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Original Article

Expression of FANCD2 is associated with prognosis in patients with nasopharyngeal carcinoma

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Abstract: The relationship between Fanconi anemia complementation group D2 (FANCD2) and early diagnosis, pathogenesis, recurrence, and prognosis in patients with nasopharyngeal carcinoma (NPC) was investigated in a retrospective case-control study. The clinicopathological data of patients with NPC were collected. The results showed that FANCD2 was significantly higher in poorly differentiated squamous cell carcinoma than in moderately and well differentiated carcinoma. FANCD2 was significantly lower in recurrent NPC tissues than in NPC tissues before treatment. FANCD2 was markedly higher in T_{1-2}, stage I-II NPC tissues with a duration of disease shorter than 6 months than in T_{3-4}, stage III-IV NPC tissues with a duration of disease longer than 6 months. Moreover, compared with patients with cervical lymph node metastases, FANCD2 was elevated in tissues from patients without cervical lymph node metastases. Furthermore, the NPC patients in the high-FANCD2-expression group exhibited a higher recurrence rate than the patients in the low-FANCD2-expression group. Finally, the disease-free survival rate of the high-expression group was significantly lower than it was in the low-expression group. Therefore, FANCD2 is associated with the occurrence, differentiation, and cervical lymph node metastasis of NPC. With the development of NPC, FANCD2 is down-regulated. FANCD2 may be a molecular marker for the early diagnosis and prognosis of NPC.

Keywords: Fanconi anemia, head and neck tumor, clinical significance, prognosis, immunohistochemistry, nasopharyngeal carcinoma

Introduction

Nasopharyngeal carcinoma (NPC) is a malignant head and neck tumor that originates from the nasopharynx, and the incidence of NPC depends on region, ethnicity and familial history. Specifically, the incidence of NPC is high in Southern China and in the Southeast Asian countries; statistical data from worldwide cancer registries on the incidence of NPC report 86,700 new cases of NPC and 50,800 deaths from NPC in 2012, which corresponds to the 6th highest incidence rate among all tumors in the developing countries of Southeast Asia. Moreover, the annual incidence rate in males was approximately twice that in females [1]. Because the nasopharynx is difficult to access, NPC is less likely to be detected at an early stage. Thus, patients with NPC often begin treatment during the advanced stages of the disease after the optimal treatment time, which negatively affects their survival and prognosis. Specifically, more than 70% of patients with NPC reportedly presented with metastases in the skull base or cavernous sinus tissues at the time of initial diagnosis, which corresponds to clinical stage III or IV disease [2]. Because NPC tumors are highly sensitive to radiotherapy, this disease is primarily treated with radiotherapy. The current clinical treatment protocols for NPC include radiotherapy alone or the combination of radiotherapy and chemotherapy; surgical resection is mainly used for local relapse and tumor remnants after radiochemotherapy. With the application of new endoscopic technology platforms and the development of radiotherapy over the last decade, many patients with NPC have received timely diagnoses and treatment. However, resistance to chemoradiotherapy and local recurrence remain the major causes of death in patients with advanced NPC. In addition to providing personalized treatments to patients, searching for new therapeutic targets to achieve an early diagnosis and improving the

sensitivity to chemoradiotherapy, survival and quality of life of patients with NPC remain important.

Fanconi anemia (FA) is an autosomal recessive genetic disorder clinically characterized by congenital developmental abnormalities, progressive bone marrow hematopoiesis failure, chromosomal instability and high cancer susceptibility. Previous studies have shown that FA occurs due to the interruption of the FA/BRCA pathway, which results in dysfunctional DNA damage repair in cells. Specifically, 15 FA genetic subtypes have been identified to date (FANCA, B, C, D1, D2, E, F, G, I, J, L, M, N, O, and P). The genes of these genetic subtypes encode related proteins, which interact and form a complex functional network called the FA pathway [3]. Because multiple breast cancer-related genes and proteins (BRCA2, BRCA1, etc.) are involved in this pathway, it is also called the FA/BRCA pathway. As a key protein of the FA/BRCA pathway, the FA complementation group D2 (FANCD2) participates in the following 3 key points in the pathway: the formation of the FA core complex, the monoubiquitination of FANCD2 and FANCI, and the formation of the Fanconi anemia I-Fanconi anemia D2 (ID) complex and FANCD2-I nuclear foci. The FANCD2 gene is located on chromosome 3p25.3 and consists of 44 exons and encodes 2 proteins that both contain 1451 amino acids (FANCD2-S and FANCD2-L). These 2 proteins can interconvert via chemical modification [4]. Moreover, multiple mechanisms are responsible for DNA damage repair in normal human cells. For example, FANCD2, a key protein involved in DNA damage repair in the FA/BRCA pathway, participates in multiple pathways to repair DNA damage, including mismatch repair, homology-directed double-strand break repair and translesion synthesis. When a normal cell responds to DNA damage in the S phase of the cell cycle, the lysine at position 561 of FANCD2 protein is monoubiquitinated, and FANCD2 then co-localizes with repair-related proteins downstream in the FA/BRCA pathway at specific sites in the nucleus to play a role in DNA damage repair [5]. Accordingly, previous studies have demonstrated elevated FANCD2 in proliferating cells [6], and subsequent studies have shown that FANCD2 is abnormally expressed in cancer tissues, including breast cancer and esophageal cancer tissues [7, 8]. In addition, silencing the expression of the FANCD2 gene in multiple cancer cell lines using RNA interference technology significantly increased the sensitivity of cancer cells to mitomycin (MMC) and γ-rays [9]. FANCD2 is important in head and neck cancer, but its expression in this setting has rarely been reported. Moreover, radiotherapy remains the current first-line treatment regimen for NPC, but the relationship between FANCD2 and the clinical prognosis of patients with NPC remains unclear.

This study analyzed the relationship between FANCD2 with the pathogenesis and prognosis of NPC by examining the FANCD2 protein in NPC tissues and considering the clinicopathological and follow-up data from patients with NPC. This analysis served to assess the value of FANCD2 in NPC diagnosis and prognosis.

Materials and methods

Patients and specimens

This study enrolled a total of 112 NPC patients (88 males and 24 females with the mean age of 51 years) who had been newly diagnosed at the Department of Otorhinolaryngology Head and Neck Surgery of the Affiliated Hospital of Southwest Medical University between January 2005 and December 2008. Complete follow-up data were available for all patients. Nasopharyngeal inflammatory tissues from 80 patients (46 males and 34 females with the mean age of 45 years) who were diagnosed with chronic nasopharyngeal inflammation based on hematoxylin-eosin (HE) staining were used as controls.

According to the criteria of the TNM staging system of the American Joint Committee on Cancer (AJCC) for NPC and the NPC pathological classification of the World Health Organization (WHO; 2nd Edition), the TNM stage and clinical stage of the NPC patients was defined. All specimens were classified as squamous cell carcinoma based on histopathology, including 5 well differentiated tumors, 14 moderately differentiated tumors and 93 poorly differentiated tumors. None of the subjects exhibited definite distant metastases at initial diagnosis, and all patients received radical radiotherapy after the diagnosis was confirmed. The start of the follow-up period was defined as the time of discharge from the hospital after completed treatment, and the follow-up period ended in January 2015. The duration of the follow-up period for

Table 1. FANCD2 in NPC and negative nasopharyngeal tissues ($\bar{x} \pm s$)

Groups	N	MOD value of FANCD2 expression	*t* value	*p* value
NPC	112	0.113 ± 0.056	6.717	0.000
Control	80	0.068 ± 0.036		

the NPC patients in this study ranged from 5 to 112 months (median 62.5 months).

All specimens were fixed with 10% formaldehyde and embedded with paraffin, followed by continuous tissue sectioning at 4 μm.

Assessment of FANCD2 in NPC tissues

The concentrations of FANCD2 in the NPC tissues were measured using streptavidin peroxidase-conjugated (SP-conjugated) immunohistochemistry. After deparaffinization, the sections were washed 3 times with phosphate-buffered saline (PBS) and treated with a 3% hydrogen peroxide solution to block endogenous peroxidases. The sections were then placed in a 0.01 M citrate buffer for antigen retrieval, followed by the sequential addition of rabbit anti-human FANCD2 primary antibody (diluted 1:50 in PBS, Santa Cruz Biotechnology), biotinylated secondary antibody and horseradish peroxidase-conjugated anti-streptavidin working solution for DAB development. PBS was used to replace the primary antibody in the negative control, and tonsillar tissue sections were used as the positive control.

The staining of the sections was examined under a microscope, and the cells whose nuclei and/or cytoplasms had been stained brownish yellow were considered positive. The FANCD2 protein was quantitatively assessed using an Olympus imaging system. Specifically, 5 non-overlapping fields of view were photographed per slide. The mean optical density (MOD = SumIOD/SumArea) of the positive region in each photograph was measured using the Image-Pro Plus 6.0 image analysis system, and the average value was taken as the relative content of FANCD2 in that specimen.

Statistical analysis

The statistical analyses were performed using the software SPSS 12.0 for Windows. The MOD value of each group was expressed as the mean ± standard deviation ($\bar{x} \pm s$). The MOD values of FANCD2 in the different groups were compared using a *t*-test for 2 independent samples and a 1-way ANOVA. The Dunnett T3 method was used for pairwise comparisons of the mean values of multiple samples. The local recurrence rate was compared between different groups using the χ^2-test. The Kaplan-Meier method was used for the univariate survival analysis, and a log-rank test was used to assess differences. A multivariate Cox regression model was used to analyze the factors influencing the patients' prognosis. The test significance level was set at $\alpha = 0.05$, and differences were considered significant when $P < 0.05$.

Results

The expression of FANCD2 in NPC tissues

Comparison of the MOD values of FANCD2 expression: The MOD value of FANCD2 in the 112 patients with NPC was 0.113 ± 0.056, but the value in the 80 patients in the control group with nasopharyngeal inflammation was 0.068 ± 0.036. Compared with control group, the MOD value of FANCD2 in the patients with NPC was increased ($t = 6.717$, $P = 0.000$). Specifically, FANCD2 is highly expressed in NPC tissues, and the expression is primarily located in the cytoplasms, but the expression in the nuclei is low (**Table 1**; **Figure 1**).

The expression of FANCD2 in different differentiation level NPC

The MOD value of FANCD2 in poorly differentiated squamous NPC tissues was 0.120 ± 0.058, but the value in the moderately and well differentiated squamous NPC tissues was 0.078 ± 0.023. Compared with the moderately and well differentiated squamous NPC, the MOD value of FANCD2 in the poorly differentiated squamous NPC was also increased ($t = 5.273$, $P = 0.000$). Specifically, the FANCD2 in the poorly differentiated squamous NPC was significantly higher than it was in the moderately and well differentiated squamous NPC (**Table 2**; **Figure 1**).

Comparison of the MOD values of FANCD2 expression in primary NPC foci before and after recurrence

There were a total of 31 patients with NPC who experienced nasopharyngeal relapse during

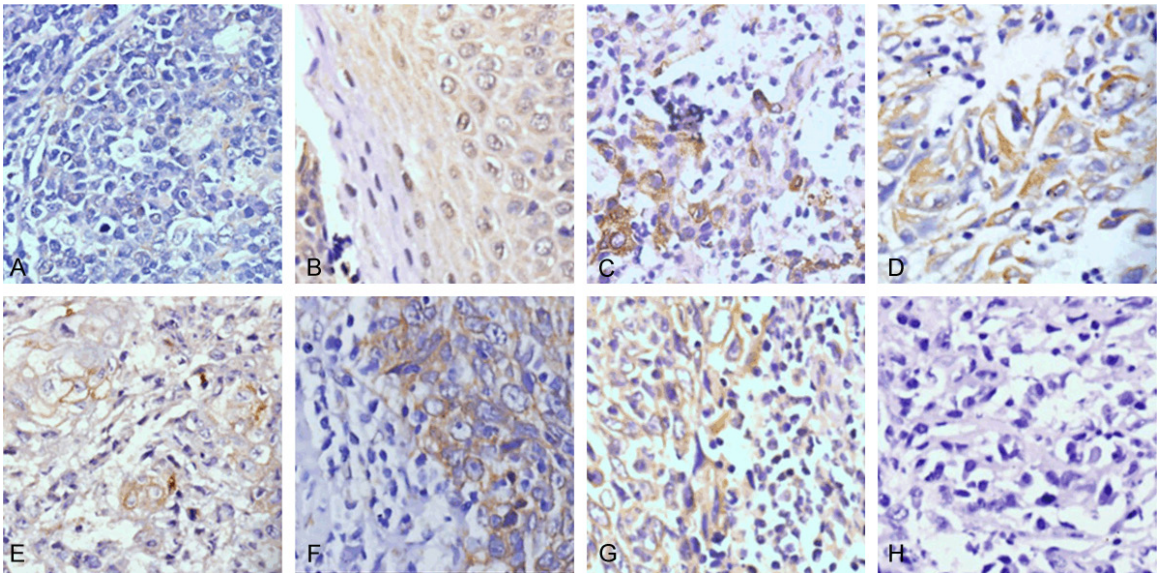

Figure 1. FANCD2 in nasopharyngeal mucosa and carcinoma tissues (SP×400); A: FANCD2 in human germinal center cells of the tonsils (positive control); B: FANCD2 in nasopharyngeal mucosa tissue; C: FANCD2 in poorly differentiated nasopharyngeal squamous cell carcinoma; D: FANCD2 in moderately differentiated nasopharyngeal squamous cell carcinoma; E: FANCD2 in well differentiated nasopharyngeal squamous cell carcinoma; F: FANCD2 in metastatic neck lymph node tissue of recurring NPC; G: FANCD2 in nasopharynx tumor tissue of recurring NPC; H: FANCD2 in nasopharyngeal carcinoma (negative control).

Table 2. FANCD2 in different differentiated NPC tissues ($\bar{x} \pm s$)

Groups	N	MOD value of FANCD2	*t* value	*p* value
Poorly differentiated	93	0.120 ± 0.058	5.273	0.000
Moderately-well differentiated	19	0.078 ± 0.023		

Table 3. FANCD2 in recurring NPC tissues ($\bar{x} \pm s$)

Groups	N	MOD value of FANCD2	*t* value	*p* value
Post-recurrence	31	0.087 ± 0.033	3.972	0.000
First diagnosis	31	0.133 ± 0.055		

the follow-up period. Among these patients, the MOD value of FANCD2 in the nasopharyngeal tumor tissues after recurrence was 0.087 ± 0.033, but the value in the nasopharyngeal tumor tissues upon initial diagnosis was 0.133 ± 0.055. Compared with nasopharyngeal tumors after recurrence, the MOD value of FANCD2 in nasopharyngeal tumor tissues upon the initial diagnosis was also increased ($t = 3.972$, $P = 0.000$). Specifically, FANCD2 in the nasopharyngeal carcinoma tissues was decreased after the recurrence of nasopharyngeal carcinoma (**Table 3**; **Figure 1**).

The relationship between FANCD2 expression and the clinicopathological characteristics of NPC patients

The FANCD2 in the NPC tissues correlated with the disease course, clinical stage, T stage and N stage ($P < 0.05$), but not with the age, gender or the drinking and smoking habits of the patients ($P > 0.05$). FANCD2 was significantly higher in T_{1-2}, stage I-II NPC tissues with a disease duration shorter than 6 months than in T_{3-4}, stage III-IV NPC tissues with a disease duration longer than 6 months. Moreover, compared with the patients with cervical lymph node metastases, the FANCD2 was elevated in the tissues from patients without cervical lymph node metastases (**Table 4**).

Relationship between FANCD2 expression and the clinical prognosis of NPC patients

Follow-up results: The duration of the follow-up period ranged from 5 to 112 months (median 62.5 months). The follow-up results showed that the overall survival rate was 33.0% among all patients in this study, and the 1-year, 3-year, and 5-year survival rates were 84.8%, 61.6% and 51.8%, respectively. The median survival

Table 4. The correlation of FANCD2 with some clinical characteristics ($\bar{x} \pm s$)

Groups	N	MOD value of FANCD2	*t* value	*p* value
Age (years)				
≤ 51	59	0.110 ± 0.053	0.588	0.558
> 51	53	0.116 ± 0.059		
Gender				
Male	88	0.113 ± 0.055	0.092	0.927
Female	24	0.112 ± 0.060		
Tobacco and alcohol habits				
Yes	56	0.118 ± 0.059	1.000	0.320
No	56	0.108 ± 0.052		
Course (months)				
≤ 6	71	0.127 ± 0.058	4.143	0.000
> 6	41	0.088 ± 0.041		
T stage				
T_{1-2}	62	0.128 ± 0.060	3.381	0.001
T_{3-4}	50	0.094 ± 0.044		
N stage				
N_0	26	0.138 ± 0.065	2.331	0.026
N_{1-3}	86	0.105 ± 0.051		
Clinical stage				
I-II	43	0.135 ± 0.060	3.334	0.001
III-IV	69	0.099 ± 0.048		

Table 5. The correlation of FANCD2 with local recurrence

Groups	N	Local recurrence N (%)	No local recurrence N (%)	χ^2 value	*P* value
FANCD2					
High	55	25 (45.5)	30 (54.5)	4.466	0.035
Low	57	15 (26.3)	42 (73.7)		

time was 67 months. The 5-year disease-free survival rate was 46.4%, and the median disease-free survival was 57 months. Among those 112 patients, 47 experienced recurrence, corresponding to a recurrence rate of 42%. Moreover, 40 of 47 patients had local recurrence and 7 had distant metastasis. Among the 40 patients with local recurrence, 28 had a recurrence of nasopharyngeal carcinoma, 9 had a recurrence of cervical cancer, and 3 had a recurrence of nasopharyngeal carcinoma with cervical metastasis.

Relationship between FANCD2 and local recurrence in NPC patients

The patients were divided into the high-FANCD2-expression group (MOD > 0.091194) and the low-FANCD2-expression group (MOD ≤ 0.091194) with the median MOD value (0.091194) of FANCD2 in the NPC tissues. During the follow-up period, among the 55 patients in the high FANCD2 expression group, 25 had local recurrence, with a corresponding local recurrence rate of 45.5%. In addition, 15 of the 57 patients in the low FANCD2 expression group had local recurrence, with a local recurrence rate of 26.3%. Finally, the local recurrence rate of the high expression group was higher than that of the low expression group (**Table 5**).

Effect of FANCD2 expression on the survival rate of NPC patients

The disease-free survival rate of the high expression group was 25.5%, significantly lower than the rate of the low expression group (31.6%, 2 = 4.289, P = 0.038), but there was no significant difference in the overall survival rate between the two groups (2 = 1.390, P = 0.238) (**Figure 2**).

Multivariate analysis of the factors affecting the prognosis of NPC patients

The results showed that T stage, clinical stage, and local recurrence were related to the overall survival rate, but N stage, course of the disease, age, drinking and smoking habits, and initial symptoms did not affect the overall survival rate. In addition, there was a correlation between clinical stage and prognosis (P = 0.000, HR = 4.884, 95% confidence interval: 2.647-9.009). However, FANCD2 expression was not an independent risk factor for the prognosis of nasopharyngeal carcinoma (P = 0.604) (**Table 6**).

Discussion

At present, the significance of the FANCD2 protein in malignant tumor tissues has not yet been conclusively determined. However, the monoubiquitination of the FANCD2 protein and the absence of nuclear foci formation have been reported in multiple squamous cell carcinoma tissues of patients with FA [10]. An examination of FA gene and protein expression in

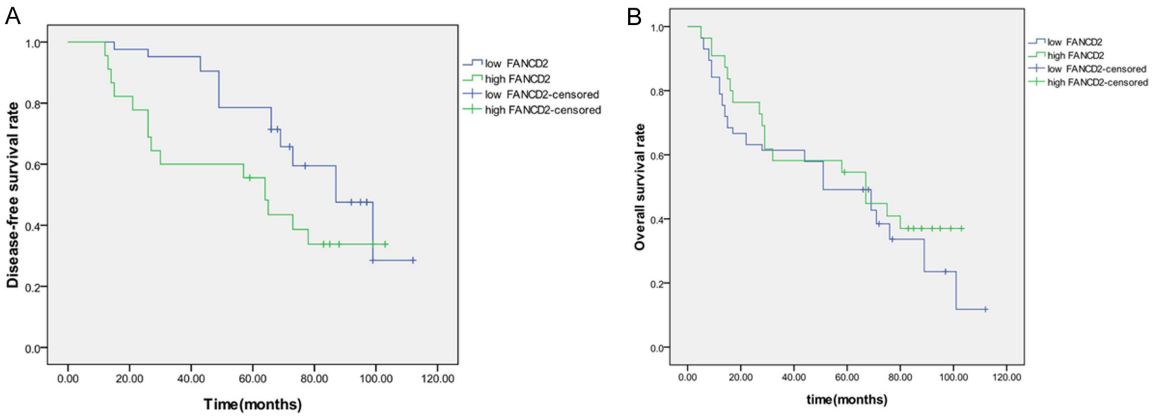

Figure 2. Correlation of FANCD2 with the prognosis of NPC; A: Correlation of FANCD2 expression with the disease-free survival rate; B: Correlation of FANCD2 with the overall survival rate.

Table 6. Multivariate analysis of the prognostic significance

Variable	*P* value	Hazard ratio	95% confidence interval
FANCD2	0.312	1.357	0.750~2.455
Age	0.701	1.121	0.625~2.012
Gender	0.916	0.969	0.539~1.741
Course	0.088	1.708	0.923~3.162
Tobacco and alcohol habits	0.081	1.657	0.940~2.919
First symptom	0.543	0.897	0.631~1.275
Grade	0.613	0.833	0.411~1.691
T stage	0.599	0.795	0.368~1.716
N stage	0.209	0.641	0.321~1.283
Clinical stage	0.000	6.620	2.712~16.159

oral squamous cell carcinoma patients showed significantly elevated FANCD2 genes in the oral squamous cell carcinoma tissues of elderly patients compared with the expressions in the normal control oral mucosa tissues [6]. A comparison of FANCD2 protein in normal breast tissues and breast cancer tissues revealed that the percentage of cells expressing FANCD2 was lower in breast cancer tissues than in normal breast tissues, and the FANCD2 protein was not expressed in some invasive breast cancers [7, 11]. However, compared to normal nervous tissues and benign neurilemmoma tissues, in which FANCD2 expression is almost absent, the FANCD2 protein was significantly upregulated in glioblastoma tissues [12]. A study of the expression levels of multiple repairing proteins in endometrial carcinoma showed that the expression of these repair proteins, including FANCD2, was negative in the normal endometrium but significantly elevated in the endometrial carcinoma tissues [13]. In the present study, the FANCD2 protein was expressed in both NPC and normal nasopharyngeal mucosal tissues, but the expression was significantly higher in the NPC tissues than it was in normal nasopharyngeal mucosal tissues. However, the relationship of differential FANCD2 between tumor tissues and the biological properties of the tumors awaits further research. The location of FANCD2 was determined in this study; FANCD2 was primarily expressed in the cytoplasms of NPC tissues but not in the nuclei. Conversely, researchers from other countries reported that FANCD2 was expressed in both the nuclei and cytoplasms of all benign breast tissues, but the expressions in the nuclei were apparently lost in most malignant breast tumor tissues, and the cytoplasmic expression was confined to early-stage breast tumors [14]. In a normal cell, monoubiquitinated FANCD2 is translocated to the nucleus in response to DNA damage, where it participates in the repair of this damage. The nuclear translocation of FANCD2 may be obstructed in NPC, leading to the accumulation of a large amount of FANCD2 in the cytoplasm. Consequently, any FANCD2 that cannot enter the nucleus will not be able to participate in DNA damage repair, which results in NPC. The retention of FANCD2 in the cytoplasm may be caused by defects in the monoubiquitination of FANCD2. However, these hypotheses need to be confirmed in further studies, and the results of such studies may elucidate the role of FANCD2 in the mechanism of NPC pathogenesis.

The treatment regimens for patients with NPC are determined primarily based on TNM stage, and the clinical pathological data of patients with NPC in this study indicated that FANCD2 was correlated with the disease course, T stage, N stage, and the patients' clinical stage. Specifically, FANCD2 is highly expressed in early-stage NPC, and its expression becomes downregulated as the disease progresses. Accordingly, elevated FANCD2 was found in early-stage breast cancer and endometrial carcinoma in reports from both China and other countries [6, 11]. This variation in FANCD2 may be related to the downregulation of the expression of genes involved in DNA repair. As cancer progresses, DNA repair genes that are active at the early stage are gradually inactivated as their expression is downregulated, leading to a decrease in the expression levels of proteins that are active in transcription or repair during the late stage of the disease. This study also found that FANCD2 was significantly higher in poorly differentiated squamous carcinoma than in moderately and well differentiated squamous carcinoma. To date, the relationship between FANCD2 and tumor differentiation has rarely been reported. Nevertheless, analyses of the FANCD2 protein in glioblastoma and endometrial carcinoma and relevant clinical pathological data revealed a correlation between FANCD2 and tumor stage [12, 13]. The monoubiquitination of FANCD2, a core protein in the FA/BRCA pathway, is the key to its activity, and FANCD2 monoubiquitination is regulated by a variety of proteins, including the FA core complex with ubiquitin ligase activity and the deubiquitinating enzyme ubiquitin-specific protease 1 (USP1) [15-17]. The expression of ubiquitin-conjugating enzyme 2C (UBE2C), an E2 ubiquitin ligase, is markedly increased in poorly differentiated or undifferentiated NPC cell lines [18], but the effect of UBE2C expression on FANCD2 ubiquitination and differential FANCD2 in NPC tissues by differentiation degree remains unclear. Moreover, a correlation between FANCD2 in NPC tissues and the degree of malignancy of tumor tissues has not been confirmed. Thus, further studies are required to address these questions.

Given the advances in radiotherapy, most patients with early-stage NPC have a good prognosis. However, local recurrence and resistance to radiochemotherapy remain the major causes of death in the majority of patients with advanced NPC. Studies of the correlation between the FANCD2 protein and prognosis following breast cancer relapse found that the 1-year postoperative recurrence rate of the high-FANCD2-expression group was significantly higher than that of the low-FANCD2-expression group, but the 3-year disease-free survival rate of the high-expression-group was markedly lower than the rate of the low-expression group [19]. Moreover, a Cox regression survival analysis revealed that FANCD2 overexpression served as an independent adverse prognosis indicator of the overall survival rate in breast cancer patients [7], and an analysis of the correlation between multiple DNA repair proteins and the prognosis of endometrial carcinoma patients revealed that the 5-year disease-free survival and overall survival rates of FANCD2 expression-positive patients were markedly lower than those of the FANCD2 expression-negative patients [13]. However, a study by Alexander [20] reported a shorter time to relapse in breast cancer patients expressing low levels of FANCD2. A more in-depth study found that the head and neck squamous cell carcinoma (HNSCC) cell line FaDu, which exhibits abnormal FNCD2 monoubiquitination, was more sensitive to MMC than HNSCC cell lines in which the FA pathway is normal [21]. Accordingly, silencing the FANCD2 gene in multiple cancer cell lines via an RNA interference technique showed that the inhibition of the FANCD2 gene significantly enhanced the sensitivity of cancer cells to MMC and γ-rays, and the ability of cancer cells to relapse was significantly reduced when FANCD2 was lost [9]. This study examined the correlation between the FANCD2 expression level and local recurrence and disease-free survival rates in patients with NPC and found that the local recurrence rate of the high-FANCD2-expression group was higher than the rate of the low-expression group. Furthermore, the disease-free survival rate of the high-expression group was lower than the rate of the low-expression group. The increased risk of cancer recurrence caused by the high expression of the FANCD2 protein was hypothesized to be related to the resistance to chemoradiotherapy associated with high FANCD2. In response to the DNA damage caused by radiochemotherapy, DNA repair proteins, such as FANCD2, are upregulated and actively participate in the repair of DNA damage in cancer cells, leading to resistance to radiochemotherapy and a consequently increased risk for can-

cer relapse. A study of sensitivity to radiochemotherapy in patients with early-stage cervical cancer found that the radiochemotherapy-resistant group exhibited an elevated FANCD2 protein compared with the radiochemotherapy-sensitive group [22]. This finding also supported the above hypothesis and indicated that the overexpression of FANCD2 can serve as a useful indicator of adverse prognosis in NPC patients. Furthermore, inhibiting FANCD2 in cancer may represent a new treatment paradigm. The mTOR pathway, as an important classical signaling pathway, is involved in tumor pathogenesis and was found to participate in the repair of double-strand DNA breaks and response to DNA damage by regulating FANCD2 [23]. Further studies demonstrated that mTOR kinase inhibitors enhanced the sensitivity of rhabdomyosarcoma to radiotherapy by inhibiting FANCD2 [24]. Celastrol is a natural compound that inhibits the chemotherapy drug cisplatin and was found to inhibit FANCD2 monoubiquitination induced by DNA damage via the ubiquitin-proteasome pathway, which results in the degradation of FANCD2 and interferes with normal DNA damage repair [25]. Related NPC studies, in which the first-line treatment is radiotherapy, have not been reported, and further studies are needed to elucidate the mechanism underlying the relationship between FANCD2 and radiotherapy sensitivity to provide a new direction for the treatment of advanced NPC. In addition, this study showed that in the 31 patients with NPC who exhibited local relapse, FANCD2 was significantly lower in the relapsed NPC tissues than it was before treatment, but the mean FANCD2 level in the NPC tissues in these patients before treatment (0.133 ± 0.055) was significantly higher than the median MOD value for FANCD2 in the NPC tissues of all the patients (0.091194). Moreover, most patients with relapse were in the high-FANCD2-expression group, which further suggests that FANCD2 directly correlates with local recurrence. However, further studies are needed to investigate the relationship between radiotherapy and the significant reduction in FANCD2 in NPC tissues after relapse as well as the mechanisms underlying this reduction.

This study showed that the FANCD2 was dominant in the cytoplasms of cells in human NPC tissues. FANCD2 is related to the course of disease, T stage, N stage and clinical stage, and may be an early event in the pathogenesis of NPC. The intensity of FANCD2 is related to the NPC local recurrence rate and the disease-free survival rate, and its high expression can be used as a useful indicator of the poor prognosis of patients with NPC. Taken as a target, inhibiting its expression in tumors can bring new ideas for the treatment of tumors.

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Disclosure of conflict of interest

None.

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