·Basic Research·

Expression of TEIF protein in colorectal tumors and its correlation with centrosome abnormalities

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[Abstract] Background and Objective: The telomerase transcriptional elements-interacting factor (TEIF) gene is a transcription factor of a kind of regulation telomere enzyme hTERT gene expression found recently in our research group and expressed in many kinds of tumor tissue. This study evaluated the expression of the TEIF protein in human colorectal tumors and explored its correlation to centrosome abnormalities. **Methods:** The expression of TEIF in 10 specimens of normal intestinal mucosa tissue, 30 specimens of colorectal cancer, and 54 specimens of colorectal adenoma were detected by immunohistochemistry. The expression of γ-tubulin was detected by immunofluorescence. **Results:** Immunohistochemistry showed that the difference of TEIF protein expression between the normal group and each tumor group was statistically significant (P<0.01), and the difference of TEIF protein expression between the malignant tumor group and the benign group was not significant (P>0.05). TEIF strong positive rate (≥ + +) was significantly higher in the carcinoma group than in either the adenoma group or the normal group (all P<0.001). The difference of the TEIF strong positive rate between Grade Ⅰ adenoma and Grades Ⅱ or Ⅲ adenoma was significant (P<0.05), and the difference of the TEIF strong positive rate between Grade Ⅱ adenoma and Grade Ⅲ adenoma was not significant (P>0.05). Immunofluorescence results showed that the positive rate of centrosome amplification was significantly higher in the colorectal cancer group than in either the normal group or the adenoma group (both P<0.01). The difference of the centrosome amplification positive rate between Grade Ⅰ and Grade Ⅲ adenoma was statistically significant (P<0.05), and the difference of the positive rate of centrosome amplification between Grade Ⅱ adenoma and Grades Ⅰ or Ⅲ adenoma was statistically significant (P> 0.05). **Conclusions:** The TEIF protein and centrosome amplification is commonly found in colorectal tumors. The expression level is related to the histologic grade of the tumor and the degree of malignancy. TEIF protein expression and centrosome amplification showed a high degree of positive correlation.

Key words: colorectal neoplasm, telomerase transcriptional elements-interacting factor, gene expression, centrosome

Colorectal cancer is the second most common cancer among all gastrointestinal tumors. As the understanding at molecular level on the pathogenic mechanisms of colorectal cancers has become more in-depth in recent years, many genes, such as adenomatous polyposis coli (APC), P53, and K-ras, jointly regulate multiple biologic processes at the molecular level during the development of colorectal cancer, including cell proliferation and apoptosis, leading to tumorigenesis.[1,2]

The centrosome is also known as the microtubule organizing center (MTOC). Research has shown that almost all human aneuploid tumors have centrosome abnormalities.[3]

The telomerase transcriptional elements-interacting factor (TEIF) gene is a transcription factor to regulate the expression of human telomerase reverse transcriptase (hTERT), a gene previously cloned by our laboratory.[4] Research showed that TEIF was highly expressed in various tumor tissues and correlated with either the histological grade or the malignancy of tumors (eg, sarcoma).[5] A recent study found that TEIF was located in the centrosome,[6] and abnormalities of centrosomes could directly result in tumorigenesis. These findings suggest that TEIF is closely related to the initiation of human tumors. In addition, telomere loss and DNA damage could cause TEIF to move toward centrosomes and lead to centrosome amplification. However, results on colorectal tumor tissue have not yet been reported.

In this study, we produced a specific polyclonal antibody to detect the expression of TEIF in human colorectal cancer tissue

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This paper was translated from Chinese into English by Beijing Xinglin Medtrans Center and edited by Hope J. Lafferty on 2009-09-30.

The Chinese version of this paper is avaiable at http://www.cjcsysu.cn/cn/article.asp?id=16061.

Submitted: 2009-03-31; Revised: 2009-06-25

(including colorectal cancer and colorectal adenomas), and tested centrosome expression in colorectal cancer through immunofluorescence, aiming to explore the correlation between TEIF-gene expression in colorectal tumors and changes in centrosomes.

Materials and Methods

Source of tissue specimens and general case information

This study used a retrospective research method. Specimens from surgical excision or biopsy were archived in paraffin blocks at Beijing Shijitan Hospital from 2005 to 2008, for which pathological diagnosis and immunohistochemical identifications were performed according to the 2002 World Health Organization (WHO) classification method for colorectal cancer. A total of 30 colorectal cancer specimens from 16 men and 14 women, aged 43 years to 85 years, with a median age of 63 years. Of the 30 cases of colorectal cancer, 5 were well-differentiated, 17 moderately differentiated, and 8 poorly differentiated. Various grades of colorectal adenomas were included in our study. A total of 18 specimens of Grade I colorectal adenomas (all tubular adenomas) from 13 men and 5 women, aged 47 years to 83 years, with a median age of 67.5 years; 17 Grade II colorectal adenomas (including 3 tubular adenomas, 1 villous adenoma, and 13 villous tubular adenomas) from 9 men and 8 women, aged 52 years to 82 years, with a median age of 68 years; 19 Grade III colorectal adenomas (including 2 tubular adenomas, 3 villous adenomas, and 14 villous tubular adenomas) from 10 men and 9 women, aged 27 years to 84 years, with a median age of 70 years; and 6 specimens of adenomas with local malignant transformation. In addition, 10 specimens of normal mucosal tissue from 6 men and 4 women, aged 47 years to 77 years, with a median age of 74 years, were used as controls.

Preparation of immunized serum

According to a published method,[6] purified fusion TEIF protein was used to immunize male New Zealand white rabbits. The initial immunization was done using an adequately emulsified mixture of 1 mg fusion protein and an equal volume of complete Freund's adjuvant to subcutaneously inject the rabbits at multiple sites. The first booster immunization was administered 14 days after the initial immunization using an emulsified mixture of 1 mg fusion protein and an equal volume of incomplete Freund's adjuvant, in the same injection sites. After another 14 days, booster immunizations of the same dose and at the same injection sites were repeated. At 56 days after the initial immunization, blood was drawn from the carotid artery, and the serum was separated, aliquoted, and frozen at -20℃. Serum from the auricular vein was taken before immunization and served as the negative control.

Western blot analysis

After blocking with nonfat milk, the nitrocellulose membrane was incubated in a prepared rabbit serum containing the first antibody (1∶2 000), 37℃ for 1 h, washed by 1 × phosphate buffer, and incubated with biotinylated goat anti-rabbit IgG (1:500) at room temperature for 1 h. Then streptavidin-alkaline phosphatase (1:500) was added to the conjugate at room temperature for 1 h and color was developed using NBT-BCIP. Rabbit serum before immunization was included as the negative control.

Immunohistochemistry

Tissue slides were processed sequentially by xylene dewaxing, graded ethanol hydration, 0.3% hydrogen peroxide treatment at room temperature for 1 h, and citric acid buffer antigen repair in a 95℃ microwave for 20 min. Then the slides were blocked by bovine serum albumin (2%) at room temperature for 1 h, an anti-TEIF serum (1:1500) was added at room temperature for 1 h. The slides were then washed and the EnVision anti-rabbit antibody (DAKO company) was added at room temperature for 30 min. Color was developed by DAB, counterstained by hematoxylin, and mounted using Balsam neutral. The negative control of rabbit serum before immunization was incubated by 1× PBS instead of the first antibody.

To determine the result in the negative control group, partial positive TEIF staining was possibly the colorectal glandular cell nucleus stained brown. Positive staining intensity of the tissue was graded according to the ratio of stained tumor cells to total cells: < 5% negative or positive cells were graded -; 5% –50% positive cells with moderate intensity were graded +; >50% but ⩽80% positive cells with strong staining intensity were graded ++; and > 80% positive cells with ultra-strong staining intensity were graded +++.

Immunofluorescence staining

Tissue slides were processed sequentially by xylene dewaxing, graded ethanol hydration, 0.3% hydrogen peroxide treatment at room temperature for 1 h, and citric acid buffer antigen repair in a 95℃ microwave for 20 min. Then the slides were blocked by bovine serum albumin (2%) at room temperature for 1 h, and anti-γ-tubulin first antibody (1:2000, Santa Cruz company) was added at room temperature for 1 h. The slides were then washed, and TRITC labeled anti-goat IgG secondary antibody (ZhongShan company) was added at room temperature for 30 min. The nuclei were counterstained by DAPI, and mounted by glycerol. The negative control of rabbit serum before immunization was incubated by 1 × PBS instead of the first antibody.

To determine the results, the slides were observed under RHOD and UV channels of a fluorescence microscope and the negative control showed no positive staining. Samples with centrosome amplification showed 2 or more red fluorescent dots or blocks around the nucleus.

Statistical analysis

Data was analyzed by SPSS version 13.0. Differences in TEIF expression and centrosome abnormalities among normal colorectal mucosa tissues, adenomas, and colorectal cancers were detected using a 2 × 2 contingency table-χ^2 test. The correlation between abnormal TEIF expression and centrosome amplification abnormalities was evaluated using correlation analysis. P<0.05 was considered significant.

Results

Expression of TEIF protein in colorectal cancer

Immunohistochemical staining that was positive for TEIF showed as nuclear staining; perinuclear staining was also seen in some cases. Most slides showed a diffuse distribution of positive tumor cells (Fig. 1). Immunohistochemical results are summarized in Table 1. There was a significant difference in the positive rate of TEIF protein between normal mucosa tissue and each group of tumors (all $P<0.001$), while no significant difference was detected between malignant tumors and benign tumors ($P>0.05$).

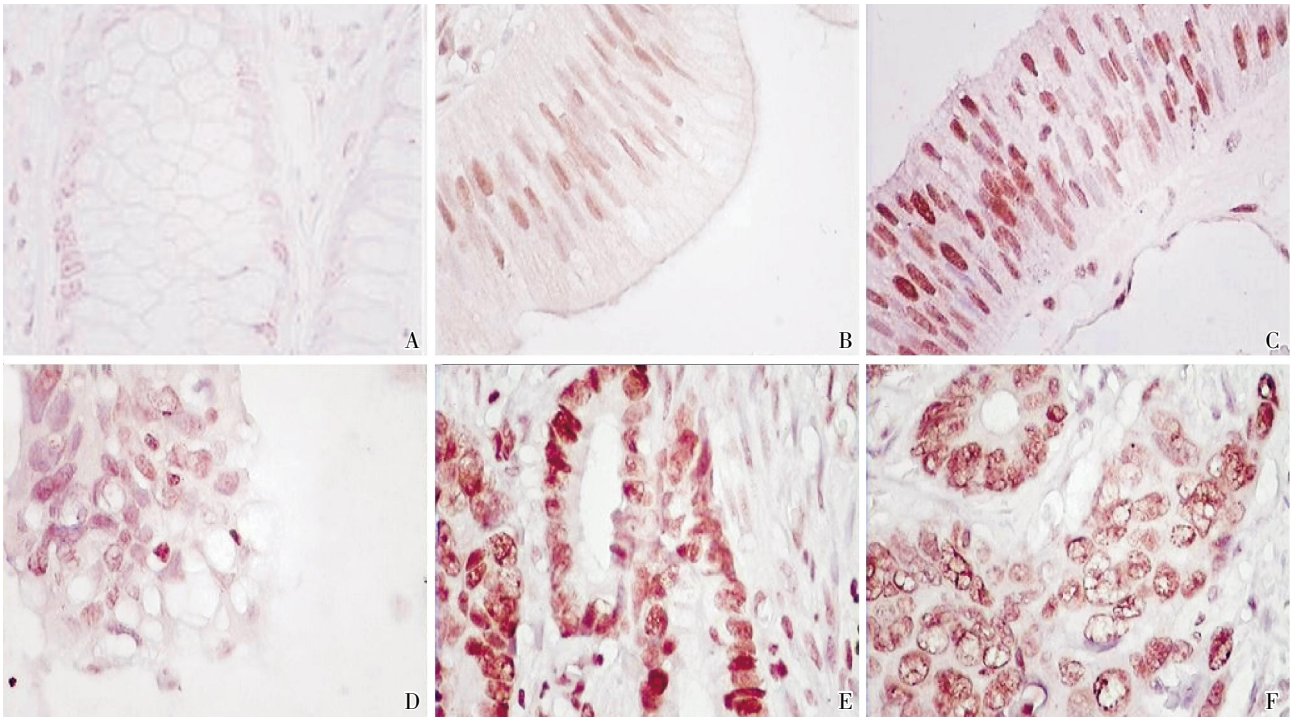

Figure 1 Expression of telomerase transcriptional elements-interacting factor (TEIF) in human colorectal tumor tissues (EnVision ×200)

Positive expression located in the nuclei is brown. A, adenoma of Grade 1 (+); B, adenoma of Grade 2 (++); C, adenoma of Grade 3 (+++); D, well-differentiated adenocarcinoma(+++); E, moderately-differentiated adenocarcinoma (+++); F, poorly-differentiated adenocarcinoma (+++).

Correlation between TEIF protein expression and types of colorectal cancer

The range of intensity of positive expression of the TEIF protein in cancerous tissue and various grades of adenoma are shown in Table 1. There was a significant difference in the expression of the TEIF protein between the normal, the colorectal adenomas, and the colorectal cancer groups (all $P<0.01$). The TEIF-positive rates of different cancer tissues were all significantly

Table 1 Positive rates and levels of transcriptional elements-interacting factor (TEIF) expression in various types of colorectal tissues

Group	Type	Number	TEIF [number (%)]			
			–	+	++	+++
Total		30	0 (0.0)	0 (0.0)	10(33.3)	20(66.7)
Colorectal cancer	Well-differentiated adenocarcinoma	5	0 (0.0)	0 (0.0)	4(80.0)	1(20.0)
	Moderately-differentiated adenocarcinoma	17	0 (0.0)	0 (0.0)	5(29.4)	12(70.6)
	Poorly-differentiated adenocarcinoma	8	0 (0.0)	0 (0.0)	1(12.5)	7(87.5)
Adenoma	Grade 1	18	1 (5.6)	15(83.3)	2(11.1)	0 (0.0)
	Grade 2	17	0 (0.0)	4(23.5)	10(58.8)	3(17.6)
	Grade 3	19	0 (0.0)	1 (5.3)	10(52.6)	8(42.1)
Normal tissue	Normal intestinal mucosa	10	9(90.0)	1(10.0)	0 (0.0)	0 (0.0)

higher than those of the various grades of adenoma (all $P<0.01$). Significant differences were detected between Grade I adenoma and Grades II or III adenoma ($P<0.05$), but not between Grade III and Grade II adenoma ($P>0.05$).

Strong expression of the TEIF protein in colorectal cancer showed that well-differentiated adenocarcinoma was significantly different from both moderately differentiated and poorly differentiated adenocarcinomas (all $P<0.05$), while no statistical difference was detected between the latter two groups ($P>0.05$).

Detection of centrosome amplification

Under a fluorescence microscope, red fluorescent dots could be seen around the cell nucleus (Fig. 2). In colorectal carcinoma tissue, more than 2 red fluorescent dots or blocks could be detected around the nucleus in the majority of tumor cells, indicating the abnormal expression of γ-tubulin protein in these cells. That is, abnormal centrosome amplification was present in these cells. Immunofluorescence staining results are summarized in Table 2. The positive rate of centrosomal amplification in the colorectal cancer group was significantly higher than that of the normal mucosa and adenoma groups (all $P<0.01$). There was a significant difference between Grade I adenoma and Grade III adenoma ($P<0.05$), while no statistical differences were detected between either Grade I and Grade II adenomas or Grade II and Grade III adenomas ($P>0.05$).

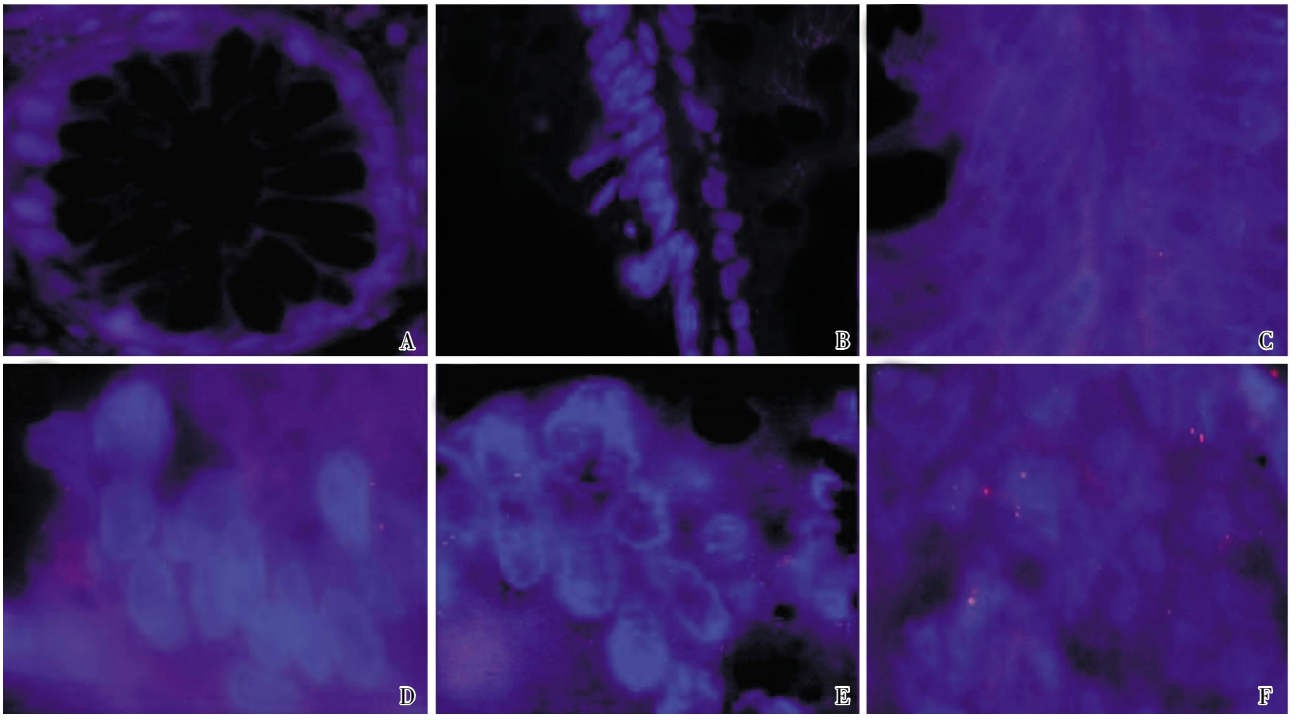

Figure 2 Expression of γ-tubulin in human colorectal tumor tissues (immunofluorescence ×1 000)

Positive expression of γ-tubulin is shown as two or more red fluorescent particles around the nuclei. A, adenoma of Grade 1 (-); B, adenoma of Grade 2 (-); C, adenoma of Grade 3(+); D, well-differentiated adenocarcinoma(+); E, moderately-differentiated adenocarcinoma(+); F, poorly-differentiated adenocarcinoma (+).

Table 2 Centrosome amplification in colorectal tissues

Group	Type	Number	Centrosome amplification	
			Positive [number(%)]	Negative (number)
Total		30	30(100.0)	0
Colorectal cancer	Well-differentiated	5	4(100.0)	0
	Moderately-differentiated	17	14(100.0)	0
	Poorly-differentiated	8	7(100.0)	0
Adenoma	Grade 1	18	8 (44.4)	10
	Grade 2	17	12 (70.6)	5
	Grade 3	19	15 (79.0)	4
Normal tissue	Normal intestinal mucosa	10	0 (0.0)	10

Correlation between centrosome amplification and types of colorectal cancer

In the colorectal cancer group, the positive rate of centrosomal amplification was 100.0% (5/5) in well-differentiated cancer tissue, while the strong positive rate of TEIF expression was 0% (0/5). In moderately differentiated colorectal cancer tissue, the γ-tubulin-positive rate was 100% (17/17), and the strong positive rate of TEIF expression was 76.5% (13/17) with an ultra-strong positive rate of 29.4% (5/17). In poorly differentiated colorectal cancer tissue, the γ-tubulin-positive rate was 100% (8/8) and the ultra-strong TEIF positive rate was 87.5% (7/8). Clearly, in less differentiated tumor tissue, it is more likely to see an increased rate of centrosome amplification.

Correlation between TEIF protein expression level and abnormal centrosome amplification

TEIF protein and centrosomal amplification showed a significant linear correlation (r = 0.628, P<0.001) (Table 3).

Table 3 Correlation of TEIF expression levels with centrosomal amplification in colorectal tissues (number)

TEIF expression	Centrosome amplification		Total
	−	+	
−	10	0	10
+	21	0	21
++	25	7	32
+++	7	24	31
Total	63	31	94

Discussion

PTEIF is a transcription factor that regulates the expression of the newly cloned hTERT gene, which activates endogenous hTERT mRNA expression and regulates its telomerase activity through specific binding to upstream regulatory regions of hTERT. Our previous studies showed that TEIF gene expression was consistent with the activity of telomerase, and a reduction in TEIF expression could significantly inhibit cancer cell growth and its malignant phenotype.[4-7] Our study detected a high expression rate of the TEIF protein in colorectal cancer tissue. Considering our previous findings, it is likely that the abnormal expression of TEIF, which is associated with the activation of telomerase during the development of colorectal cancer or the biologic characteristics of colorectal cancer, appears at the early stage of development of benign colorectal lesions. Studies have shown that the C-terminus of the TEIF protein is located at the centrosome, suggesting that TEIF is not only involved in the regulation of telomerase activity, but its gene product may also serve as an important regulatory protein of centrosomes to participate in regulating the centrosome responses induced by the abnormal telomere.[8]

Our study showed that the TEIF protein in colorectal cancer expressed at a level significantly higher than in colorectal adenoma of all grades or in normal mucosal tissue. In addition, the expression of the TEIF protein in Grade III colorectal adenoma with a local malignant transformation was significantly higher than in Grade I adenoma, suggesting that the TEIF protein might be a marker for the malignant transformation in the development of colorectal cancer. Therefore, TEIF protein expression could be used as a marker for the differential diagnosis of colorectal tumors, especially for detecting the malignant transformation of colorectal adenomas. This study tested the expression of the TEIF protein in colorectal cancer tissue and preliminarily confirmed its high expression therein, which further suggests the possibility of using this protein as a therapeutic target for colorectal cancer.

Recent studies have detected centrosome amplification in many other tumors, such as bladder cancer and soft tissue tumors.[9,10] Centrosome defects can also increase changes of other cancer-related genes to cause further genetic changes, thereby affecting tumor development.[2] Results of this study showed that centrosome abnormalities in colorectal cancer tissue were significantly higher than in various grades of colorectal adenoma (Grades Ⅰ, Ⅱ, and III were 44.4%, 70.6%, and 79.0%, respectively). The strong positive rate of TEIF in poorly differentiated adenocarcinoma was as high as 80%, significantly higher than the 0% strong positive rate in well-differentiated adenocarcinoma. These data suggest that centrosome abnormalities (that is, increases in centrosome number, volume, and so on) clearly exist in colorectal cancer tissue and are closely related to the grading and biologic behaviors of colorectal cancer. Analyzing the high expression of the TEIF protein and γ-tubulin-related centrosome abnormalities could identify a significant positive correlation between these two. That is, high expression of TEIF in colorectal cancer tissue was often accompanied by centrosome abnormalities and vice versa, indicating a link between the two.

Chromosome 11q13 where the TEIF gene mapped is a breakpoint of chromosomal translocation or an amplification region associated with many human tumors,[10-12] which also contains many tumor and cell proliferation-related genes such as cyclin D1, EMS1, FGF3 (Int-2), and FGF4.[6,13,14] This suggests that TEIF overexpression possibly relates to mutations of its gene in tumor cells. The mechanisms for TEIF overexpression may be a result of gene regulation. Since its high expression preceded centrosome abnormalities, it is likely that the TEIF gene is in the position to regulate centrosome abnormalities. Studies have shown that[11] the TEIF protein could be located at the centrosome during the whole cell cycle and it indeed participated in centrosome regulation.

Our study indicated that high expression levels of the TEIF protein could affect the status of centrosome and other activities such as cell cycle and aging, causing abnormalities in the cell centrosome. That is, telomere loss could induce centrosome abnormalities. TEIF is not only involved in regulating telomerase activity; the TEIF protein may also serve as a regulatory protein of the centrosome to participate in a telomere abnormality-induced modulated response of the centrosome. Experiments using tumor cell lines also proved that cells with

TEIF overexpression had centrosome amplifications and an increased number of multinucleated cells. Changes in TEIF expression can induce abnormal amplification of centrosomes, while centrosome amplifications also directly or indirectly correlate with the degree of malignancy of colorectal cancers.

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