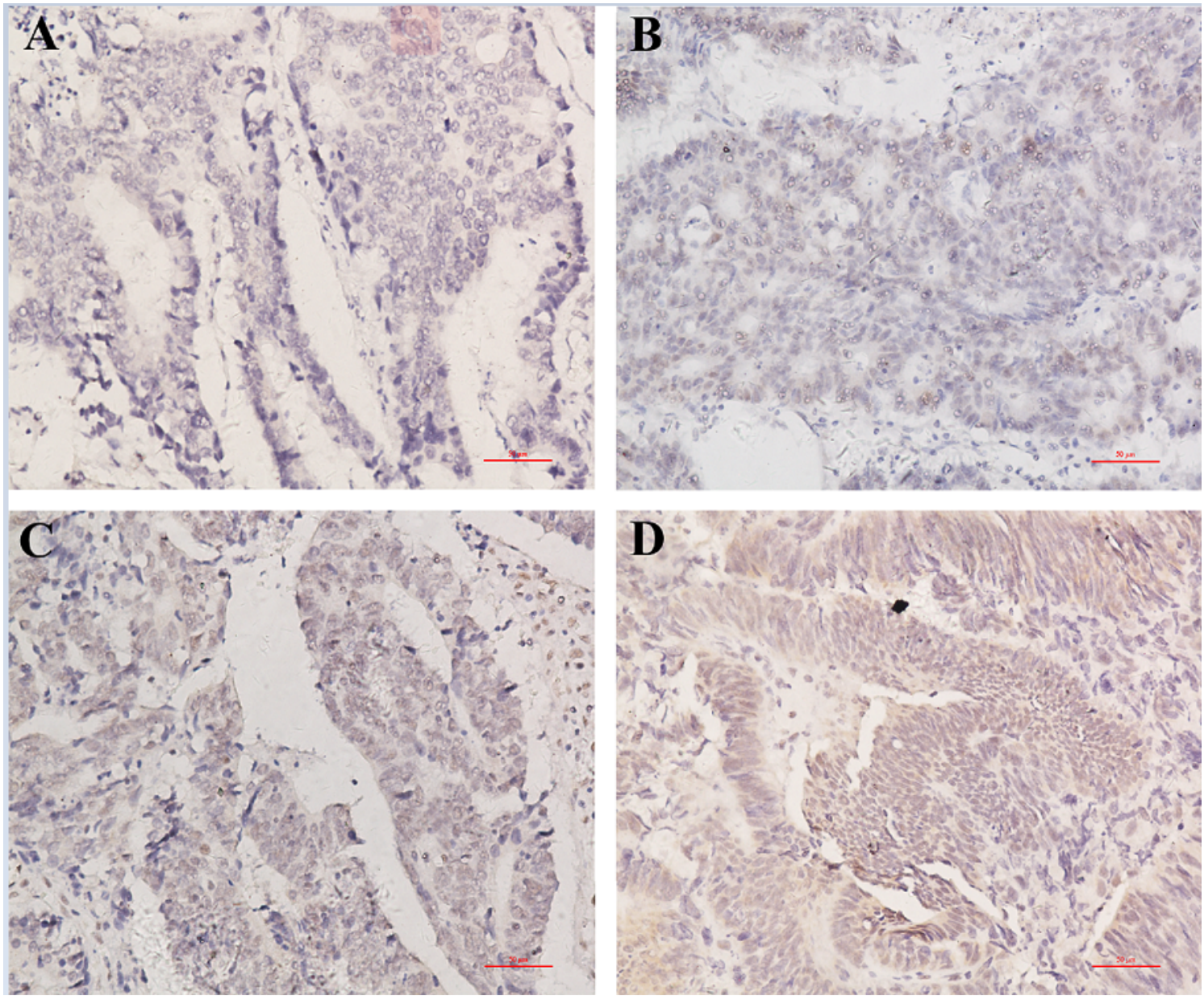

Figure 3

Different levels of XPF expression in CRC tissues. (A) Negative (−), (B) weakly positive (+), (C) moderately positive (++), (D) strongly positive (+++).

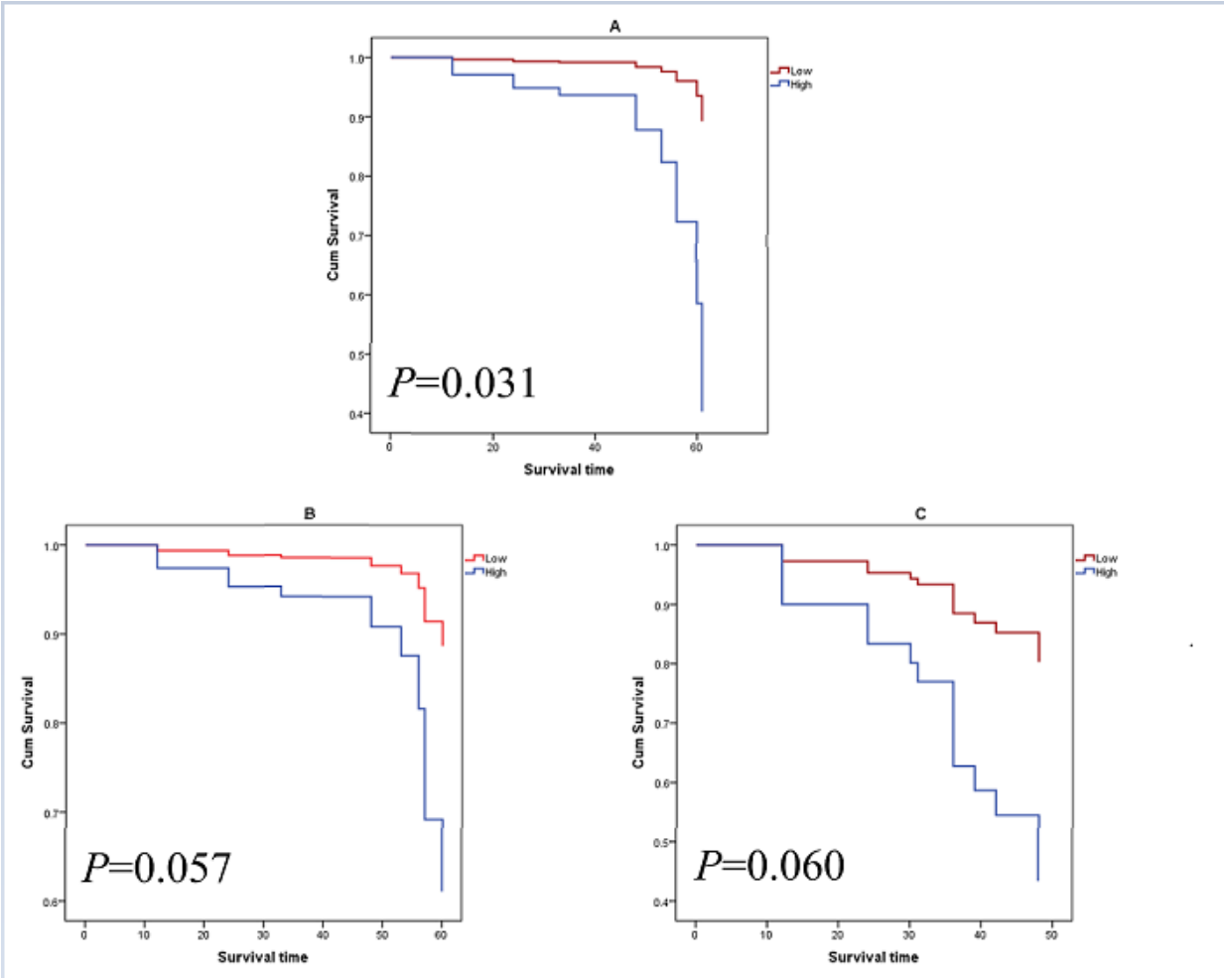

Figure 4

Low expression of XPF was correlated with the prognosis in CRC patients. (A) patients in T1-2 invasive extent with low XPF expression exhibited longer survival time than those with high XPF expression; (B) TII individuals who expressed lower XPF protein demonstrated better prognosis; (C) patients with vessel carcinoma embolus also identified XPF expression as a bad indicator for CRC prognosis.

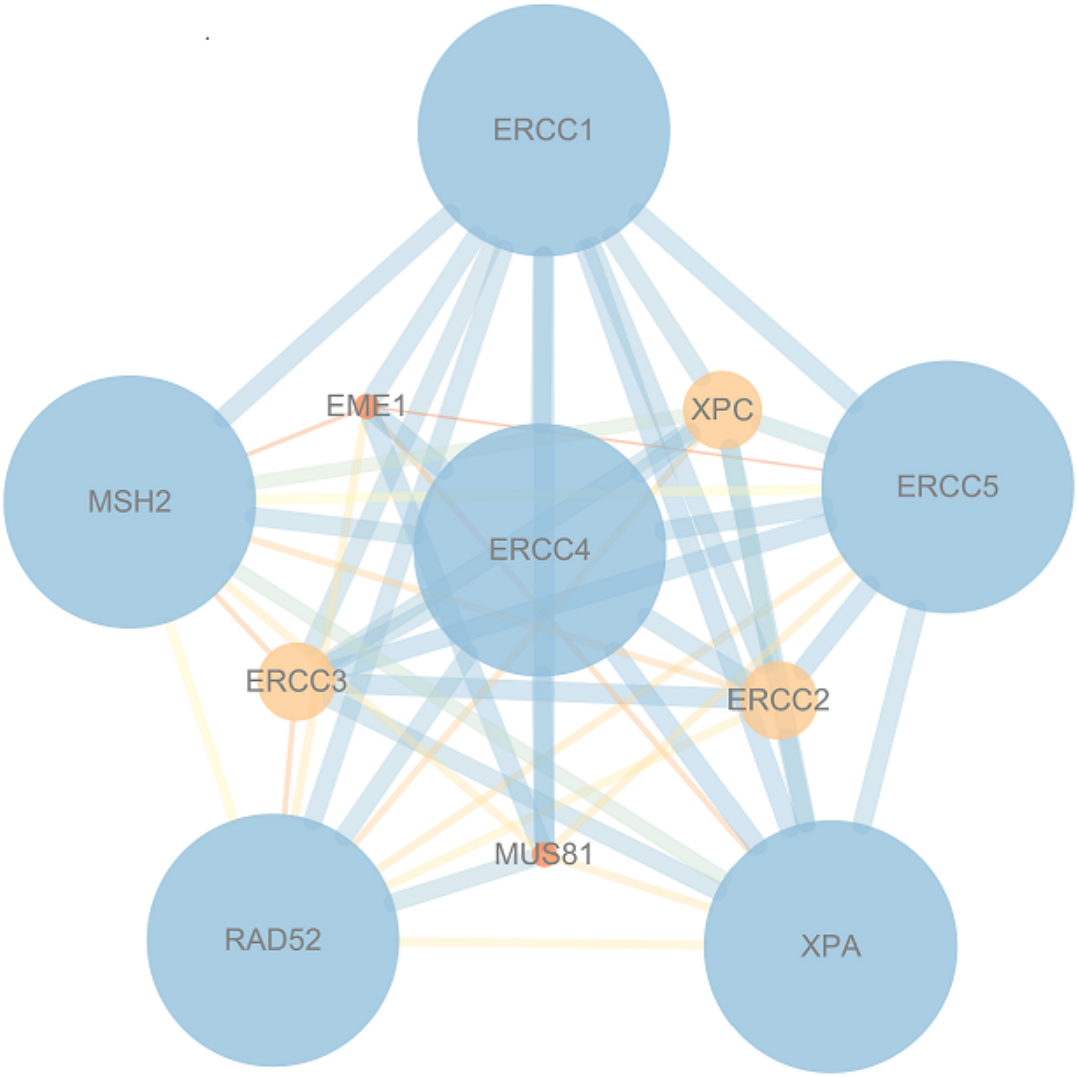

Figure 5

PPI network of XPF.

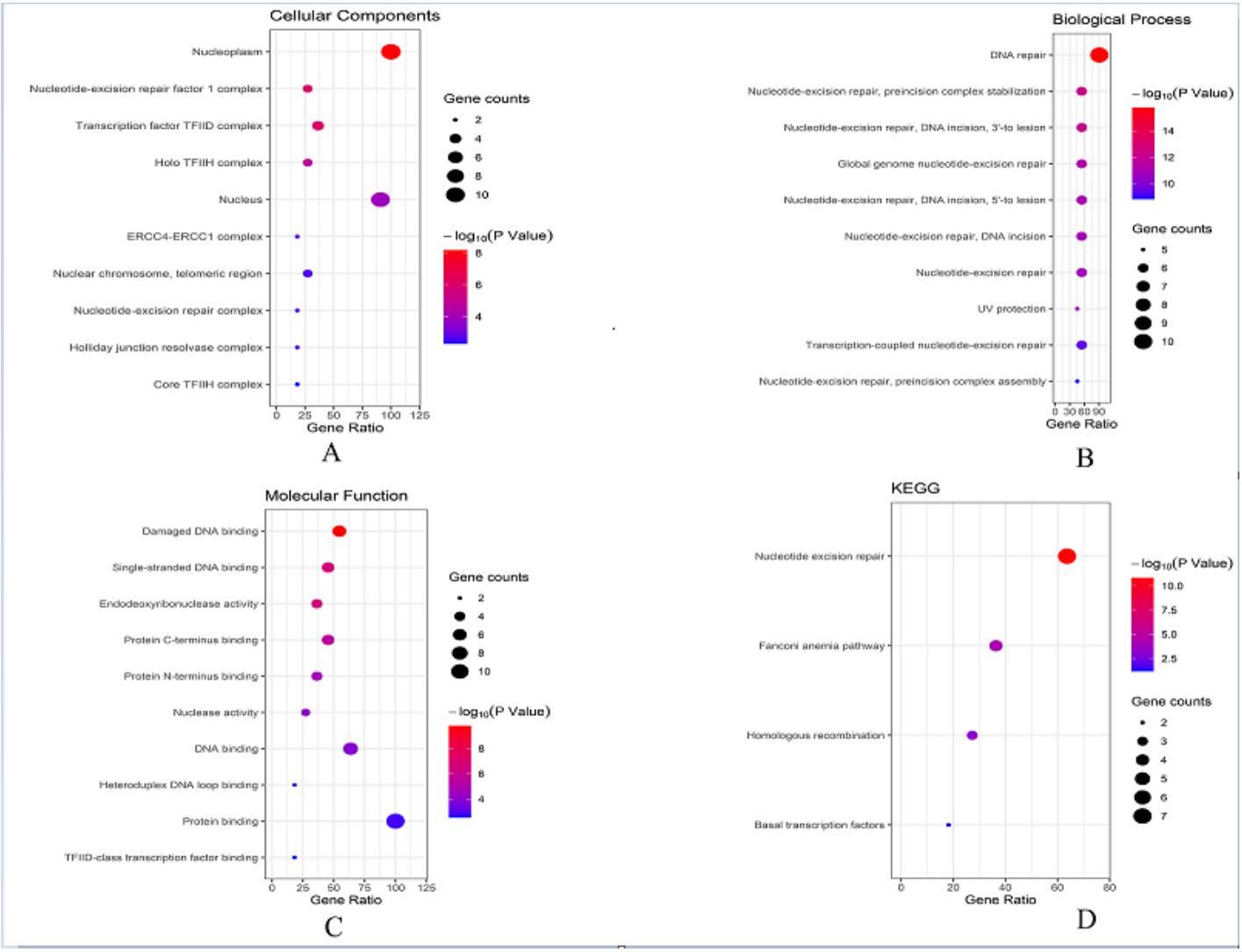

Figure 6

GO and KEGG enrichment of XPF and its interacting genes.

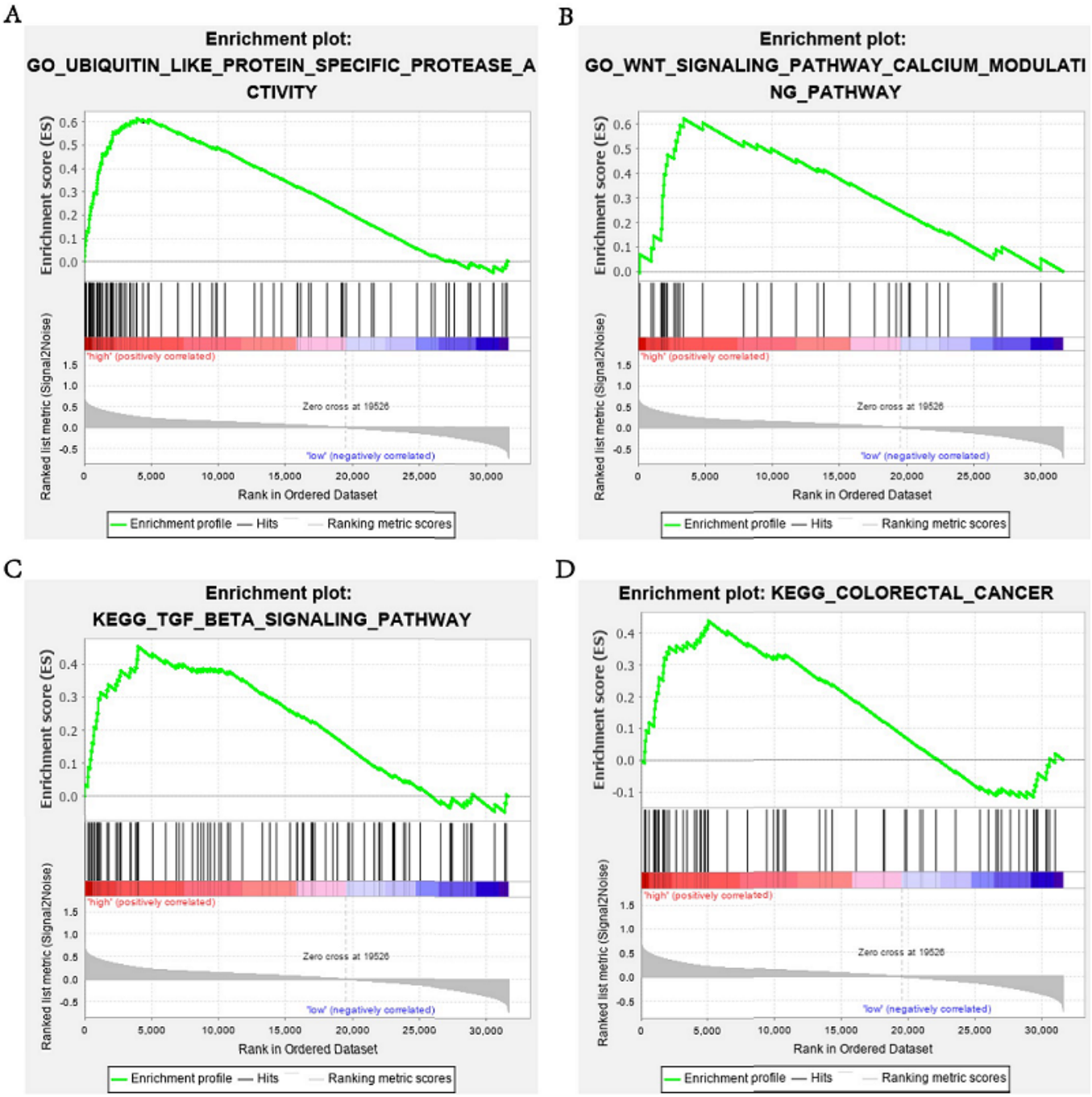

Figure 7

Enrichment analysis results of GSEA. A. GO-Ubiquitin like protein specific protease activity; B. GO-Wnt signaling pathway calcium modulating pathway; C. KEGG-TGF-BETA signaling pathway; D. KEGG-Colorectal cancer.

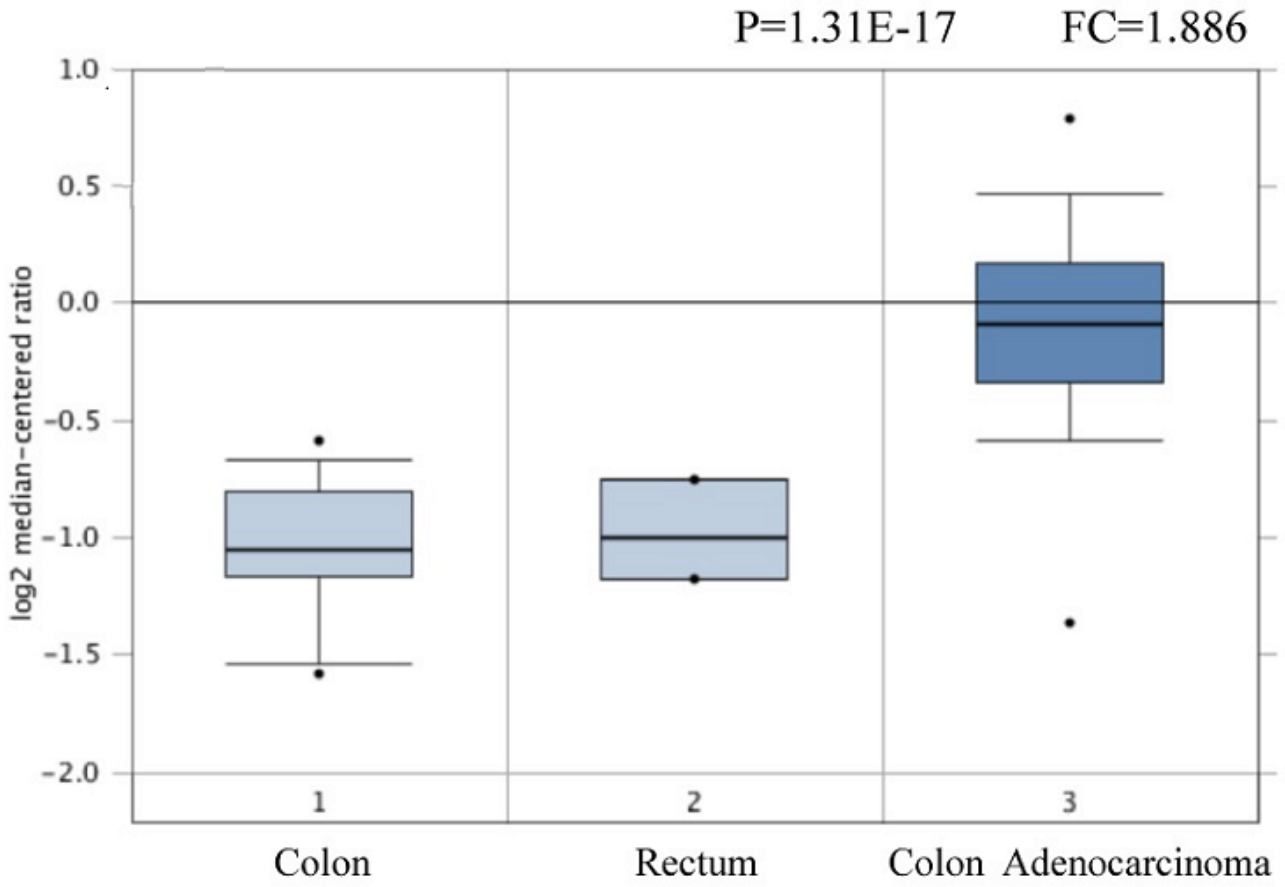

Figure 8

XPF expression in TCGA colorectal cancer cohort

Supplementary Files

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- FigureS1.png