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Original Article

Correlation between HP infection-induced peptic ulcers accompanied with gastric precancerous lesions and LC3B

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Abstract: Autophagy is one lysosome-dependent degradation process. It can degrade damaged structures inside cytoplasm, producing amino acids and free fatty acids, thus acclimating cells for anorexia and hungry environments. Autophagy is modulated by various signaling pathways, whose dysregulation is closely correlated with tumor, aging and degeneration. This study aims to investigate the correlation between *Helicobacter pylori* (HP) infection accompanied with gastric precancerous lesions and autophagy. A total of 263 peptic ulcers patients who were diagnosed by gastroscopy and mucosal biopsy were recruited. Semi-quantitative RT-PCR and Western blotting were employed to quantify expression of autophagy factor LC3B. HP assay was performed by ^{14}C inhalation test. The correlation between HP positive rate and expression level of LC3B was analyzed, along with the correlation between precancerous lesion in gastric ulcers by HP infection and LC3B level. LC3B was significantly correlated with HP infection, atypical atrophy of gastric epithelial cells, chronic atrophic gastritis and intestinal metaplasia. Logistic regression revealed correlation between LC3B level with HP infection (OR=5.05, 95% CI: 1.14~23.45, P=0.02). There was a correlation between autophagy factor LC3B over-expression and HP infection along with gastric ulcers accompanied with atypical atrophy of gastric mucous.

Keywords: Helicobacter pylori, LC3B, precancerous disease

Introduction

Gastric ulcer is common disease in digestive tract. Since the discovery of *Helicobacter pylori* (HP), its correlation with gastric ulcer has been confirmed. HP is also one important factor causing gastric cancer and precancerous disease, as proved by various correlation studies [1-3]. HP infection cause gastric mucus damage secondary after gastric ulcers via a series of complicated pathological alternations, among which autophagy is one important regulatory pathway. Autophagy plays dual roles in mediating inflammation, as it can degrade damaged organelles and larger molecules by lysosome. Autophagy can also inhibit cytokine activation, thus depressing inflammation via clearing endogenous stimulus. The absence of autophagy thus accelerates inflammation via endogenous factors [4]. Autophagy has been recognized as the critical factor maintaining cell homeostasis, and is also one self-rescue mechanism of cells via degrading organelles and proteins, thus exerting important roles in various inflammatory responses and modulating tumors [5, 6]. Microtubule associated protein 1 light chain 3 (LC3B) on the autophagy vacuole membrane is one marker molecule reflecting autophagy, which is activated during inflammatory injury of gastric mucous, and regulates regeneration and repair of gastric mucous, making the molecular mechanism of autophagy as one hot topic in digestive tract [7]. Therefore, the radical treatment of HP has been recommended in gastric ulcer patients, but little has been known regarding the correlation between autophagy regulation and HP infection along with incidence of precancerous disease [8]. This study thus investigated such correlation by analyzing the expression of autophagy factor level and HP infection in various types of precancerous diseases, in order to

Table 1. Correlation between LC3B relative expression and clinical features

Clinical feature	N	Percentage (%)	LC3B mRNA level (x±s)	*P* value	LC3B protein level (x±s)	*P* value
Sex						
Male	148	56.27	0.47±0.16	0.32	0.56±0.21	0.33
Female	115	43.73	0.38±0.20		0.42±0.24	
Age (years)						
>60	126	47.91	0.43±0.21	0.75	0.47±0.28	0.76
≤60	137	52.09	0.46±0.15		0.50±0.15	
Atypical hyperplasia	80	30.42	0.49±0.18	0.01	0.52±0.22	0.01
Atrophic gastritis	76	28.90	0.30±0.15		0.34±0.18	
Intestinal metaplasia	107	40.68	0.26±0.09		0.29±0.11	
Disease location						
Antrum	105	39.92	0.49±0.17	0.42	0.52±0.20	0.45
Body	16	6.08	0.28±0.13		0.31±0.14	
Cardia	71	27.00	0.46±0.23		0.50±0.24	
Whole	71	27.00	0.41±0.15		0.45±0.17	
Size of mucosal lesion						
≤3 cm	121	46.00	0.41±0.18	0.12	0.44±0.19	0.14
>3 cm	142	54.00	0.48±0.21		0.53±0.18	
HP infection						
Yes	71	27.00	0.44±0.18	0.03	0.46±0.19	0.03
No	192	73.00	0.88±0.21		0.92±0.23	

provide clinical evidences for regulation of autophagy in HP infection-induced precancerous disease in gastric mucous.

Materials and methods

General information

A total of 263 cases of gastric ulcer patients (148 males and 115 females, average age =46.25±10.23 years) in The 3rd Xiangya Hospital of CSU from January 2014 to May 2015 were recruited in this study, which has been pre-approved by the ethical committee and has obtained written consents from all participants.

Inclusive criteria

All participants were complied with those criteria: (1) Aging between 18 and 75 years; (2) Being diagnosed with upper digestive tract ulcers, including gastric ulcer, duodenal ulcer and complex ulcer, and were manifested with/without active bleeding; (3) No other severe systemic disease or tumors; (4) Not taken any medicines that protect stomach or affecting HP test results; Not take steroid, antibiotics or Chinese herbs within 1 month; (5) Accomplished gastroscopy and biopsy; (6) Willing to finish questionnaire.

Exclusive criteria

(1) Aging less than 18 years or older than 75 years; (2) Complicated with systemic disease; (3) Alcoholics or drug abuse history; (4) Zollinger-Ellison syndrome, having gastric surgery, gastric carcinoma, or varicose veins in esophagus; (5) With auditory, visual dysfunction, mental disorder or diseases, or taken psychiatric medicines.

Gastroscopy and biopsy

Under gastroscopy, gastric ulcer was found with biopsy for collecting pathological tissues for routine examination. Forrest grade system was utilized to classify morphology and size of ulcers.

^{14}C inhalation assay

^{14}C-uera capsule was given to fasted patients. 30 min later inhalation test was performed using HP analyzer (model HUBT-01) with the help of nurses.

Pathological staining

Pathological samples were examined under HE staining using routine pathological and history approaches. Those tissues with HP negative were further confirmed by Warthin-Starry staining.

Mucous staining

Using HID-AB (pH2.5)-PAS method, acidic mucous was stained as dark brown, neutral mucous was shown as red-purple, while sialic

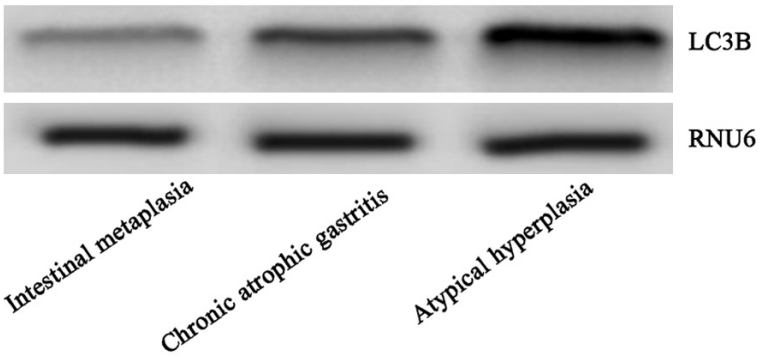

Figure 1. LC3B mRNA expressions in different pathological conditions.

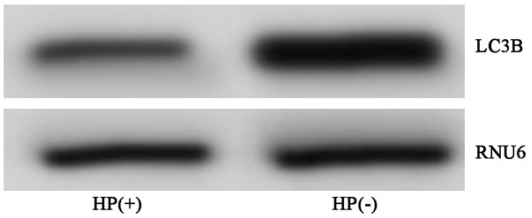

Figure 2. LC3B mRNA expression and HP infection.

acid was indicated by blue. Based on morphology standard [9], mucosal staining was used to classify various precancerous diseases including chronic atrophic gastritis, intestinal metaplasia and atypical atrophy of gastric epithelial.

RT-PCR

Total RNA was extracted from gastric tissues by RNA extraction kit. TaqMan RNA test kit was used to quantify the expression of autophagy factor LC3B using internal reference. 10 ng RNA was used in each reaction of RT-PCR. Due to the variation of reverse transcription efficiency, RNA concentration measurement error and variable cDNA contents in each sample, RNU6 was used as the internal reference. PCR amplification was performed under the following conditions: 95°C pre-denature, followed by 20 cycles each containing 95°C 30 s, 67.5°C 45 s and 72°C 60 s, followed by 20 cycles each containing 95°C 30 s, 58°C 30 s and 72°C 40 s, and ended with 72°C elongation for 6 min. PCR products were separated by 3% agarose gel electrophoresis in 1XTBE buffer under 150 V electrical field for 30 min. Images of gel were taken to analyze relative expression level.

Western blotting

100 mg total proteins were extracted from gastric tissues in all patients. After SDS-PAGE separation, proteins were transferred onto PVDF membrane by semi-quantitative method. Primary antibody against autophagy factor LC3B was added (1:500) for 4°C overnight incubation. On the next day, the membrane was washed in PBS and incubated with horseradish peroxidase (HRP)-labelled goat anti-rabbit secondary antibody (1:1 000) for 1 h at room temperature. ECL reagent was added for developing the membrane. All protein bands were determined for absorbance values using imaging analysis system.

Statistical analysis

All collected data were analyzed by SPSS21.0 software package. All patients were divided into LC3B low-expression group and high-expression group based on the average value. Stepwise regression approach was employed to screen out parameters with statistical significance based on the correlation analysis between LC3B expression and disease incidence. Logistic regression method was used to analyze the risk between clinical factors. Between-group-comparison was performed by analysis of variance (ANOVA), followed by LSD post-hoc approach in those samples with equal variance. Dunnett T3 method was applied in those samples with unequal variance. Chi-square analysis was employed to compare ratios. Mann-Whitney U test was used to compare group medians. A statistical significance was defined when $P<0.05$.

Results

LC3B expression and clinical features

The relative expression level of LC3B was 0.436 (95% CI, 0.323-0.534). There were 143 cases having lower-than-average LC3B level, while 120 cases with higher-than-average level. LC3B relative level was not significantly different regarding age, sex, disease site and size of

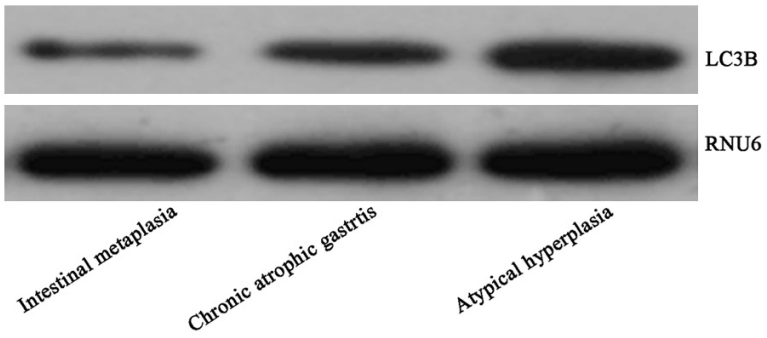

Figure 3. LC3B protein expressions in different pathological conditions.

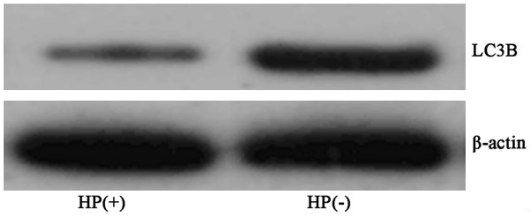

Figure 4. HP infection and LC3B protein level.

mucosal injury (P>0.05). HP infection, atypical atrophy of gastric epithelial, chronic atrophic gastritis and intestinal metaplasia were all related with LC3B relative expression (P<0.05, **Table 1**; **Figures 1-4**).

Relative level of LC3B and clinical factors

Logistic regression analysis showed the correlation between LC3B relative expression level and clinical factors. LC3B level was correlated with HP infection, with an OR value at 5.05 (95% CI, 1.14~23.45, P=0.02). See **Table 2** for details.

Discussion

Gastric ulcer is occurred in gastric and duodenum, and is one common disease in digestive tract. Its formation involves multiple factors, mainly attributed to the digestion of mucous by acidic gastric fluid [10-12]. Study has established the close correlation between gastric ulcer and HP infection, which is thus attributed as the major reason causing ulcers. The discovery of HP is one hallmark in upper digestive tract ulcer. With advancement of related studies, HP is found to be involved in the whole process of occurrence and progression of digestive disease, and is major reason causing mucosal injury, as it can induce chronic gastritis, gastric ulcer, precancerous lesion or even gastric carcinoma. HP infection invades gastric mucous, thinning mucosal layer and change the type of epithelial cells, thus weakening barrier of mucous, thus leading to precancerous lesion. Meanwhile, the lower self-clearance ability of the body against HP further accelerates HP infection. The interaction between these two events elevates the incidence of upper digestive tract disease [13-17].

Autophagy is one protective mechanism by various tissues and cells. Cells can form autophagosome via degradation. These vacuoles were transported to lysosome forming autophagy lysosomes, thus accomplishing self-renewal and metabolism of organelles by enzyme digestion. Recent study showed the involvement of autophagy in the whole process of inflammation and mucosal injury repair, thus playing a critical role in the repair of gastric mucous, making cellular repair and injury of gastric mucous and autophagy as one research hotspot currently [18-20]. This study recruited 243 cases of gastric ulcer patients, who received gastroscopy and biopsy along with ^{14}C-urea inhalation, along with semi-quantitative RT-PCR method for detecting autophagy factor LC3B level. The correlation between HP positive rate and LC3B expression level was compared, along with the correlation between HP infection-induced gastric ulcer accompanied with precancerous lesion and LC3B level. Results showed relative expression level of LC3B was 0.436 (95% CI, 0.323-0.534). There were 143 cases having lower-than-average LC3B level, while 120 cases with higher-than-average level. LC3B relative level was not significantly different regarding age, sex, disease site and size of mucosal injury (P>0.05). HP infection, atypical atrophy of gastric epithelial, chronic atrophic gastritis and intestinal metaplasia were all related with LC3B relative expression. These results suggested the correlation with LC3B mRNA and protein with HP positive and chronic gastritis with atypical hyperplasia. HP infection could induce damage

Table 2. LC3B expression level and clinical factors

Clinical feature	OR (95% CI)	*P* value	Adjusted OR (95% CI)[a]	*P* value[adjusted OR]
Sex				
Male	0.92 (0.25~3.17)	0.90	0.45 (0.09~2.01)	0.93
Female	1.00		1.00	
Age (years)				
>60	1.08 (0.45~3.03)	0.97	0.91 (0.25~3.19)	0.32
≤60	1.00		1.00	
HP infection				
Yes	1.00	0.65	1.00	0.03
No	1.48 (0.59~4.85)		0.73 (0.15~3.82)	
Disease site				
Antrum	1.00	0.41	1.00	0.91
Others	1.79 (0.81~6.59)		1.16 (0.21~6.82)	

on gastric mucous by gastric acid, altering cell type of mucosal membrane. HP-induced gastric acid secretion inhibition could cause atypical hyperplasia, atrophic gastritis, intestinal metaplasia and precancerous disease. Meanwhile HP infection produces inhibitory protein on parietal cells, inhibiting gastric acid secretion. Therefore the over-expression of autophagy factor LC3B, HP infection and precancerous lesion were closely inter-acted [21-23].

In summary, this study observed HP infection and precancerous disease from the perspective of autophagy, and found the close correlation between atypical hyperplasia of mucous and HP infection along with over-expression of autophagy factor LC3B. This study demonstrated certain relationship between over-expression of LC3B and precancerous lesion of gastric cancer. Moreover, over-expression of LC3B had potential modulatory role on HP infection and precancerous disease, suggesting the importance of autophagy in self-repair of gastric mucous during the formation and aggravation of gastric mucous. In clinical treatment, LC3B thus may act as the potential drug target, providing new insights for gene therapy.

Disclosure of conflict of interest

None.

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