RESEARCH ARTICLE

FEN1 Status and Its Correlation with Clinicopathologic Characteristic in Colorectal Cancer

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Abstract: Objective: The goal of this study was to investigate the status of FEN1 in colorectal cancer (CRC) and determine the potential correlation between FEN1 expression level and clinicopathological parameters in CRC patients.

Methods: Expression of FEN1 in CRC tissue on tissue microarray was detected using immunohistochemistry (IHC). The relationship between FEN1 expression status and clinicopathological characteristics of CRC was analyzed by the Chi-square test. The survival data of TCGA Colon Cancer (COAD) were obtained from ucsc xena browser (https://xenabrowser.net/). Patients were separated into higher and lower expression groups by median FEN1 expression. The association with prognosis of CRC patients was determined by Kaplan-Meier survival analysis with Log-rank test.

Results: FEN1 expression level and cellular localization had wide variability among different individuals; we classified the staining results into four types: both positive in nucleus and cytoplasm, both negative in nucleus and cytoplasm, only positive in the nucleus, only positive in the cytoplasm. Moreover, FEN1 expression status only correlated with patients’ metastasis status, and the patients in the NLCL group showed more risk of cancer cell metastasis.

Conclusion: Our results indicate that FEN1 expression level and cellular localization had wide variability in CRC and is not a promising biomarker in CRC.

Keywords: Colorectal cancer, FEN1, Clinicopathologic characteristic, Prognosis.

1. INTRODUCTION

Colorectal cancer (CRC) was rather rare in 1950 but has become the second-most and third-most common cancer in women and men in the world, respectively[1-4]. Based on the data of global cancer statistics in 2018 by Bray’s group, CRC accounts for approximately 10% of all annually diagnosed cancers and cancer-related deaths worldwide[5].

Biomarkers play important roles in the detection, treatment, and risk stratification of patients with CRC[6-12]. Among them, mutation of P53 gene, KRAS gene and BRAF gene, expression of PTEN gene and ezrin protein, chromosome 18q loss of heterozygosity (18qLOH), and microsatellite instability (MSI) have been shown to be prognostic factors for CRC patients[2, 13-20]. However, these biomarkers are too few in number to establish a complete risk stratification system; more biomarkers with prognostic values are still needed to assess the outcome of CRC patients.

Human flap endonuclease-1 (FEN1) is a structure-specific DNA nuclease[21, 22]. It is essential in Okazaki fragment maturation during DNA replication and DNA repair pathways like base excision repair (BER) and polymerase α error editing[23-26]. In human tumors,
overexpression of FEN1 has been widely reported, and the expression level of FEN1 has prognostic value in breast cancer, pancreatic cancer, ovarian cancer, and lung cancer[27-37]. However, the expression status and prognostic value of FEN1 in CRC are still unclear. In the present study, we analyzed the expression level and cellular localization of FEN1 in CRC tissue and then evaluated the correlations with clinicopathologic characteristics and prognosis.

2. MATERIALS AND METHODS

2.1. Patients and Samples

One hundred and eighty-three patients were selected from January 2003 to December 2007 with primary CRC and no evidence of malignancy in other organs, with the mean age of 62.82±12.45. All patients underwent curative resection before radiotherapy and chemotherapy, and all the paraffin specimens were confirmed diagnosis by two pathologists. All patients’ care and experimental procedures were approved by the committee on patient care and the committee on the ethic of Suzhou municipal hospital. All methods were performed in accordance with the relevant guidelines and regulations. All patients had signed the informed consent form.

2.2. Immunohistochemistry

The CRC tissues were fixed by 10% neutral formalin and were embedded with paraffin. Each sample contained two specimens from the center and periphery of the tumor tissues and with a diameter of 1.6 mm. After stained with H&E, the tumor tissue localizations were labeled via microscopy. FEN1 primary antibody (GTX70185) was purchased from Genetex company.

2.3. Evaluation of Immune Staining

The immunohistochemical result of FEN1, was detected by a pathologist who was blinded to the clinicopathological characteristics of patients. Positive expression of FEN1 was sub-located in the cytoplasm and nucleus, respectively. The whole field of inspection of the sample was scored, and the intensity of staining was grouped as follows: “-” stands for no staining, “+” stands for weak positive staining, “++” stands for moderately positive staining, “+++” stands for strong positive staining; “low” stands for the sum of no staining and weak positive staining samples, “high” stands for the sum of moderately positive staining and strong positive staining samples.

2.4. Statistical Analysis

The relationship between FEN1 expression status and clinicopathologic characteristics of CRC was analyzed by Chi-square test and Fisher's Exact Test. The survival data of TCGA Colon Cancer (COAD) were obtained from ucsc xena browser (https://xenabrowser.net/). Patients were separated into higher and lower expression groups by median FEN1 expression. By Kaplan-Meier survival analysis, Log-rank p-value < 0.05 was considered to be significantly associated with the prognosis of patients. All tests were two-sided, and a p-value of 0.05 was considered statistical significance. All analyses were performed using SAS 9.4 and R (3.6.0).

3. RESULTS

3.1. The Heterogeneity of FEN1 Expression Level and Cellular Localization in CRC

Immunohistochemistry results showed that FEN1 expression level and cellular localization had wide variability among different individuals. In order to clarify the heterogeneity of FEN1 expression in CRC, we classified the staining results of 183 patients into four types: both positive in nucleus and cytoplasm, both negative in nucleus and cytoplasm, only positive in the nucleus, only positive in the cytoplasm (Fig. 1). Furthermore, based on the expression level, we sub-divided the only positive in nucleus type and only positive in cytoplasm type into six sub-types (Fig. 1). From the statistical data of FEN1 expression level (Fig. 2), there were only 91 patients (91/183), and 79 patients (79/183) respectively showed the same FEN1 expression staining level in cell cytoplasm and nucleus between the center and periphery of tumor tissue, which indicated that the heterogeneity expression of FEN1 in a patient sample is quite common. Surprisingly, 6 patients showed no FEN1 staining in cytoplasm, nor in the nucleus, 37 patients showed FEN1 staining only in the cytoplasm, and 24 patients showed FEN1 staining only in the nucleus (patients with the center and periphery of tumor tissue were both positively expressed FEN1), which suggests that FEN1 is probably not essential in CRC cell survival and DNA proliferation.

3.2. FEN1 expression Status and its Association with Clinicopathologic Characteristics of CRC

To investigate the correlation between expression status of FEN1 and clinicopathologic characteristics of CRC, FEN1 expression status was divided into 4 groups, NLCL: low expression in nucleus and low expression in the cytoplasm, NLCH: low expression in nucleus and high expression in the cytoplasm, NHCL: high expression in nucleus and low expression in the cytoplasm, NHCH: high expression in nucleus and high expression in the cytoplasm. The results showed that FEN1 expression status only correlated with patient’s metastasis status, and the patients in the NLCL group showed more risk of cancer cell metastasis (Table 1).

3.3. FEN1 Expression Level is Not a Good Clinical Prognostic Factor in CRC

In order to determine the relationship between the expression level of FEN1 and prognosis of CRC, we downloaded the data of CRC patients from ucsc xena browser database and performed a Kaplan-Meier analysis. Based on the analysis, patients with high FEN1 expression level showed better overall survival (OS); however, the difference was not statistically significant; The disease-free survival (DFS) results showed patients with low FEN1 expression level had better DFS; again, the difference was not statistically significant (Fig. 3).
Fig. (1). Representative microphotographs of FEN1 expression status in CRC tissue (magnification x200).

Fig. (2). FEN1 expression level in nucleus and cytoplasm of CRC patient cells in replicated specimens. “0” stands for no staining, “1” stands for weak positive staining, “2” stands for moderately positive staining, “3” stands for strong positive staining; Numbers in blue shadow showed the expression level in the nucleus in replicated specimens, numbers in green shadow showed the expression level in the cytoplasm in replicated specimens, numbers with white background stands for patient numbers; 00-03, 10-13, 20-23, 30-33 stands for the staining results of the center or periphery of each tumor tissue.
Table 1. FEN1 expression status and its association with clinicopathologic characteristics of CRC. TNM stands for Tumor, Node, Metastasis; T describes the size of cancer, N describes whether cancer has spread to the lymph nodes, M describes whether cancer has spread to a different part of the body, the numbers stand for the amount of tumor progress.

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*The p-value was calculated by Fisher's exact test.

Fig. (3). Kaplan-Meier analysis of OS and DFS of CRC patients based on the FEN1 expression level. A. Kaplan-Meier analysis of OS of CRC patients based on the FEN1 expression level. B. Kaplan-Meier analysis of DFS of CRC patients based on the FEN1 expression level.
4. DISCUSSION

Our present study showed that FEN1 expression level and cellular localization had wide variability among different individuals, and some patients showed no FEN1 staining in the cytoplasm or expressed only in the cytoplasm. We further determined that FEN1 expression level had no correlation with prognosis and clinicopathologic characteristics of CRC, except for patient’s metastasis status. Our results indicate that FEN1 expression level is not a good clinical prognostic factor in CRC.

FEN1 is well known in DNA metabolism, including DNA replication and DNA repair pathways, and these processes mainly conduct in the nucleus. However, our immunohistochemistry results showed nonstaining or only cytoplasm staining of FEN1 in CRC tissues, similar to previous results in breast and ovarian cancer tissues[29], indicate that FEN1 may have a backup in the DNA replication process in types of cancer cells.

It was shown that FEN1 is overexpressed in various types of cancer and the expression levels of FEN1 have the potential as a valuable biomarker for cancer diagnosis and prognosis. However, in CRC, Saffi’s group analyzed the FEN1 expression level of 72 patients and failed to determine the relationship between expression level and clinicopathologic characteristics or prognosis[38]. Our study showed FEN1 expression status only correlated with patient’s metastasis status; the patients in the NLCL group showed more risk of cancer cell metastasis. Moreover, FEN1 expression status didn’t show a significant correlation with the prognosis; actually, the correlation analysis results of FEN1 expression status with OS and DFS seems paradoxical, which need to be explored and analyzed further in the future.

AUTHORS’ CONTRIBUTIONS

Song-Bai Liu, Yundi Guo., Zixuan Du., designed and performed research, analyzed the data, and wrote the manuscript; Yuanshuai Zhou, Haijun Sun, Rui Liang, Min-Xuan Sun, Taixiang Tang, performed research, analyzed the data, and reviewed the manuscript.

CONCLUSION

In summary, our results indicate that FEN1 expression level and cellular localization had wide variability in CRC and is not a promising biomarker in CRC.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES


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