Abstract. - OBJECTIVE: This study aimed to investigate the mechanism of Helicobacter pylori (HP) mediated PI3K/AKT/GSK3β signal pathways in the occurrence of gastric cancer.

PATIENTS AND METHODS: For this purpose, a total of 90 patients were selected; 30 were suffering from gastric cancer and gastric ulcer (observation group I), 30 were suffering from pure gastric ulcer (observation group II) and 30 were with chronic gastritis (control group), and the lesion tissues were extracted by using endoscopy. Then, rapid urease test, RT-PCR and Western blot tests were conducted to detect the positivity rate of HP infection, the expression levels of PI3K/AKT/GSK3β mRNA and protein respectively.

RESULTS: The results showed that the positivity rate of HP infection, expression level of the PI3K/AKT/GSK3β mRNA and protein from the samples of the observation group I were significantly higher than other two groups (p-value <0.05).

CONCLUSIONS: HP may mediate the PI3K/AKT/GSK3β signal pathways in the occurrence of gastric cancer.

Key Words: Helicobacter pylori, PI3K/AKT/GSK3β signal pathways, Gastric cancer.

Introduction

The occurrence rate of gastric cancer ranks 4th among malignant cancers. The patients of gastric cancer in China are 40 to 50% of the world total cases. Development of gastric cancer is a gradual process, starting from the occurrences of normal gastric mucosa, and then gradually development of atrophic gastritis, intestinal metaplasia, and finally atypical hyperplasia, that leads to the gastric cancer. Biological behaviors of HP infection, such as malignant proliferation, differentiation, invasion, migration and apoptosis play an important role in gastric cancer. Studies show that the degree of HP infection is correlated with the clinical staging of gastric cancer, and further with postoperative reoccurrence of gastric stump cancer, the chemotherapy efficacy of gastric cancer and hence with the survival outcome. Therefore, resistance against the HP infection is not only important for gastric ulcer, but also for prevention and treatment of gastric cancer. It is found that the PI3K/AKT/GSK3β, an important signal transduction pathways in cells, plays an important role in the occurrence and development of various malignant tumors, including breast cancer, ovarian cancer, non-small cell lung cancer and glioma. The main aim of this research was to study the mechanism of Helicobacter pylori (HP) mediated PI3K/AKT/GSK3β signal pathways in the occurrence of gastric cancer.

Patients and Methods

Patients

In this study, a total of 90 patients admitted to our hospital from January 2014 to January 2016 were selected, from which 30 (18 male, 12 female) with average age (52.6±13.3) years were diagnosed with gastric cancer and ulcer (observation group I), 30 (16 male, 14 female) with average age (54.3±15.6) years were of pure gastric ulcer (observation group II) and 30 were with chronic gastritis (control group). Exclusion criteria included operation, radiotherapy, chemotherapy, and medication that cause resistance against HP, PPI (proton pump inhibitors), gastric acid or protection of gastric mucosa within 3 months. The study was approved by the Ethics Committee of Daqing Oilfield General Hospital. Signed written informed consents were obtained from all participants before the study.
Data Collection Procedure

Firstly, the lesion tissues were extracted by using endoscopy. Then, urease test, RT-PCR and Western blot tests were conducted to detect the HP positivity rate, the expression level of PI3K/AKT/GSK3β mRNA and protein. The detailed procedure of these tests is elaborated as follow.

Rapid Urease Test

Rapid urease test (RUT) is based on the principle of the enzyme urease (HarveyBio, Beijing, China). The procedure of RUT was as follows: a tissue sample was taken at the time of gastroscopy and placed in the center of round-yellow test paper (Hangzhou Yisha Science and Technology Development Co., Ltd, Hangzhou, China), which were able to detect HP positivity rate within 1 min. After sealing of lining paper, the sample was observed and the results were classified into strong positivity or weak positivity rate of HP if the yellow test paper turned into cherry-red in 1 min or in 3 min, respectively. Also, HP rate was considered negative, if no change in color of yellow test paper was observed after 3 min.

RT-PCR Procedure

Total RNA was isolated from cells by using conventional TRIZol reagent (Yifeixue, Nanjing, China). Then ultraviolet spectrophotometer was used to detect concentration and purity of isolated RNA and reverse transcription kit was used to synthesize cDNA. The PCR primer sequence was synthesized (Sangon Biotech, Shanghai, China) and thus the cDNA primers, according to the sequence of Gene Bank, were as follows: for PI3K (p110), forward 5’-ACTTTAGAATGCCTCCGTG-3’, reverse 5’-TGCTTCTTGGTGAAC-3’; for AKT, forward 5’-CAGTGGCACAATGTCAGC-3’, reverse 5’-TCCACTCTTCCCCGCTCC-3’; for GSK3, forward 5’-CTTCAGGACAAGCGATTTA-3’, reverse 5’-CCAGCACCAGGTTAAGGTAG-3’ and finally for β-actin, forward 5’-GGTGAATCCTCAGTCAAAC-3’, reverse 5’-CTTCAGAGCAGCAGGTGA-3’. Of the resulting cDNA, 2-3 μl in the upper primer and 3 μl in the lower primer were amplified by reaction (PCR). Each PCR tube contained the following reagents: 0.5 μl TaqDNA polymerase, 1 μl dNTPs 3 μl MgCl2, 5 μl 10×Buffer and 2.5 μl ddH2O. The PCR reaction of incubation for 5 min at 95°C followed by 35 cycles of incubation again at 95°C for 30 s, 58°C for 30 s, 75°C for 50 s, and finally 70°C for 5 min. PCR products were separated by agarose gel electrophoresis. Furthermore, gray value was analyzed by spectroscopic imaging of gel imaging system and digital photography, and the results were expressed by 2-ΔΔCt method.

Western Blot Analysis

Main procedures of Western blot detection were as follows: the total protein of cells was extracted by conventional methods, and then BCA method (kits were obtained from Jiangsu, China) was used to check the concentration and the purity level. Before detection of protein, β-actin antibody (Abcam, Cambridge, MA, USA) was used to conduct dose standardization detection of protein level from samples. In this way, a total of 30 g protein was extracted by 8% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) separation. Proteins were transferred to polyvinylidene fluoride (PVDF) membrane and incubated overnight with mouse anti-PI3K/AKT/GSK3β monoclonal antibody (1:2000) (Abcam, Cambridge, MA, USA). Rabbit membrane was further incubated with antimouse polyclonal antibody (1:500) at room temperature for 4 h, then washed with phosphate buffered saline (PBS) and diaminobenzidine DAB color were developed. Results were scanned and reserved. Data were analyzed using 4.5 gel imaging software (Invitrogen, Carlsbad, CA, USA) and the results were expressed in term of integral optical density (IOD). Relative IOD was computed by using: Relative IOD = (IOD of p38MAPK)/ (IOD of β-actin).

Statistical Analysis

The data from different groups were presented as the mean ± standard deviation and in %. Differences among groups were analyzed by using one-way analysis of variance (ANOVA) test followed by LSD method comparing the difference between two groups. Also, Student t-test was applied for pair wise comparison between groups. Finally, p<0.05 was considered to indicate a statistically significant difference.

Results

Comparison of HP Positivity Rate in Tissues Among Different Groups

The positivity rate of HP infection was observed 83.3% from the samples of observation group I following 40.0%, and 13.3% from observation group II and the control group, respec-
Research on the mechanism of HP mediated PI3K/AKT/GSK3β pathways in gastric cancer

tively. This indicated high positivity rate of HP infection among the patients of group I that was also significantly different than the positivity rate of HP infection observed among patients of other two groups ($\chi^2=30.194, p<0.001$).

**Comparison of Expression Level of PI3K/AKT/GSK3β mRNA Among Different Groups**

The results of expression levels of PI3K, AKT and GSK3β mRNA from the sample tissues of three groups are presented in Table I. One-way ANOVA test showed that the expression levels of PI3K, AKT and GSK3β mRNA in observation group I were significantly higher ($p<0.05$) when compared with the other two groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Observation group I</th>
<th>Observation group II</th>
<th>Control group</th>
<th>F-Statistic</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI3K</td>
<td>0.6527±0.1632</td>
<td>0.3251±0.1124</td>
<td>0.1023±0.0635</td>
<td>8.625</td>
<td>0.000</td>
</tr>
<tr>
<td>AKT</td>
<td>0.5428±0.1524</td>
<td>0.2469±0.1322</td>
<td>0.1241±0.0524</td>
<td>7.629</td>
<td>0.000</td>
</tr>
<tr>
<td>GSK3β</td>
<td>0.7214±0.1825</td>
<td>0.2585±0.1251</td>
<td>0.1165±0.0432</td>
<td>9.524</td>
<td>0.000</td>
</tr>
</tbody>
</table>

**Comparison of Expression Levels of PI3K/AKT/GSK3β Protein Among Different Groups**

Table II presents the expression levels of PI3K, AKT and GSK3β protein in the sample tissues obtained from different groups. Based on one-way ANOVA, observation group I showed significantly higher expression level ($p<0.05$) of PI3K, AKT and GSK3β protein than other two groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Observation group I</th>
<th>Observation group II</th>
<th>Control group</th>
<th>F-Statistic</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI3K</td>
<td>0.58±0.15</td>
<td>0.34±0.12</td>
<td>0.12±0.05</td>
<td>9.632</td>
<td>0.000</td>
</tr>
<tr>
<td>AKT</td>
<td>0.53±0.16</td>
<td>0.25±0.14</td>
<td>0.13±0.07</td>
<td>8.657</td>
<td>0.000</td>
</tr>
<tr>
<td>GSK3β</td>
<td>0.62±0.18</td>
<td>0.26±0.16</td>
<td>0.11±0.06</td>
<td>12.221</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Discussion

Research on the mechanism of HP in the occurrence of gastric cancer has shown that HP infection can induce intestinal metaplasia, increase in cell proliferation and inhibit cell apoptosis\(^{10}\). Acceleration of cell proliferation, increase in DNA replication and transcription, and other processes may cause damage and wrong repairing that may further cause carcinogenesis. Moreover, bile reflux and HP infection can have worse effect on the inflammatory response of gastric tissues, atrophy of gastric mucosa and intestinal metaplasia among infected patients compared to patients who are not infected. Giuliani et al\(^{11}\) have found that the degree of gastric intestinal metaplasia in HP-infected patients was 4 times higher than the patients with negative HP rate. Leivonen et al\(^{12}\) have detected the proliferation index of gastric cancer Ki-67 and found a correlation between HP infection and the proliferation index of gastric cancer. Furthermore, HP infection has synergistic effect with bile reflux, which increases carcinogenic risk. Moreover, HP infection can degrade gastric mucus barrier and increase oxidative damage of gastric mucosa, which as a result releases bacteria antigen in a large amount, an important pathogenic factor that makes worse the body inflammatory response and caused a large increase of endogenous carcinogens. Meanwhile, intragastric ascorbic acid continues decreasing, with average decrease by 80.0-95.0%; thus, the ability to eliminate the free radicals is also decreased\(^{13}\). Mashimo et al\(^{14}\) showed that HP infection caused increase in the expressions of many oxygen molecules in the peripheral blood of patients, and the level decreased after complete elimination of HP infection. Also, HP infections can upregulate the expression level of the nitroso compounds in gastric juice that induces the occurrence of gastric cancer. Moreover, HP infection also induces the activation of proto-oncogene and protein of gastric mucosa.
and the deactivation of tumor suppressor gene have proved by immunohistochemical staining that the positive expression rate of p53 protein was 49.0-62.5%. Wei et al have proved that HP can promote AKT to degrade the wild-type p53 in gastric mucosa cells. PI3K/AKT/GSK3β signal pathways were regulated by various factors and there are mainly two activation ways: one is to promote the change of dimer conformation to activate by combination with the growth factor receptor of phosphotyrosine residues or connexin and the other is to activate the PI3K effector molecule by the directional combination of RAS and p110. Then, activated Akt can activate or inhibit downstream target proteins, including GSK3β, NF-κB, forkhead transcription factors, p21, etc. by phosphorylation. Besides, it can regulate cell proliferation, differentiation, apoptosis, migration, etc. PI3K/AKT/GSK3β signal pathways can regulate the proliferation of gastric cancer. \textit{In vitro} cell experiment adopts plasmid to trans infect the MGC-830 cell line of human gastric adenocarcinoma and has proved that the gastric cancer cells of N24P55γ, the regulation subunit of PI3K show decreased proliferation speed, slow growth, reduced formation rate of cell colony, prolonged G0/G1 stage of cell cycle and increased apoptosis rate, suggesting the N24P55γ of PI3K can inhibit the proliferation of gastric cancer cells and promote apoptosis. LY294002, the specific PI3K inhibitor, can inhibit the phosphorylation of bad protein of apoptosis molecules and its combination with antibody CH-11 can increase the apoptosis degree of MKN-45, a gastric cancer cell line. It should be noted that PI3K/AKT/GSK3β signal pathways play an important role in regulating the apoptosis process of gastric cancer. Expressions of HGF, upper stream regulator of Akt-PKB, and its receptor, c-Met, are unusually increased in tissues of gastric cancer. PHA-665752, small molecular weight c-Met inhibitor can decrease the activation degree and the expression levels of Akt/PKB signal and decrease the tumor cell number in gastric cancer xenograft model and reduce the volume. PI3K/AKT/GSK3β signal pathways can regulate the angiogenesis of gastric cancer. VEGF plays an important role in the invasion and metastasis of tumors. There are binding sites of specific HIF-1 in the promoter sequence of VEGF, which is regarded as an important downstream effector of HIF-1. Research has confirmed that PI3K blockers of cells can inactivate HIF-1α and reduce the expression level of VEGF; p85 subunit, the dominant negative mutant of p85, can reduce the transcription regulation function of HIF-1α and result in reduction of VEGF level. Trans infection of wild-type p110 subunit of PI3K can increase the VEGF level. PI3K/AKT/GSK3β signal pathways are correlated with chemotherapy resistance of gastric cancer. Liu et al has found in their research that etoposide, a chemotherapy drug, can activate the PI3K-Akt and MAPK-ERK signal pathways in time-based way, upregulate p53 expression, inhibit c-myc expression, reduce the sensitivity to chemotherapy of SGC7901 and BGC823 gastric cancer cell lines; maybe, it is an important molecule mechanism of gastric cancer drug resistance.

Conclusions

We showed that the positivity rate of HP infection among the patients of observation group I was higher than the other two groups and the expression levels of PI3K/AKT/GSK3β mRNA and protein were also higher too. Thus, we conclude that HP may mediate PI3K/AKT/GSK3β signal pathways in the occurrence of gastric cancer.

Conflict of Interests:

The authors declare no conflict of interest.

References

Research on the mechanism of HP mediated PI3K/AKT/GSK3β pathways in gastric cancer


