Expressions of E-cadherin, p120ctn, β-catenin and NF-κB in Ulcerative Colitis

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Summary: This study was aimed to investigate the expressions of E-cadherin, p120ctn, β-catenin and NF-κB in ulcerative colitis (UC) tissues and the implications of their expressions in the pathogenesis of UC. The expressions of E-cadherin, p120ctn, β-catenin and NF-κB were detected by immunohistochemistry, and those of p120ctn and NF-κB by Western blotting in 23 cases of UC and 17 cases of normal colonic tissues. The relationship between the expression of E-cadherin or NF-κB and that of p120ctn was analyzed by Spearman rank correlation analysis. The results showed that in UC and normal colonic tissues, the abnormal expression rate of E-cadherin, p120ctn, β-catenin and NF-κB was 52.2% vs. 0 (P<0.05), 73.9% vs. 23.5% (P<0.05), 65.2% vs. 17.6% (P<0.05) and 78.4% vs. 23.5% (P<0.05), respectively. p120ctn expression was positively correlated with E-cadherin expression (r=0.404, P<0.05), but negatively with nuclear NF-κB expression (r=−0.347, P<0.05). Western blotting showed that as compared with the normal controls, the p120ctn protein level was significantly decreased (P<0.05), whereas the NF-κB protein level was increased (P<0.05) in UC tissues. It was concluded that in the colonic tissues of UC patients, the expressions of E-cadherin, p120ctn and β-catenin are decreased, suggesting that mucosal barrier is impaired in UC. Moreover, NF-κB is increased and activated in the UC tissues, resulting in the inflammation in UC. p120ctn may influence the UC development through modulating intercellular adhesion and inflammatory response.

Key words: ulcerative colitis; E-cadherin; p120ctn; β-catenin; NF-κB

Ulcerative colitis (UC) is a non-specific chronic inflammation involving the colonic mucosa. UC lesions are confined to the colonic mucosa and submucosa, and they start in the rectum, gradually extend to the proximal part of, and even the entire colon. Unlike Crohn’s disease, another inflammatory bowel disease, UC seldom develops intestinal perforation and intestinal fistula, but relapse and difficulty to cure which lead to intestinal bowel fibrosis and shortening also expose UC patients to loss of labor capacity[1, 2]. The incidence of UC is increasing in Europe and America[3]. With the dietary westernization, UC incidence has also been rising in China, particularly in the past 10 years. Its incidence rate has reached 11.6/100 000 in China, far higher than that of Crohn’s disease[2].

The pathogenesis of UC has not been fully elucidated. Although hormone, salicylic acid preparations and immunosuppressive agents can effectively alleviate the clinical symptoms of acute UC, they do not change its clinical course[1, 2]. Recently, a number of studies have noted that the destruction of colonic mucosal barrier may be the first event of inflammatory bowel disease[3–5]. Mucosal healing has become the ultimate goal of treatment of inflammatory bowel diseases[3–5]. The concept of deep remission including clinical remission and mucosal healing updates the treatment of inflammatory bowel disease, and this objective can significantly improve the prognosis and quality of life of patients[6].

E-cadherin is an important regulator of epithelial cell adhesion. It is a single-pass transmembrane glycoprotein, and belongs to the classical cadherin family[7]. Mainly located in the adherens junction (AJ), it regulates intercellular adhesion through formation of calcium-dependent homodimers by the extracellular segment. Its cytoplasmic domains associate with p120 catenin (p120ctn), α-, β- and γ-catenin, and maintain the polarization and integrity of epithelial cells through cadherin-catenin complex[8].

p120ctn is initially found by the study of Src substrate[9]. Highly conserved armadillo (ARM) domain of p120ctn binds to the E-cadherin juxtamembrane domain (JMD) to form cadherin-catenin complex. p120 controls the stability and turnover of E-cadherin[10]. Moreover, several diverse mechanisms control AJ recycling. p120ctn plays an important role as the key regulator of AJ formation, stability and turnover. Recent studies show that p120ctn down-regulation can enhance RhoA activity, and it is related to the activation of its downstream transcription factor NF-κB[11].

Nuclear factor κB (NF-κB) is initially detected from B lymphocytes as a nuclear transcription factor. Subse-
quent studies have confirmed the expression of NF-κB in a wide variety of cells in the body, which is correlated with a number of important biological processes, such as cell proliferation, differentiation and survival, innate and acquired immune response. NF-κB abnormal activation leads to release of pro-inflammatory cytokines, adhesion molecules, growth factors, and overexpression of cell proliferation- and survival-related genes. Its activation is involved in many inflammatory diseases, including inflammatory bowel diseases[12].

In this study, immunohistochemistry was performed to detect the expression and distribution of E-cadherin, p120ctn, β-catenin, NF-κB in 23 cases of UC tissues and 17 cases of normal colonic tissues; the protein level of p120ctn and NF-κB in UC tissues and normal colonic tissues were detected by Western blotting. Our study was aimed to examine the relationship between cadherin-catenin complex and transcription factor NF-κB and gain new insights in the pathogenesis of UC in an attempt to provide novel therapeutic clues for UC.

1 MATERIALS AND METHODS

1.1 Patients and Specimens

In this study, the tissue blocks of 23 patients with UC who underwent colectomy between January 2001 to May 2013 at Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, were obtained from the archives. There were 13 males and 10 females, aged 15 to 62 years (median: 43 years old). In addition, 17 cases of normal colonic tissue blocks were obtained by surgical resection of tissues distal to tumor sites, including 11 males and 6 females, aged 28 to 70 years (median: 56 years old).

1.2 Immunohistochemistry

Both UC and normal tissue blocks were sliced into 4-μm serial sections. Immunohistological staining was performed by streptavidin-peroxidase (SP) method. Firstly, antigens were retrieved in preheated 0.1 mol/L citrate buffer (pH 6) in a pressure cooker at 90 kpa for 90 s. Subsequently, endogenous peroxidase activity was blocked by 3% hydrogen peroxide at room temperature for 10 min, and nonspecific antigens were blocked with 2% bovine serum albumin (BSA, Sigma, USA) in TBS for 1 h. The sections were then incubated with primary antibodies overnight at 4°C. The dilutions of primary antibodies were as follows: E-cadherin rabbit monoclonal antibody (Cell Signaling Technology, USA), 1:200; p120ctn goat polyclonal antibody (Santa Cruz, USA), 1:200; β-catenin rabbit monoclonal antibody (Cell Signaling Technology, USA), 1:200; NF-κB p65 rabbit monoclonal antibody (Cell Signaling Technology, USA), 1:1000; β-actin mouse monoclonal antibody (ProteinTech Group, USA), 1:2500. Horseradish peroxidase-conjugated secondary antibodies were added the next day, and an enhanced chemiluminescent substrate was used to detect the protein bands.

1.3 Western Blotting

Protein lysates of the samples were prepared by using a paraffin-embedded tissue protein extraction kit (Qproteome FFPE Tissue Kit, QIAGEN Company, Germany) according to the instructions of the manufacturer. Protein was quantified by using a Bradford protein quantification kit (Beyotime Institute of Biotechnology, China). Total protein (60 μg) from each sample was separated by SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose (NC) membrane. After being blocked with 5% skim milk powder diluted in TBS containing 0.1% Tween-20 for 1 h, the membrane was incubated with primary antibodies at 4°C overnight. The dilutions of primary antibodies were as follows: p120ctn goat polyclonal antibody (Santa Cruz, USA), 1:300; NF-κB p65 rabbit monoclonal antibody (Cell Signaling Technology, USA), 1:1000; β-actin mouse monoclonal antibody (ProteinTech Group, USA), 1:2500. Horseradish peroxidase-conjugated secondary antibodies were added the next day, and an enhanced chemiluminescent substrate was used to detect the protein bands.

1.4 Evaluation of Immunostaining

All sections were observed under the optical microscope at the same conditions. E-cadherin, β-catenin and p120ctn are normally expressed on the cell membrane in colonic mucosa. NF-κB positive staining is supposed to be brown or dark-brown granules in the cytoplasm or nucleus, and the negative control to have no brown reaction product. A representative high power field (×400) was selected. The percentage of positive cells was counted, which was combined with the staining intensity to calculate the staining score.

Staining score evaluation criteria[13] were as follows: (1) the percentage of positive cells fell into five categories: negative for 0, 1%–25% for 1, 26%–50% for 2, 51%–75% for 3, 76%–100% for 4; (2) the staining intensity was scored as follows: negative for 0, weakly for 1, moderately for 2, strong for 3. The final score of each slice was defined as (1)×(2).

To facilitate statistical analysis, for E-cadherin, β-catenin and p120ctn staining score, 0 to 3 points was classified as abnormal expression, and 4 points and above as normal expression; for NF-κB staining score, 0 to 3 points was classified as normal expression, 4 points and above as abnormal expression.

1.5 Statistical Analysis

Data analysis was performed by using SPSS 19.0 statistical software. The expression of E-cadherin, p120ctn, β-catenin and NF-κB was compared between UC group and normal colonic group by using chi-square test and Fisher’s exact test. Spearman rank correlation analysis was used to assess the correlation between the expression of E-cadherin or NF-κB and that of p120ctn. P<0.05 was considered to be statistically significant.

2 RESULTS

2.1 Expression of E-cadherin in UC Tissues

A uniform continuous membranous E-cadherin staining was found in all normal colonic epithelial tissues (fig. 1A). Compared with normal colonic tissues, in UC tissues, heterogeneous staining with loss of normal membranous E-cadherin immunoreactivity and presence of cytoplasmic staining was observed in the cuboidal non-polarized cells, which formed a regenerating monolayer adjacent to the ulcerated mucosa. Epithelial cells with a columnar shape and polarized nuclei distant