DNA repair protein XPA is differentially expressed in colorectal cancer and predicts better prognosis

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Abstract
As an indispensable factor in DNA damage recognition step of nucleotide excision repair, XPA interacts with a series of proteins to initiate repair process. The expression characteristics of XPA in colorectal cancer (CRC) and its influence on CRC prognosis remain elusive. Tissue specimens of CRC and nontumor adjacent tissues from 283 patients were collected. XPA protein expressions were detected by immunohistochemistry staining. Nonparametric test was used to investigate the difference of XPA expression between CRC and nontumor adjacent tissues, as well as the correlation between XPA expression and clinicopathological parameters of CRC. Univariate and multivariate Cox proportional hazards models were applied to estimate the relationship between XPA expression and CRC prognosis. Meanwhile, we analyzed TCGA data to investigate the relation between XPA mRNA expression and survival of CRC. XPA protein expression was significantly decreased in CRC tissues compared with nontumor adjacent tissues (P = 0.001). Subgroup analysis indicated consistently significant down-regulation of XPA in CRC tissues in age > 60 (P = 0.026), age ≤ 60 (P = 0.008), colon cancer (P = 0.009), and rectal cancer (P = 0.015) patients and males (P = 0.004). For clinicopathological parameters, CRC patients with drinking habits revealed XPA overexpression than nondrinkers (P = 0.032). For prognosis, CRC patients with high XPA protein expression had longer overall survival (OS) (HR = 0.62, 95%CI: 0.39–0.97, P = 0.037). Stratified analysis suggested a better prognosis in relation to high XPA protein expression in patients over 60 years (adjusted HR = 0.48, P = 0.021), with rectal cancer (HR = 0.56, P = 0.037), without distant metastasis (HR = 0.58, P = 0.033), without tumor deposits (HR = 0.40, P = 0.006; adjusted HR = 0.44, P = 0.028), and with tumor diameter over 4 cm (HR = 0.49, P = 0.023). DNA repair protein XPA is significantly decreased in colorectal cancer tissues than in adjacent nontumor tissues. High expression of XPA protein showed significant relationship with better survival of CRC, especially rectal cancer. XPA might be a novel biomarker but might not be an independent factor to predict prognosis of CRC patients.

Introduction
Various human diseases, particularly cancer, mainly derive from an imbalance between DNA damage and repair [1, 2]. DNA damage is induced by endogenous or exogenous stimuli [3, 4], while DNA repair is accomplished by systems including nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR), and double-strand break repair (DSBR) [5]. NER system, which is versatile and crucial, monitors and restores multiple DNA damage of ultraviolet-induced cyclobutane pyrimidine dimers, bulky adducts as well as DNA cross-links [6, 7]. And four key procedures participated in the NER pathway are as follows: damage recognition, damage...
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Demarcation and unwinding, damage incision, and new strand ligation [8, 9].

*Xeroderma pigmentosum group A* (XPA) gene, mapped to chromosome 9q22.3, includes six exons and encodes a zinc finger protein of 273 amino acids [10]. As an indispensable factor in DNA damage recognition, XPA interacts with a series of NER proteins to initiate repair process [6, 11, 12]. It has been revealed that cells or animals lacking XPA cannot accomplish NER [9, 13–15]. Considering the critical role of XPA in NER, a number of studies have been conducted to investigate the effect of XPA on cancer [16–18]. Xiang Fu et al. [19] found that high expression of XPA correlated with poor prognosis in 129 nasopharyngeal carcinoma patients treated with platinum-based chemoradiotherapy using immunohistochemistry. In metastatic ovarian carcinoma, the results of 67 malignant effusion specimens showed that the overexpression of XPA was associated with better (progression-free survival) PFS and (overall survival) OS [20]. So far, however, the expression characteristics of XPA in CRC, which is the fourth most common cause of cancer mortality and third most frequently diagnosed cancers in both males and females in China [21], and its influence on CRC prognosis remain elusive.

In this study, we detected XPA protein expression levels in the colorectal mucosa tissues and their adjacent non-tumor tissues from 283 CRC patients by immunohistochemical staining. Meanwhile, the association between XPA expression with clinicopathological parameters and prognosis in CRC patients was analyzed to clarify the latent effect of XPA on the progression and prognosis of CRC.

**Materials and Methods**

**Patients and tissue specimens**

The study was approved by the Institute Research Medical Ethics Committee of the First Affiliated Hospital of China Medical University, and written informed consents were obtained from all individuals. Patients were enrolled from the First Affiliated Hospital of China Medical University who experienced surgical operation between October 2012 and July 2015. Tissue specimens including 283 CRC tissues and the corresponding nontumor adjacent tissues were collected in our study.

On the basis of the World Health Organization criteria, the tissue samples of CRC diagnosed on the account of histological results. International Union Against Cancer (UICC)/American Joint Committee on Cancer (AJCC) (seventh edition, 2010) was used to confirm TNM staging of CRC in the following of postoperative pathological diagnosis. Three criteria were made to exclude CRC patients (1) having XP disease, (2) accepting preoperative chemotherapy or radiation, and (3) having hereditary nonpolyposis colorectal cancer (HNPCC). The follow-up was performed until August 2017. A total of 266 cases were included to analyze the prognosis (mean survival time was 37.9 months; the time of follow-up ranged from 1 month to 56 months; 79 of them died), while the rest 17 cases were not included for the OS analysis because loss of follow-up. The study defines overall survival (OS) as the period from the date of operation to death. The patients who smoke at least one cigarette daily for at least 1 year were regarded as the cases with history of smoking. Meanwhile, the study defines history of drinking as the mean alcohol ingest per day for at least 50 g and lasting for at least 1 year.

**Immunohistochemistry**

Immunohistochemistry was performed mainly as previously described [22]. Tissues, which were fixed with formalin and embedded with paraffin, were cut into 4-μm-thick sections and mounted in a poly-1-lysine-coated glass slides. After routine deparaffinization, rehydration in a graded alcohol series and washing in tap water, the tissue sections were exposed to 10 mmol/L citrate buffer (PH 6.0) for 90 sec in a steam pressure cooker for antigen retrieval. Endogenous peroxidase was blocked using 3% hydrogen peroxide for 10 min, and then, the tissue sections were washed with phosphate-buffered saline (PBS, PH 7.4). To lessen the nonspecific binding, 10% normal goat serum was subsequently used to block tissue collagen for 10 min. The mouse monoclonal antibody anti-XPA (ab-2352, 1:200 dilution; Abcam, Cambridge, U.K.) was used as the primary antibody to detect XPA protein expression and incubated for 60 min at room temperature (24–27°C). After that, the sections were rinsed by PBS for 10 min each and then incubated with biotinylated secondary antibody (goat anti-rabbit antibody, Maixin Inc., Fujian, China) and streptavidin–biotin–peroxidase for 10 min each at temperature (24–27°C). Slides were stained with DAB (DAB-0031, Maixin Inc., Fujian, China) chromogenic reagent for 80 sec. At last, the slides were dehydrated and fixed by resin. Meanwhile, we used three ways to control the quality of IHC. First, we used negative (PBS was used to substitute primary and secondary antibodies, respectively) and positive controls in the IHC staining to avoid false-negative or false-positive results. Second, the DAB staining was observed by microscope in case that the staining was overestimated or underestimated. Third, two pathologists independently scored the XPA expression level in a double-blind manner.

**Evaluation of immunohistochemistry**

XPA protein expressions in the different tissues were read and scored independently by two pathologists, in accordance with the double-blind principle. On the basis of
immunohistochemistry semiquantification method, the pathologists evaluated the area and intensity of the staining results. And if the differences between the results of the pathologists were more than one grade, more scopes would be selected and the final scores would be discussed and concluded by the two pathologists. Semiquantitative scoring criterion was used to evaluate the expression of XPA in nucleus. The staining intensity of cancer cells was graded on a scale of 0–3(I0–I3): I0 (no staining), I1 (light brown), I2 (brown staining), and I3 (heavy brown staining) (intermediate intensity between two levels was defined as I0.5, I1.5, and I2.5); the proportion of stained cells were recorded as (P0–P3): 0–5% (P0), 6–25% (P1), 26–50% (P2), 51–75% (P3), and 76–100% (P4). The final IS scores were accumulated by the formula: IS score = I n × P m . At last, the XPA protein expression was graded as follows: negative (−), score = 0; weak expression (+), score = 0.5–4; moderate expression (++), score = 4.5–8; and strong expression (+++), score = 9–12. As the median for immunohistochemistry score, score 4.5 was selected as the cutoff value to distinguish high or low expression for XPA protein.

Obtainment of data from TCGA database

The Cancer Genome Atlas (TCGA) is a publicly available database that has generated comprehensive, multidimensional maps of the important genomic changes in 33 types of cancer. In this study, data of 478 colon adenocarcinoma cases (TCGA-COAD, provisional) with expression and clinicopathological information were downloaded. Additionally, data of 166 rectum adenocarcinoma cases (TCGA-READ, provisional) were obtained to analyze the relationship of XPA mRNA expression with CRC prognosis.

Statistical analysis

All statistical analyses were performed using SPSS software, Chicago, IL (version 18.0). The comparison of XPA expression between CRC and nontumor adjacent tissues was assessed by nonparametric test. The correlation between XPA expression and clinicopathological parameters of CRC was also conducted by nonparametric test. The study applied Kaplan–Meier method to visualize the patient survival time and employed log-rank tests to analyze the difference between groups. Univariate Cox proportional hazards model was applied to estimate the relationship between the expression of XPA and CRC prognosis, and multivariate Cox proportional hazards model was used to evaluate the association adjusted by age, gender, TNM stage, and differentiation degree. P values <0.05 were considered statistically significant.

Results

Baseline characteristics

The baseline characteristics of the 283 CRC patients included are shown in Table 1. Altogether, 165 males

| Characteristics | CRC | Cases of events | MST | P   |
|-----------------|-----|-----------------|-----|-----|
| Gender          |     |                 |     |     |
| Male            | 165 | 52              | 44.55 | 0.252 |
| Female          | 118 | 30              | 47.14 |     |
| Age (years)     |     |                 |     |     |
| >60             | 152 | 50              | 43.64 | 0.171 |
| ≤60             | 131 | 32              | 47.25 |     |
| Smoking         |     |                 |     |     |
| Yes             | 72  | 19              | 46.66 | 0.578 |
| No              | 209 | 63              | 45.19 |     |
| Drinking        |     |                 |     |     |
| Yes             | 31  | 8               | 46.03 | 0.615 |
| No              | 237 | 72              | 45.18 |     |
| Tumor location  |     |                 |     |     |
| Colon           | 80  | 24              | 44.41 | 0.889 |
| Rectum          | 202 | 57              | 46.29 |     |
| TNM stage       |     |                 |     |     |
| I               | 73  | 14              | 26.62 | <0.001 |
| II              | 69  | 30              | 41.32 |     |
| III             | 121 | 32              | 49.72 |     |
| IV              | 20  | 6               | 53.35 |     |
| Invasive extent |     |                 |     |     |
| T1–2            | 86  | 11              | 52.04 | <0.001 |
| T3–4            | 197 | 71              | 42.77 |     |
| Lymph node metastasis |     |                 |     |     |
| Positive        | 135 | 62              | 39.29 | <0.001 |
| Negative        | 148 | 20              | 51.26 |     |
| Distant metastasis |     |                 |     |     |
| Positive        | 20  | 14              | 26.62 | <0.001 |
| Negative        | 263 | 68              | 46.98 |     |
| Tumor deposit   |     |                 |     |     |
| Positive        | 31  | 17              | 29.16 | <0.001 |
| Negative        | 184 | 44              | 44.49 |     |
| Perineural invasion |     |                 |     |     |
| Positive        | 148 | 49              | 39.75 | 0.004 |
| Negative        | 71  | 13              | 49.13 |     |
| Vessel carcinoma embolus |     |                 |     |     |
| Positive        | 65  | 27              | 38.92 | 0.006 |
| Negative        | 218 | 55              | 47.20 |     |
| Growth pattern  |     |                 |     |     |
| Infiltrative    | 163 | 61              | 42.07 | <0.001 |
| Nestcd/eddy     | 119 | 21              | 50.28 |     |
| Differentiation degree |     |                 |     |     |
| Poor/mucinous   | 79  | 39              | 36.41 | <0.001 |
| Well/moderate   | 191 | 37              | 49.98 |     |
| Maximum diameter(cm) |     |                 |     |     |
| >4              | 133 | 45              | 42.92 | 0.036 |
| ≤4              | 149 | 36              | 48.10 |     |
| Family history  |     |                 |     |     |
| Positive        | 57  | 15              | 45.65 | 0.478 |
| Negative        | 226 | 67              | 45.43 |     |
| Chemotherapy    |     |                 |     |     |
| Yes             | 107 | 27              | 48.14 | 0.409 |
| No              | 111 | 30              | 44.53 |     

CRC, colorectal cancer; MST, median survival time.

1Incomplete information.
and 118 females were enrolled with a median survival time (MST) of 44.55 months and 47.14 months, respectively. Totally, 152 CRC patients were over 60 years of age, while 131 cases were younger than 60. The location of colorectal cancer included colon (80 cases) and rectum (202 cases). TNM staging was as follows: stage I, 73; stage II, 69; stage III, 121; and stage IV, 20.

**Down-regulation of XPA in CRC tissues than nontumor adjacent tissues**

The representative immunohistochemistry staining of CRC tissue and nontumor adjacent tissue is shown in Figure 1 (Figure 1A and B), respectively. Figure 2 demonstrated four different staining grades as negative (−), light positive (+), positive (++) and strong positive (+++). The detailed results of the expression profile of XPA in CRC and nontumor adjacent tissues are summarized in Table 2. According to the Mann-Whitney U-test, XPA protein expression was significantly decreased in CRC tissues compared with nontumor adjacent tissues ($P = 0.001$), which is visualized by scatter plots in Figure 3.

Subgroup analysis based on age and tumor location suggested consistently significant down-regulation of XPA in CRC tissues than in their adjacent tissues in age > 60 ($P = 0.026$), age ≤ 60 ($P = 0.008$), colon cancer ($P = 0.009$), and rectal cancer ($P = 0.015$). In addition, male patients showed low XPA expression in CRC tissues compared with adjacent tissues ($P = 0.004$), but no significant difference was observed in female individuals ($P = 0.067$).
Association between XPA protein expression and clinicopathological parameters of CRC patients

CRC patients were stratified according to variables including gender, age, smoking, drinking, TNM stage, and tumor invasion depth, and Mann–Whitney U-test was performed to explore the differential expression of XPA between groups (Table 3). The results indicated that XPA protein expression correlated with drinking status: CRC patients with drinking habits revealed XPA overexpression than nondrinkers ($P = 0.032$). However, most comparisons of other clinicopathological parameters of CRC did not demonstrate significant difference ($P > 0.05$).

Relationship between XPA expression and CRC prognosis

The cutoff value of IS was 4.5 in this study as it was the median score for immunohistochemistry staining of XPA in CRC (IS $\geq 4.5$ means high expression, and IS $< 4.5$ means low expression). To investigate whether XPA protein expression could indicate CRC prognosis, Cox proportional hazards model was applied to estimate the relationship between the expression of XPA and CRC survival (Table 4). Univariate Cox proportional hazards model revealed that CRC patients with high XPA protein expression had longer overall survival (OS) (HR = 0.62, 95%CI: 0.39–0.97, $P = 0.037$, Fig. 4A). Multivariate Cox proportional hazards model adjusting for age, gender, TNM stage, and differentiation degree did not show significant relation with CRC survival (adjusted HR = 0.68, 95% CI: 0.42–1.09, $P = 0.107$).

Stratified analysis based on age and tumor location suggested that patients over 60 years of age with high XPA expression exhibited longer survival time than those with low XPA expression (adjusted HR = 0.48, 95% CI: 0.26–0.89, $P = 0.021$, Fig. 4B); rectal cancer individuals who expressed higher XPA protein demonstrated favorable prognosis (HR = 0.56, 95% CI: 0.32–0.97, $P = 0.037$, Fig. 4C). In the subgroup without distant metastasis, high XPA expression showed significant association with better OS (HR = 0.58, 95% CI: 0.35–0.96, $P = 0.033$, Fig. 4D). Both univariate and multivariate analyses indicated significant correlation between high XPA expression and decreased hazards of death in the CRC cases without tumor deposits (HR = 0.40, 95% CI: 0.21–0.77, $P = 0.006$, adjusted...

![Figure 3](image-url). XPA protein expression was significantly decreased in CRC tissues compared with nontumor adjacent tissues. According to the Mann–Whitney U-test, the differential expression of XPA between CRC specimens and nontumor adjacent specimens was visualized by scatter plots. *: $P<0.05$
Table 3. Association between XPA expression and clinicopathological parameters in CRC.

| Variables                        | Cases | (−) | (+)  | (++) | (+++) | PR (%) | P     |
|----------------------------------|-------|-----|------|------|-------|--------|-------|
| Gender                           |       |     |      |      |       |        |       |
| Male                             | 165   | 8 (4.8) | 73 (44.2) | 61 (37.0) | 23 (13.9) | 95.2  | 0.734 |
| Female                           | 118   | 5 (4.2) | 50 (42.4) | 50 (42.4) | 13 (11.0) | 95.8  |       |
| Age (years)                      |       |     |      |      |       |        |       |
| >60                              | 152   | 8 (5.3) | 57 (37.5) | 68 (44.7) | 19 (12.5) | 94.7  | 0.218 |
| ≤60                              | 131   | 5 (3.8) | 66 (43.4) | 43 (28.3) | 17 (13.0) | 96.2  |       |
| Smoking                          |       |     |      |      |       |        |       |
| Yes                              | 72    | 2 (2.8) | 7 (9.7) | 31 (20.4) | 12 (16.7) | 97.2  | 0.102 |
| No                               | 209   | 11 (2.1) | 95 (45.5) | 80 (38.3) | 23 (11.0) | 94.7  |       |
| Drinking                         |       |     |      |      |       |        |       |
| Yes                              | 31    | 1 (3.2) | 9 (29.0) | 14 (45.2) | 7 (22.6) | 96.8  | 0.032 |
| No                               | 237   | 12 (5.1) | 108 (45.6) | 92 (38.8) | 25 (10.5) | 94.9  |       |
| Tumor location                   |       |     |      |      |       |        |       |
| Colon                            | 80    | 2 (2.5) | 39 (48.8) | 30 (37.5) | 9 (11.3) | 97.5  | 0.372 |
| Rectum                           | 202   | 1 (5.4) | 84 (41.6) | 80 (39.6) | 27 (13.4) | 94.6  |       |
| TNM stage                        |       |     |      |      |       |        |       |
| I                                | 73    | 2 (2.7) | 33 (45.2) | 30 (31.1) | 8 (11.0) | 97.3  | 0.863 |
| II                               | 69    | 5 (7.2) | 25 (36.2) | 31 (44.9) | 8 (11.6) | 92.8  |       |
| III                              | 121   | 4 (3.3) | 58 (47.9) | 44 (36.4) | 15 (12.4) | 96.7  |       |
| IV                               | 20    | 2 (10.0) | 7 (35.0) | 6 (30.0) | 5 (25.0) | 90.0  |       |
| Invasive depth                   |       |     |      |      |       |        |       |
| T1–2                             | 86    | 2 (2.3) | 38 (44.2) | 37 (43.0) | 9 (10.5) | 97.7  | 0.653 |
| T3–4                             | 197   | 11 (5.6) | 85 (43.1) | 74 (37.6) | 27 (13.7) | 94.4  |       |
| Lymph node metastasis            |       |     |      |      |       |        |       |
| Positive                         | 135   | 6 (4.4) | 64 (47.4) | 48 (35.6) | 17 (12.6) | 95.6  | 0.552 |
| Negative                         | 148   | 7 (4.7) | 59 (39.9) | 63 (42.6) | 19 (12.8) | 95.3  |       |
| Distant metastasis               |       |     |      |      |       |        |       |
| Positive                         | 20    | 2 (10.0) | 7 (35.0) | 6 (30.0) | 5 (25.0) | 90.0  | 0.997 |
| Negative                         | 263   | 11 (4.2) | 116 (44.1) | 105 (39.9) | 31 (11.8) | 95.8  |       |
| Tumor deposits                   |       |     |      |      |       |        |       |
| Positive                         | 31    | 1 (3.2) | 11 (35.5) | 13 (41.9) | 6 (19.4) | 96.8  | 0.098 |
| Negative                         | 184   | 11 (6.0) | 81 (44.0) | 73 (39.7) | 19 (10.3) | 94.0  |       |
| Perineural invasion              |       |     |      |      |       |        |       |
| Positive                         | 148   | 10 (6.8) | 67 (45.3) | 53 (35.8) | 18 (12.2) | 93.2  | 0.146 |
| Negative                         | 71    | 2 (2.8) | 28 (39.4) | 33 (46.5) | 8 (11.3) | 97.2  |       |
| Lymphatic/venous invasion        |       |     |      |      |       |        |       |
| Positive                         | 65    | 3 (4.6) | 27 (41.5) | 25 (38.5) | 10 (15.4) | 95.4  | 0.729 |
| Negative                         | 218   | 10 (4.6) | 96 (44.0) | 86 (39.4) | 26 (11.9) | 95.4  |       |
| Growth pattern                   |       |     |      |      |       |        |       |
| Infiltrative                     | 163   | 11 (6.7) | 74 (45.4) | 59 (36.2) | 19 (11.7) | 93.3  | 0.085 |
| Cloddy/hested                    | 119   | 2 (1.7) | 49 (41.2) | 51 (42.9) | 17 (14.3) | 98.3  |       |
| Differentiation degree           |       |     |      |      |       |        |       |
| Poor/mucinous                    | 79    | 7 (8.9) | 35 (44.3) | 30 (38.0) | 7 (8.9) | 91.1  | 0.332 |
| Well/moderate                    | 191   | 6 (3.1) | 83 (43.5) | 76 (39.8) | 26 (13.6) | 96.9  |       |
| Maximum diameter (cm)            |       |     |      |      |       |        |       |
| >4                               | 133   | 8 (6.0) | 53 (39.8) | 54 (40.6) | 18 (13.5) | 94.0  | 0.521 |
| ≤4                               | 149   | 5 (3.4) | 69 (46.3) | 57 (38.3) | 18 (12.1) | 96.6  |       |
| Family history                   |       |     |      |      |       |        |       |
| Positive                         | 57    | 2 (3.5) | 26 (45.6) | 21 (36.8) | 8 (14.0) | 96.5  | 0.911 |
| Negative                         | 226   | 11 (4.9) | 97 (42.9) | 90 (39.8) | 28 (12.4) | 95.1  |       |

PR, positive rate. Negative (−), light positive (+), positive (++) , strong positive (+++) staining.

The association of XPA 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
Discussion

XPA, containing a zinc-finger domain, displays a damaged DNA-binding activity, which is essential for assembly of the preincision complex during nucleotide excision repair [11, 23]. It has been reported that XPA exerts regulatory role not only by recognizing the existence of DNA damage, but, along with its interaction partner RPA, also in monitoring proper three-dimensional arrangement of NER complex ahead of activation of endonuclease subunits [3, 24–26]. Considering its important function in NER pathway, XPA is probably implicated in diseases related to imbalance.

Table 4. Correlation between XPA expression and survival in CRC.

| XPA expression | Cases of events | MST | Univariate HR | 95% CI | P     | Multivariate HR | 95% CI | P     |
|----------------|----------------|-----|---------------|--------|-------|-----------------|--------|-------|
| Low (IS < 4.5) | 129            | 46  | 42.25         | 0.62   | 0.39–0.97 | 0.037           | 0.68   | 0.42–1.09 | 0.107 |
| High (IS ≥ 4.5) | 134            | 32  | 47.52         | 0.58   | 0.33–1.01 | 0.055           | 0.48   | 0.26–0.89 | 0.021 |

For stratification:

| Stratification | Cases of events | MST | Univariate HR | 95% CI | P     | Multivariate HR | 95% CI | P     |
|----------------|----------------|-----|---------------|--------|-------|-----------------|--------|-------|
| Age >60        | 63             | 27  | 39.76         | 0.56   | 0.32–0.97 | 0.037           | 0.59   | 0.33–1.05 | 0.072 |
| Age ≤ 60       | 82             | 22  | 45.74         | 0.58   | 0.33–1.01 | 0.055           | 0.48   | 0.26–0.89 | 0.021 |
| Location Rectum Low | 90   | 32  | 43.06         | 0.56   | 0.32–0.97 | 0.037           | 0.59   | 0.33–1.05 | 0.072 |
| Location Rectum High | 96    | 21  | 48.54         | 0.56   | 0.32–0.97 | 0.037           | 0.59   | 0.33–1.05 | 0.072 |
| Location Colon Low  | 39       | 14  | 40.24         | 0.70   | 0.31–1.57 | 0.390           | 0.85   | 0.36–2.02 | 0.710 |
| Location Colon High | 37       | 10  | 45.20         | 0.70   | 0.31–1.57 | 0.390           | 0.85   | 0.36–2.02 | 0.710 |
| Distant metastasis Positive Low | 9  | 7  | 20.44         | 0.58   | 0.19–1.74 | 0.330           | 0.71   | 0.19–2.72 | 0.618 |
| Distant metastasis Positive High | 11  | 7  | 27.18         | 0.58   | 0.19–1.74 | 0.330           | 0.71   | 0.19–2.72 | 0.618 |
| Distant metastasis Negative Low | 120  | 25  | 43.66         | 0.58   | 0.35–0.96 | 0.033           | 0.61   | 0.36–1.04 | 0.072 |
| Distant metastasis Negative High | 123  | 39  | 49.02         | 0.58   | 0.35–0.96 | 0.033           | 0.61   | 0.36–1.04 | 0.072 |
| Tumor deposits Positive Low  | 10       | 6   | 27.90         | 0.89   | 0.32–2.45 | 0.823           | 1.22   | 0.43–3.47 | 0.711 |
| Tumor deposits Positive High | 19   | 10  | 28.47         | 0.89   | 0.32–2.45 | 0.823           | 1.22   | 0.43–3.47 | 0.711 |
| Tumor deposits Negative Low | 88    | 29  | 41.34         | 0.40   | 0.21–0.77 | 0.006           | 0.44   | 0.21–0.92 | 0.028 |
| Tumor deposits Negative High | 85   | 13  | 46.72         | 0.40   | 0.21–0.77 | 0.006           | 0.44   | 0.21–0.92 | 0.028 |

| Max diameter (cm) | Cases of events | MST | Univariate HR | 95% CI | P     | Multivariate HR | 95% CI | P     |
|-------------------|----------------|-----|---------------|--------|-------|-----------------|--------|-------|
| >4                | 58             | 27  | 37.37         | 0.49   | 0.26–0.91 | 0.023           | 0.62   | 0.32–1.18 | 0.143 |
| ≤4                | 61             | 16  | 45.91         | 0.49   | 0.26–0.91 | 0.023           | 0.62   | 0.32–1.18 | 0.143 |

CI, confidence interval; HR, hazard ratio; MST, median survival time. IS, the immunohistochemistry score.
The bold values: P<0.05

HR = 0.44, 95% CI: 0.21–0.92, P = 0.028). Besides, the subgroup with tumor diameter over 4 cm also identified XPA expression as a good indicator for CRC prognosis (HR = 0.49, 95% CI: 0.26–0.91, P = 0.023). However, no significant relation was observed according to the subgroup analysis of TNM stage, invasion depth, lymph node metastasis, growth pattern, differentiation degree, and chemotherapy after initial surgical operation (Table S1). According to the TCGA results, the association between XPA mRNA expression and survival of CRC was not statistically significant (Table S2).
between DNA damage and repair. However, the specific role of XPA in the progression and prognosis in CRC was still ambiguous. In this study including 283 CRC patients in China, we, for the first time, elucidated that DNA repair protein XPA is significantly decreased in colorectal cancer tissues than adjacent nontumor tissues. Univariate Cox proportional hazards model revealed that CRC patients with high XPA protein expression had longer overall survival (OS), but the association was not statistically significant in multivariate analysis. Besides, no significant relation was observed between XPA mRNA expression and survival of CRC according to TCGA results. According to the results of multivariate analysis and TCGA data, we suggested that XPA might be a promising biomarker but might not be an independent factor to predict prognosis of CRC patients.

In the present study, differential expression of XPA between colorectal cancer and nontumor adjacent tissues was explored. We found that XPA protein expression was significantly decreased in CRC tissues compared with non-tumor adjacent tissues. Subgroup analysis suggested

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**Figure 4.** High expression of XPA correlates with the prognosis in CRC patients. (A) Kaplan–Meier analysis and log-rank test for overall survival according to XPA expression level; (B) patients over 60 years of age with high XPA expression exhibited longer survival time than those with low XPA expression; (C) rectal cancer individuals who expressed higher XPA protein demonstrated favorable prognosis; (D) subgroup without distant metastasis also identified XPA expression as a good indicator for CRC prognosis.
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Considering the core position of XPA in NER pathway, we assumed that the down-regulation of XPA in CRC tissues might arise from the impairment of NER capacity upon colorectal carcinogenesis and the low XPA protein expression, which indicates degraded nuclear expression repair in CRC patients, might help create poor prognosis. On the contrary, sufficient NER activity did not benefit cancer patients from the aspect of chemotherapy, because platinum-based chemotherapeutic regimens destroy cancer cells mainly via DNA damage. As the one of the toughest challenges for cancer treatment, chemotherapeutic resistance for platinum has been detected in XPA-overexpressed nasopharyngeal cancer [19]. Whether XPA contributes to CRC chemotherapeutic resistance remains to be clarified in the future. Biomarkers that could predict survival of cancer patients are urgently in need for clinical doctors to make individualized treatment plans and follow-up management. In this study, the cutoff value (4.5) we used was based on our group of patients. More reliable cutoff value should be explored by multiple investigations based on different ethnicities. The obvious relation between XPA protein overexpression and favorable CRC prognosis in our study might provide useful clues for elucidating colorectal development, offering novel idea for effective treatment and improving survival.

Conclusion

In summary, DNA repair protein XPA is significantly decreased in colorectal cancer tissues than adjacent nontumor tissues. High expression of XPA protein showed significant relationship with better survival of CRC, especially rectal cancer. XPA might be a novel biomarker but might not be an independent factor to predict prognosis of CRC patients.

Conflict of Interest

All authors declare that there is no conflict of interest.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1. Correlation between XPA expression and survival in CRC.
Table S2. Correlation between XPA mRNA expression and survival in colon cancer and rectal cancer (based on TCGA).