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Nrf−2 expression in ulcerative colitis lesions and its correlation with antioxidant enzyme levels and tissue injury

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ABSTRACT

Objective: To study the Nrf-2 expression in ulcerative colitis lesions and its correlation with antioxidant enzyme levels and tissue injury. **Methods:** Patients who were diagnosed with ulcerative colitis and colon polyp by colonoscopy and pathology biopsy in the Yan'an People′s Hospital between May 2013 and April 2016 were selected and enrolled in UC group and control group respectively. Lesion tissue was collected to determine the mRNA expression of Nrf-2, antioxidant enzymes, intestinal mucosa function molecules and intestinal mucosa apoptosis molecules as well as the levels of antioxidant enzymes. **Results:** Nrf-2, SOD, GSH-Px, CAT, Fas, FasL, NF-kB, TNF-α and Bak mRNA expression in lesions of UC group were significantly higher than those of control group while SOD, GSH-Px and CAT levels as well as cingulin, claudin-2, galectin-1, galectin-3 and galectin-9 mRNA expression were significantly lower than those of control group; Nrf-2 mRNA expression in lesion of UC group was positively correlated with SOD, GSH-Px, CAT, Fas, FasL, NF-kB, TNF-α and Bak mRNA expression, and negatively correlated with SOD, GSH-Px and CAT levels as well as cingulin, claudin-2, galectin-1, galectin-3 and galectin-9 mRNA expression. **Conclusions:** Compensatory high Nrf-2 expression in ulcerative colitis is closely related to oxidative stress and intestinal mucosa tissue injury.

1. Introduction

Ulcerative colitis (UC) is a kind of nonspecific inflammatory bowel disease whose pathogenesis is unclear, and its main clinical manifestations include abdominal pain, diarrhea and mucopurulent bloody stool[1,2]. Intestinal mucosa damage is a local important intestinal mucosal pathological feature in patients with UC, and the inflammation, oxidative stress and the resulting secondary apoptosis are thought to be closely related to the intestinal mucosa injury[3]. Nuclear factor-E2 related factor 2 (Nrf-2) plays a key role in the process of oxidative stress, the external damage factors can activate Nrf-2 and make it transfer into the nucleus, which starts the transcription of a variety of antioxidant enzymes, enhances the antioxidant capacity of local tissue, and helps to reduce the oxidative stress levels caused by external damage factors[4]. In order to define the role of oxidative stress reaction mediated by Nrf-2 in the development and change of UC, the Nrf-2 expression in UC lesions and its correlation with antioxidant enzyme levels and tissue injury were analyzed in the following study.

2. Subjects and methods

2.1 Research subjects

Patients who were diagnosed with ulcerative colitis and colon polyp by colonoscopy and pathology biopsy in the Yan′an People′s Hospital between May 2013 and April 2016 were selected as the research subjects, all the patients were pathologically diagnosed by the same group of pathologists, and the patients signed informed consent. A total of 48 patients with ulcerative colitis were enrolled in the UC group, including 31 men and 17 women that were 32-54 years old; a total of 36 patients with colon polyp were enrolled in the control group, including 22 men and 14 women that were 30-52 years old. There was no significant difference in general information between the two groups of patients (P>0.05).

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2.2 Research methods

2.2.1 Lesion tissue collection

During colonoscopy, ulcerative colitis lesion tissue was collected from UC group, polyp lesion tissue was collected from control group, the tissue was cleaned with saline for 2-3 times, then divided into two, put in EP tubes and placed in a -80 ℃ refrigerator under test.

2.2.2 Gene mRNA expression detection

Right amount of lesion tissue was taken, added in Trizol lysis buffer to separate the RNA in the tissue, reverse transcription kit was used to synthesize RNA into cDNA, fluorescence quantitative PCR reaction was conducted to amplify Nrf2, SOD, GSH-Px, CAT, cingulin, claudin-2, galectin-1, galectin-3, galectin-9, Fas, FasL, NF-kB, TNF-α and Bak respectively, and the mRNA expression were calculated.

2.2.3 Molecule content detection

Right amount of lesion tissue was taken, added in PBS lysis buffer and fully grinded, the grinded tissue suspension was centrifuged in the 4 ℃ centrifuge for 20 min at 12 000 r/min to separate supernatant, and radioimmunoprecipitation kits were used to determine the contents of SOD, GSH-Px and CAT.

2.3 Statistical methods

SPSS 20.0 software was used to input and analyze data, data analysis between two groups was by t test, correlation analysis was by Pearson test and $P<0.05$ indicated statistical significance in differences.

3. Results

3.1 Nrf-2 expression in lesion tissue

Nrf-2 mRNA expression in lesion tissue of UC group and control group were (2.52±0.41) and (1.06±0.17) respectively. After t test, Nrf-2 mRNA expression in lesion tissue of UC group was significantly higher than that of control group. Differences in Nrf-2 expression in lesion tissue were statistically significant between two groups of patients ($P<0.05$).

3.2 Antioxidant enzyme levels and expression in lesion tissue

Analysis of antioxidant enzymes SOD, GSH-Px and CAT levels and expression in lesion tissue between two groups of patients was as follows: SOD, GSH-Px and CAT levels in lesions of UC group were significantly lower than those of control group while SOD, GSH-Px and CAT mRNA expression were significantly higher than those of control group. Differences in SOD, GSH-Px and CAT levels and expression in lesion tissue were statistically significant between two groups of patients ($P<0.05$). Pearson test showed that Nrf-2 mRNA expression in lesion of UC group was positively correlated with SOD, GSH-Px and CAT mRNA expression, and negatively correlated with SOD, GSH-Px and CAT levels.

Table 1.

Antioxidant enzyme levels in lesion tissue of two groups of patients (U/L).

Groups	*n*	SOD	GSH-Px	CAT
UC group	48	32.15±4.47	26.57±3.52	16.75±1.89
Control group	36	58.96±7.14	48.51±6.79	29.21±3.48
T		8.297	9.876	8.112
P		<0.05	<0.05	<0.05

Table 2.

Antioxidant enzyme mRNA expression in lesion tissue of two groups of patients.

Groups	*n*	SOD	GSH-Px	CAT
UC group	48	2.31±0.36	1.89±0.24	2.65±0.36
Control group	36	1.04±0.15	1.02±0.14	1.06±0.17
T		13.498	8.598	16.412
P		<0.05	<0.05	<0.05

3.3 Intestinal mucosa function molecule expression in lesion tissue

Analysis of intestinal mucosa function molecules cingulin, claudin-2, galectin-1, galectin-3 and galectin-9 expression in lesion tissue between two groups of patients was as follows: cingulin, claudin-2, galectin-1, galectin-3 and galectin-9 mRNA expression in lesions of UC group were significantly lower than those of control group. Differences in cingulin, claudin-2, galectin-1, galectin-3 and galectin-9 expression in lesion tissue were statistically significant between two groups of patients ($P<0.05$). Pearson test showed that Nrf-2 mRNA expression in lesion of UC group was negatively correlated with cingulin, claudin-2, galectin-1, galectin-3 and galectin-9 mRNA expression.

Table 3.

Intestinal mucosa function molecule mRNA expression in lesion tissue of two groups of patients.

Groups	*n*	Cingulin	Claudin-2	Galectin-1	Galectin-3	Galectin-9
UC group	48	0.39±0.06	0.42±0.07	0.28±0.05	0.48±0.07	0.24±0.04
Control group	36	1.07±0.13	0.98±0.11	1.03±0.16	1.04±0.18	0.94±0.12
T		17.298	11.272	26.582	13.214	19.318
P		<0.05	<0.05	<0.05	<0.05	<0.05

Table 4.

Intestinal mucosa apoptosis molecule mRNA expression in lesion tissue of two groups of patients.

Groups	*n*	Fas	FasL	NF-kB	TNF-α	Bak
UC group	48	2.09±0.37	2.64±0.47	1.89±0.23	2.24±0.38	2.83±0.47
Control group	36	1.05±0.17	1.02±0.14	0.98±0.11	0.95±0.14	1.06±0.18
T		9.378	16.427	9.019	13.128	19.382
P		<0.05	<0.05	<0.05	<0.05	<0.05

3.4 Intestinal mucosa apoptosis molecule expression in lesion tissue

Analysis of intestinal mucosa apoptosis molecules Fas, FasL, NF-kB, TNF-α and Bak expression in lesion tissue between two groups of patients was as follows: Fas, FasL, NF-kB, TNF-α and Bak mRNA expression in lesions of UC group were significantly higher than those of control group. Differences in Fas, FasL, NF-kB, TNF-α and Bak expression in lesion tissue were statistically significant between two groups of patients ($P<0.05$). Pearson test showed that Nrf-2 mRNA expression in lesion of UC group was positively correlated with Fas, FasL, NF-kB, TNF-α and Bak mRNA expression.

4. Discussion

The etiology and pathogenesis of ulcerative colitis are not clear, and the inflammatory response and oxidative stress response are thought to be closely related to the occurrence of disease[5,6]. The Nrf-2 is an important effector molecule in the process of oxidative stress response, which can respond to exogenous damage factors and pathogenic factors. In physiological conditions, the Keap-1 is combined with Nrf-2 and makes the Nrf-2 inhibited; under the pathologic state of oxidative stress, the Keap-1 is dissociation with Nrf-2 and causes the Nrf-2 transposition into the nucleus, and the Nrf-2 in the nucleus can be combined with the response elements of a variety of antioxidant gene promoter regions and start the gene expression, thus increase the contents of antioxidant enzymes and reduce tissue damage caused by oxidative stress[7,8]. In order to define the role of oxidative stress reaction in the development and changes of ulcerative colitis, Nrf-2 expression in ulcerative colitis lesions were analyzed in the study at first, and the results showed that Nrf-2 mRNA expression in lesion tissue of UC group was significantly higher than that of control group. This means that the Nrf-2 shows compensatory activation in ulcerative colitis lesions, and it shows that the excessive activation of oxidative stress is closely related to the occurrence and development of ulcerative colitis.

SOD, GSH-Px, and CAT are important antioxidant enzymes in the pathogenesis of oxidative stress. SOD and GSH-Px can catalyze the reduction of oxygen free radicals and produce hydrogen peroxide, which is further reduced to water molecules under the catalysis of CAT[9–11]. In order to define the effect of compensatory Nrf-2 activation on antioxidant enzymes SOD, GSH-Px and CAT expression in the pathological process of ulcerative colitis, the expression of above antioxidant enzymes in ulcerative colitis lesions were analyzed in the study, and the results showed that SOD, GSH-Px and CAT mRNA expression in lesions of UC group were significantly higher than those of control group and positively correlated with the Nrf-2 mRNA expression. This shows that the compensatory activation of Nrf-2 can significantly increase the expression of the antioxidant enzymes SOD, GSH-Px and CAT in ulcerative colitis. Further analysis of the SOD, GSH-Px and CAT levels in ulcerative colitis lesions showed that SOD, GSH-Px and CAT levels in lesions of UC group were significantly lower than those of control group. It illustrates that although the antioxidant enzymes showed a trend of high expression in ulcerative colitis lesions, the actual levels of antioxidant enzymes decrease significantly, which is associated with the oxidative stress reaction activation and the mass consumption of antioxidant enzymes by oxygen free radical generation.

Oxidative stress in the intestinal mucosal tissue can cause damage to the mucosal barrier. Intestinal mucosal barrier includes mechanical barrier, biological barrier, immune barrier and chemical barrier, and the mechanical barrier formed by epithelial intercellular tight junction is most important. cingulin is combined with ZO-1, ZO-2, ZO-3, actin and myosin to maintain intestinal mucosal epithelial intercellular tight junction; claudin-2 is involved in the intracellular and extracellular calcium ion transport and affects the intercellular polarity of the cells, helping to enhance the intestinal mucosal epithelial intercellular tight junction[12]; galectin-1, galectin-3 and galectin-9 are important members of the lectin family that regulate cell proliferation and adhesion, and they can enhance the intestinal mucosal epithelial intercellular adhesion[13,14]. In the study, analysis of the intestinal mucosa function molecule expression found that cingulin, claudin-2, galectin-1, galectin-3 and galectin-9 mRNA expression in lesions of UC group were significantly lower than those of control group and negatively correlated with Nrf-2 mRNA

expression. This indicates that there is intestinal mucosa barrier injury in ulcerative colitis lesions and it is closely related to the activation of oxidative stress response.

The damage of intestinal mucosa is not only characterized by the damage to the mucosa barrier function, but also the abnormal apoptosis of the cells. In the pathologic state of overactivation of oxidative stress, the oxygen free radicals can act on cells and cause cell apoptosis through multiple pathways. Fas/FasL is the core regulator of death receptor apoptosis pathways, Fas is tumor necrosis factor receptor superfamily member on the cell membrane, and can be combined with FasL to cause apoptosis[15]. NF-kB is an important transcription factor that regulates inflammation in cells and has been proven to be able to regulate cell apoptosis in recent years; oxygen free radicals promote the NF-kB activation and transposition into nucleus, which can start after the TNF-α expression, then influence the expression of Bak on mitochondrial membrane, and promote the release of cytochrome C in the mitochondria and the activation of apoptosis through the biological effect of TNF-α[16,17]. In the study, the analysis of above intestinal mucosa apoptosis molecule expression showed that Fas, FasL, NF-kB, TNF-α and Bak mRNA expression in lesions of UC group were significantly higher than those of control group and positively correlated with Nrf-2 mRNA expression. This shows that there is apoptosis of intestinal mucosal epithelial cells in ulcerative colitis and it is closely related to the activation of oxidative stress.

The Nrf-2 shows the trend of compensatory high expression in ulcerative colitis and can mediate the high expression of antioxidant enzymes; the activation of oxidative stress can cause the injury of intestinal mucosa barrier function and induce apoptosis in ulcerative colitis lesions.

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