Journal of Hainan Medical University

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Effect of helicobacter pylori L−form infection on proliferation, apoptosis and invasion molecule expression in gastric cancer tissue

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ARTICLE INFO

Article history:
Received 3 May 2017
Received in revised form 7 May 2017
Accepted 15 May 2017
Available online 24 May 2017

Keywords:
Gastric cancer
Helicobacter pylori L-form
Proliferation
Apoptosis
Invasion

ABSTRACT

Objective: To study the effect of Helicobacter pylori L-form infection on proliferation, apoptosis and invasion molecule expression in gastric cancer tissue. **Methods:** The gastric cancer tissues surgically removed in our hospital between May 2013 and October 2016 were collected and divided into Hp negative, Hp-L negative and Hp-L positive according to the condition of helicobacter pylori infection. The proliferation, apoptosis and invasion gene expression were detected. **Results:** LOXL2, PCNA, CyclinD1, Rab1A, Bcl-2, Snail, N-cadherin, UHRF1 and AnnexinII mRNA expression in Hp-L-positive gastric cancer tissues were significantly higher than those in Hp-L-negative and Hp-negative gastric cancer tissues while ING5, PTPN13, Beclin1 and Mst1 mRNA expression were significantly lower than those in Hp-L-negative and Hp-negative gastric cancer tissues; LOXL2, PCNA, CyclinD1, Rab1A, Bcl-2, ING5, PTPN13, Beclin1, Mst1, Snail, N-cadherin, UHRF1 and AnnexinII mRNA expression in Hp-L-negative gastric cancer tissues were not different from those in Hp-negative gastric cancer tissues. **Conclusion:** Helicobacter pylori L-form infection can influence the proliferation, apoptosis and invasion gene expression to promote cell proliferation and invasion, and inhibit cell apoptosis.

1. Introduction

Gastric cancer is the malignant digestive tract tumor with the highest incidence in our country, and its development is a complex process involving many factors, many links and many phases. Helicobacter pylori (Hp) infection is a basic independent risk factor for gastric ulcer, gastric cancer and so on[1]. In gastric cancer lesions, under the influence of the strong cancer cell metabolism, Hp growth and reproduction are in a relatively adverse environment, and Hp is prone to cell wall defect and forms helicobacter pylori L-form (Hp-L-form). Study has confirmed that the Hp-L is more infectious and aggressive and hard to be cleared than traditional Hp[2]. In the occurrence and development of gastric cancer, persistent Hp-L infections can influence the expression of multiple genes to cause the abnormal proliferation and invasion of cancer cells. At present, it is not yet clear about the genes affected by Hp-L infection in gastric cancer lesions. In the following study, the effect of helicobacter pylori L-form infection on proliferation, apoptosis and invasion molecule expression in gastric cancer tissue was analyzed.

2. Materials and methods

2.1 Experimental materials

4% paraformaldehyde, Giemsa staining kits and ABC immunohistochemical staining kits were bought from Beijing Zhongshan Golden Bridge Company, RNA extraction and real-time quantitative PCR detection kits were bought from Beijing ComWin Company, and Hp antibody was bought from Santa-Cruz Company.

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Fund Project: Science and Technology Plan Projects of Qianjiang City Hubei Province in 2010 No: 2010012.

2.2 Clinical materials

Clinical gastric cancer samples were taken from gastric cancer patients who received surgical resection in our hospital between May 2013 and October 2016, moderate amount of gastric cancer lesions were collected from all patients within 1 h after surgery and divided into two, one was fixed by 4% paraformaldehyde and then made into paraffin sections, and the other was frozen with liquid nitrogen and then preserved in a -80 ℃ refrigerator. All gastric cancer samples were used for subsequent research after the properties were confirmed by pathological examination. 85 cases of samples were collected, the corresponding patients were 47-65 years old, 59 cases were male and 26 cases were female.

2.3 Experimental methods

2.3.1 Hp–L infection detection methods

The paraffin sections of gastric cancer tissue were taken, stained with Giemsa kit and observed under 1 000 magnified visual field, those with Hp and Hp-L count more than 20 were judged as Hp positive, and those with count less than 20 were judged as Hp negative; Hp antibody was further used for immunohistochemical staining of the paraffin sections, the samples with fine grained tan gastric cancer cells and normal mucosa epithelial cells were judged as Hp-L positive, and the rest was judged as Hp-L negative.

2.3.2 Gene expression detection methods

Gastric cancer lesions frozen by liquid nitrogen were taken to extract RNA, and then real-time quantitative PCR detection kits were used to detect LOXL2, PCNA, CyclinD1, Rab1A, Bcl-2, ING5, PTPN13, Beclin1, Mst1, Snail, N-cadherin, UHRF1 and AnnexinII mRNA expression.

2.4 Statistical methods

SPSS 17.0 software was used to input gene expression data in gastric cancer tissue, and comparison of gene expression between Hp-L-positive and Hp-L-negative gastric cancer tissue was by t test. $P<0.05$ indicated statistical significance in the differences.

3. Results

3.1 Effect of helicobacter pylori L–form infection on proliferation gene expression

Analysis of proliferation genes LOXL2, PCNA, CyclinD1, Rab1A and Bcl-2 mRNA expression in the Hp-L-positive, Hp-L-negative and Hp-negative gastric cancer tissue was as follows: LOXL2, PCNA, CyclinD1, Rab1A and Bcl-2 mRNA expression in Hp-L-positive gastric cancer tissues were significantly higher than those in Hp-L-negative and Hp-negative gastric cancer tissues, and LOXL2, PCNA, CyclinD1, Rab1A and Bcl-2 mRNA expression in Hp-L-negative gastric cancer tissues were not different from those in Hp-negative gastric cancer tissues. Differences were statistically significant in LOXL2, PCNA, CyclinD1, Rab1A and Bcl-2 mRNA expression in Hp-L-positive and Hp-L-negative gastric cancer tissue as well as Hp-L-positive and Hp-negative gastric cancer tissue ($P<0.05$).

3.2 Effect of helicobacter pylori L–form infection on apoptosis gene expression

Analysis of apoptosis genes ING5, PTPN13, Beclin1 and Mst1 mRNA expression in the Hp-L-positive, Hp-L-negative and Hp-negative gastric cancer tissue was as follows: ING5, PTPN13, Beclin1 and Mst1 mRNA expression in Hp-L-positive gastric cancer tissues were significantly lower than those in Hp-L-negative and Hp-negative gastric cancer tissues, and ING5, PTPN13, Beclin1 and Mst1 mRNA expression in Hp-L-negative gastric cancer tissues were not different from those in Hp-negative gastric cancer tissues. Differences were statistically significant in ING5, PTPN13, Beclin1 and Mst1 mRNA expression in Hp-L-positive and Hp-L-negative gastric cancer tissue as well as Hp-L positive and Hp-negative gastric cancer tissue ($P<0.05$).

Table 2.

Effect of helicobacter pylori L-form infection on apoptosis gene expression.

Hp infection	n	ING5	PTPN13	Beclin1	Mst1
Hp negative	33	1.05±0.15	1.01±0.17	0.97±0.13	1.07±0.19
Hp-L negative	25	0.98±0.11	1.03±0.15	1.01±0.14	1.03±0.15
Hp-L positive	27	0.37±0.06*&	0.41±0.07*&	0.48±0.04*&	0.52±0.09*&
F		18.105	12.485	11.082	9.585
P		<0.05	<0.05	<0.05	<0.05

*: compared with Hp-negative gastric cancer tissue, $P<0.05$; &: compared with Hp-L-negative gastric cancer tissue, $P<0.05$.

Table 1.

Effect of helicobacter pylori L-form infection on proliferation gene expression.

Hp infection	n	LOXL2	PCNA	CyclinD1	Rab1A	Bcl-2
Hp negative	33	1.03±0.15	1.05±0.12	0.98±0.11	1.01±0.17	1.07±0.22
Hp-L negative	25	1.15±0.18	0.99±0.12	1.08±0.16	1.05±0.12	1.04±0.19
Hp-L positive	27	2.66±0.52*&	3.20±0.55*&	2.76±0.45*&	2.18±0.39*&	2.39±0.41*&
F		13.382	22.428	17.604	11.039	14.679
P		<0.05	<0.05	<0.05	<0.05	<0.05

*: compared with Hp-negative gastric cancer tissue, $P<0.05$; &: compared with Hp-L-negative gastric cancer tissue, $P<0.05$.

3.3 Effect of helicobacter pylori L–form infection on invasion gene expression

Analysis of invasion genes Snail, N-cadherin, UHRF1 and AnnexinII mRNA expression in the Hp-L-positive, Hp-L-negative and Hp-negative gastric cancer tissue was as follows: Snail, N-cadherin, UHRF1 and AnnexinII mRNA expression in Hp-L-positive gastric cancer tissues were significantly higher than those in Hp-L-negative and Hp-negative gastric cancer tissues, and Snail, N-cadherin, UHRF1 and AnnexinII mRNA expression in Hp-L-negative gastric cancer tissues were not different from those in Hp-negative gastric cancer tissues. Differences were statistically significant in Snail, N-cadherin, UHRF1 and AnnexinII mRNA expression in Hp-L-positive and Hp-L-negative gastric cancer tissue as well as Hp-L positive and Hp-negative gastric cancer tissue (P<0.05).

Table 3.

Effect of helicobacter pylori L-form infection on invasion gene expression.

Hp infection	n	Snail	N-cadherin	UHRF1	AnnexinII
Hp negative	33	1.03±0.12	0.99±0.11	1.05±0.16	1.07±0.19
Hp-L negative	25	0.98±0.14	1.02±0.15	1.02±0.17	1.04±0.17
Hp-L positive	27	2.43±0.08*&	2.39±0.05*&	2.31±0.07*&	2.46±0.08*&
F		13.282	15.695	17.028	12.105
P		<0.05	<0.05	<0.05	<0.05

*: compared with Hp-negative gastric cancer tissue, P<0.05; &: compared with Hp-L-negative gastric cancer tissue, P<0.05.

4. Discussion

The occurrence of gastric cancer is a complex process involving many factors, many links and many phases, and the changes in the expression of a variety of genes are closely related to the occurrence of gastric cancer. Helicobacter pylori (Hp) infection is one of the risk factors for gastric cancer, Hp-L is the Hp with cell wall defect, and the hypoxia environment caused by strong local cancer cell metabolism will increase the generation of Hp-L. Compared with the conventional Hp, Hp-L is more infectious and aggressive, it can evade the scavenging effect of body's defense mechanism and drugs, and it is long-standing within host and causes tissue damage[3]. Persistent Hp-L infections in local gastric mucosa can cause the changes in the expression of a variety of genes so as to cause the changes in a variety of cellular biological behaviors. Although the relationship between Hp-L infection and gastric cancer has received more and more recognition, and the genes affected by Hp-L infection during the development of gastric cancer are not yet clear.

LOXL2 and Rab1A are the important genes regulating gastric cancer cell proliferation. LOXL2 is a kind of lysyloxidase involved in cell cycle regulation[4]; highly expressed LOXL2 can increase the expression of PCNA and cyclinD1, the former plays an important role in the process of DNA replication and cell cycle progression from G1 phase to S phase, and the latter can form complexes with CDK4 and CDK6 and then accelerate the cell cycle progression from G1 phase to S phase, and promote cell proliferation[5,6]. Rab1A belongs to the GTPases Ras superfamily, participates in transport of a variety of proteins among organelles, and can regulate the mitochondrial function, increase the expression of Bcl-2 and prevent mitochondrial apoptosis[7,8]. In the study, the analysis of the Hp-L effect on the proliferation gene expression showed that LOXL2, PCNA, CyclinD1, Rab1A and Bcl-2 mRNA expression in Hp-L-positive gastric cancer tissues were significantly higher than those in Hp-L-negative and Hp-negative gastric cancer tissues. This means that Hp-L can increase the pro-proliferation gene expression in gastric lesions so as to promote the gastric cancer cell proliferation.

In addition to being regulated by the proliferation genes, the gastric cancer cell proliferation is also related to the expression deletion of a variety of pro-apoptosis genes. ING5, PTPN13, Beclin1 and Mst1 are the pro-apoptosis genes closely related to gastric cancer cell apoptosis. ING5 can enhance the activity of tumor suppressor gene p53, then increase the Bax and p21WAF1 protein and induce apoptosis[9,10]; PTPN13 is a member of the PTPs family that can interact with PTEN, Fas and other proteins to promote apoptosis and inhibit cell proliferation[11]; Beclin1 is a protein necessary for autophagosome formation, and it induces autophagy process to cause apoptosis[12,13]; Mst1 is a kind of serine/threonine protein kinase, it is involved in the composition of Hippo death receptor pathway, it causes YAP1 phosphorylation to make it in inactive state and thus cause the cells in a state of low proliferation, and it has the anti-proliferation activity[14,15]. In the study, analysis of Hp-L effect on the apoptosis gene expression showed that ING5, PTPN13, Beclin1 and Mst1 mRNA expression in Hp-L-positive gastric cancer tissues were significantly lower than those in Hp-L-negative and Hp-negative gastric cancer tissues. This means that Hp-L can reduce the pro-apoptosis gene expression in gastric cancer lesions so as to inhibit gastric cancer cell apoptosis.

On the basis of malignant proliferation, gastric cancer cells will show the characteristics of invasive growth, and Snail, N-cadherin, UHRF1, AnnexinII and other invasion genes are closely related to the invasive growth of cancer cells. Snail is a transcription factor adjusting the epithelial-mesenchymal transition, and can increase the expression of mesenchymal cell marker N-cadherin, reduce the adhesion between the cells and strengthen cell's movement characteristics to promote cell invasion[16,17]; UHRF1 is epigenetic regulatory molecule that can cause the methylation of p16INK4A, RB1 and other tumor suppressor genes, weaken the tumor suppressor gene function and promote cancer cell invasion[18,19]; AnnexinII is a kind of calcium-binding protein, and it can be combined with the cell membrane phospholipids to regulate cell skeleton ingredients and

promote cell movement[20]. In the study, analysis of Hp-L effect on the above invasion gene expression showed that Snail, N-cadherin, UHRF1 and AnnexinII mRNA expression in Hp-L-positive gastric cancer tissues were significantly higher than those in Hp-L-negative and Hp-negative gastric cancer tissues. This means that Hp-L can increase the pro-invasion gene expression in gastric lesions so as to promote gastric cancer cell invasion.

Helicobacter pylori L-form infection can affect the expression of proliferation genes LOXL2, PCNA, CyclinD1, Rab1A and Bcl-2, apoptosis genes ING5, PTPN13, Beclin1 and Mst1 as well as invasion genes Snail, N-cadherin, UHRF1 and AnnexinII to promote cell proliferation and invasion, and inhibit cell apoptosis.

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