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# The Relationship between the Clinical Stage and Survival of the Postoperation and Mutations of the p16, p21 and p53 Genes in Gastric Cancer

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**Abstract** After dissected paraffin blocks by 5㎛ and deparaffinisation in xylene and proteinase K digestion, the genom DNA was extracted with phenol-chloroform. The mutation of p16, p21and p53 genes were assessed by polymerase chain reaction (PCR), PCR-single strand conformation polymorphism (PCR-SSCP). We detected the mutation rate of p16 gene was 44.3%(30/69), the mutation rate of p21 gene was 70%(48/69), the mutation rate of p53 was 61.4%(42/69) and common mutation rate in genes was 91.4%(63/69). The individual and complex mutation rate of these genes had associated with pTNM stage and postoperation survival in patients with gastric cancer. The findings of this study suggest these genes have played an important role in the development of gastric cancer and may serve as a new prognostic marker for the risk of gastric cancers.

**Key words** gastric cancers, p16, p21, p53, pTNM stage, survival

## Introduction

The great leader Comrade **Kim Jong Il** said as follows.

**"Medical science should bend its energy to research on the prevention of diseases whose incidence and mortality rate are high, among them those of the cardiovascular system and cancerous diseases. It should also strive to place traditional Korean medicine on a scientific basis and combine it with modern medicine properly, prevent pollution, and increase the variety of medical supplies and appliances and improve their quality. At the same time a long-term plan should be carried out for research to advance basic medicine including virology and genetics."**("ON THE FURTHER IMPROVEMENT OF THE HEALTH SERVICE" P. 18)

Recently, according to the rapid development of science and technique, morbidity rate of several diseases has been decreased, but cancerous diseases have still a high mortality and it is important to find the way of prevention and treatment of this disease [3].

Gastric adenocarcinoma is the second most common cancer in the rate of mortality and morbidity around the world and nearly one million new cases of this tumor develop annually and well over 700 000 individuals die from neoplasm each year [2, 5, 6]. It is conventionally accepted that diet and nutrition play a critical role in gastric carcinogenesis. *Helicobacter pylori*

infection has been established as a risk factor for gastric cancer through the development of atrophic gastritis and precancerous lesions [4, 8].

A number of factors to suppress or promote gastric cancer are known; smoking, salted, alchol ingestion, history of subtotal gastrectomy.

The intestinal type is more common and more often distal. In contrast, the disuse type has a poorer prognosis and tends to occur in the youngest patients [1, 10].

In Asia, gastric cancer accounts for 31% of all cancer incidence cases in male and for 23% in female [6, 7, 9].

According to the advance of molecular genetics of gastric cancer we hope to successfully address this major global health problem. Mutations of p21 oncogenes, p16 and p53 tumor suppressor genes are observed in various kinds of cancers, especially gastric cancers, and gene therapies apply to cancers by these mutations, especially to evaluate the molecular diagnosis, treatment and prognostic value of tumors [4].

Therefore we have studied the relationship between mutations of p16, p21 and p53 genes and gastric carcinogenesis, pTNM stage and progress of post operation in patients with gastric cancer.

## 1. The Object and Methods

### 1.1. The object

The paraffin-embedded surgical specimens from 69 patients with gastric cancer which were histological diagnosised by requesting to the Institute of Medical Science and Pyongyang Medical College Hospital of **KIM IL SUNG** University from 1990 to 2004 (average age is (46.9 ± 1.6)years old). Among them, the survival in 42 patients was followed up and the advanced gastric cancer (Ⅲa, Ⅲb) was 23 cases.

### 1.2. Materials

The paraffin-embedded surgical specimens, H-E dyeing specimens of them are used in study.

DNA analysis reagent is extration solution [pH 8.0, 4℃ keeping composition; 0.1mol/L Tris-HCl, 0.01mol/L EDTA, 0.1mol/L NaCl, 5% SDS(W/V)] and TE buffer solution(pH 8.0, 4℃ keeping, composition; 10mmol/L Tris, 1mmol/L EDTA).

PCR reagent using primers are p16 gene primer(exon 1～2), p21 gene primer(exon 1～2) and p53 gene primer(exon 5～8), TaqDNA polymerase(200U/100$\mu$L), liquid, −20℃ keeping, 10 × PCR buffer solution (pH 8.8, liquid, −20℃ keeping composition; 200mmol/L Tris, 100mmol/L $(NH_4)_2SO_4$, 100mmol/L KCl, 1% Triton X-100, 20mmol/L $MgCl_2$,) 10mmol/L dNTPs(pH 7.5, liquid, −20℃ keeping).

### 1.3. Methods

DNA isolation was performed by phenol-chloroform method. The gene mutations were analyzed by PCR, PCR-single strand conformation polymorphism (PCR-SSCP). The contents of PCR solution was 1.0$\mu$L of genome DNA, 1.0$\mu$L of 10×PCR buffer, 0.25$\mu$L of 10mmol/L dNTP, 1U of Taq DNA polymerase(0.5$\mu$L), 10pmol(1.0$\mu$L) of forward and backward

primer respectively and finally whole volume of reaction solution was adjusted to 10$\mu$L by $dH_2O$.

The PCR condition was 5 min at 94℃, 35 cycles of 30s at 94℃, 30s at 54～57℃ and 50s at suitability temperature of each gene and 1min at 72℃. 3$\mu$L of PCR products was mixed with 7$\mu$L of denature buffer and after it was denatured for 8min at 95℃, it was incubated for 5 min on ice immediately. It was electrophoresed on 8% PAG for 2h at 200V. The extraction gel was incubated for 5～10 min in fixation solution, for 20 min in staining solution and for 7～10 min in developer and it was washed in $dH_2O$. In every step the gel was washed in $dH_2O$ for 20 seconds.

We used "/", when the complex mutations in one specimen are observed. For example, the mutations of p16, p21 and p53 genes together are observed, "p16/p21".

## 2. Result and Analysis

### 2.1. The relationship between pTNM stage and the mutation of p16, p21 and p53 genes

#### 2.1.1. The relationship between pTNM stage in gastric cancer and the individual mutations

The relationship between pTNM stage in gastric cancer and the individual mutations were analysed in table 1.

Table 1. The relationship between TNM stage and the individual mutations of p16, p21 and p53 genes ($n$=69)

| pTNM stage | | | Ⅰa | Ⅰb | Ⅱ | Ⅲa | Ⅲb |
|---|---|---|---|---|---|---|---|
| Observating number | | | 22 | 15 | 9 | 13 | 10 |
| p21 | Total mutation | | 16(72.7) | 9(60.0) | 6(66.7) | 9(69.2) | 9(90.0) |
| | exon | 1 | 12(54.5) | 8(53.3) | 6(66.7) | 9(69.2) | 8(80.0) |
| | | 2 | 4(18.2) | 2(13.3) | 1(11.1) | 0(0.0) | 2(20.0) |
| p16 | Total mutation | | 5(22.7) | 5(33.3) | 1(11.1) | 11**(84.6) | 8**(80.0) |
| | exon | 1 | 3(13.6) | 2(13.3) | 1(11.1) | 8**(61.5) | 7**(70.0) |
| | | 2 | 2(9.1) | 3(20.0) | 0(0.0) | 3(23.1) | 1(10.0) |
| p53 | Total mutation | | 10(45.5) | 9(60.0) | 5(55.6) | 12(92.3) | 7(70.0) |
| | exon | 5 | 7(31.8) | 4(26.7) | 0(0.0) | 9*(69.2) | 4(40.0) |
| | | 6 | 4(18.2) | 3(20.0) | 0(0.0) | 2(15.4) | 2(20.0) |
| | | 7 | 3(13.6) | 6(40.0) | 2(22.2) | 8(61.5) | 3(30.0) |
| | | 8 | 3(13.6) | 2(13.3) | 2(22.2) | 2(15.4) | 2(20.0) |

* $p$<0.05, ** $p$<0.01 (Ⅰa, Ⅰb, Ⅱ comparative)

As shown in table 1, the total and exon mutation of p21 gene were not associated with pTNM stage in gastric cancer, but the total and exon Ⅰ mutation of p16 gene were highly associated in stage Ⅲa, Ⅲb and the frequency of exon 2 mutation were not associated with pTNM. And the mutation of exon 5 of p53 gene was significantly related to stage Ⅲa.

### 2.1.2. The relationship between pTNM stage in gastric cancer and the complex mutations of p16, p21 and p53 genes

The relationship between pTNM stage in gastric cancer and the complex mutations of p16, p21 and p53 genes was analysed in table 2.

Table 2. The relationship between pTNM stage in gastric cancer and the complex mutations of p16, p21 and p53 genes ($n$=69)

| pTNM stage | Non mutation | Only one mutation | | | Two mutation | | | All mutation | Both |
|---|---|---|---|---|---|---|---|---|---|
| | | p21 | p16 | p53 | p16/p21 | p16/p53 | p21/p53 | p16/p21/p53 | |
| Ⅰa | 4(18.2) | 7(31.8) | 0(0.0) | 0(0.0) | 1(4.5) | 2(9.1) | 5(22.7) | 3(13.6) | 22(100) |
| Ⅰb | 2(13.3) | 3(20.0) | 0(0.0) | 2(13.3) | 1(6.7) | 2(13.3) | 3(20.0) | 2(13.3) | 15(100) |
| Ⅱ | 0(0.0) | 2(22.2) | 0(0.0) | 2(22.2) | 2(22.2) | 1(11.1) | 2(22.2) | 0(0.0) | 9(100) |
| Ⅲa | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 1(7.7) | 4**(30.8) | 2(15.4) | 6**(46.2) | 13(100) |
| Ⅲb | 0(0.0) | 0(0.0) | 0(0.0) | 1(10.0) | 3(30.0) | 0(0.0) | 1(10.0) | 5**(50.0) | 10(100) |

** $p$<0.01 (comparative to Ⅰa, Ⅰb, Ⅱ)

As shown in table 2, the frequency of the complex mutations of genes were associated with the stage and the frequency of the total mutation in pTNM stage Ⅲa and Ⅲb was higher than other stages. And the frequency of the mutation of p16/p53 in stage Ⅲa was significantly higher than others.

## 2.2. Change of the postoperative survival of the gastric cancer according to the mutations of p16, p21 and p53 genes

### 2.2.1. The relationship between the postoperative survival in gastric cancer and individual mutations of p16, p21 and p53 genes

The relationship between the postoperative survival (early and advanced gastric cancer) and individual mutations of p16, p21 and p53 genes was analyzed in table 3.

As seen in table 3, the survival was not related to the presence of p21 gene mutation, but the survival was significantly shorter in the mutation of p16 gene as (2.8±0.7) years than in no mutation (8.4±1.2)years). And the survival in the mutation of p53 gene as (3.9±0.9) years was also significantly shorter than in no mutation.

Table 3. The relationship between the postoperative survival and the individual mutation p16, p21 and p53 genes ($n$=42)

| Division | Mutation | Number | Survival years/y |
|---|---|---|---|
| p16 | Neg | 23 | 8.4±1.2 |
| | Pos | 19 | 2.8**±0.7 |
| p21 | Neg | 14 | 5.6±1.5 |
| | Pos | 28 | 6.0±1.0 |
| p53 | Neg | 14 | 9.8±1.2 |
| | Pos | 28 | 3.9**±0.9 |

** $p$<0.01(comparative to neg)

### 2.2.2. The relationship between the postoperative survival and complex mutations of p16, p21 and p53 genes

The relationship between the postoperative survival and complex mutations of p16, p21 and p53 genes was analyzed in table 4.

Table 4. Survival of the post operation in gastric cancer by complex mutations of p16, p21 and p53 genes ($n$=42)

| Division | No mutation | Only p21 mutation | Tumor suppressor genes mutation | | | | | All mutations |
|---|---|---|---|---|---|---|---|---|
| | | | p16 | p53 | p16/p53 | p21/p16 | p21/p53 | p16/p21/p53 |
| Case | 3 | 9 | — | 4 | 7 | 2 | 7 | 10 |
| Survival | 13.0±1.7 | 8.9±1.6 | — | 7.3±2.6 | 1.6**±0.5 | 9.0±3.0 | 6.4±2.8 | 2.4**±0.5 |

** $p$<0.01 (comparative to no mutation)

As shown in table 4, the survival was significantly associated with the mutation of p16/p53(1.6 ± 0.5 years) and p16/p21/p53 (2.4+0.5)years) than with no mutation(13.0±1.7 years). And the survival in mutations of p16/p21/p53 and p16/p53 was significantly shorter than in the mutation of only oncogene. (8.9±1.6 year)

**2.2.3. The relationship between the postoperative survival in advanced gastric cancer (Ⅲa, Ⅲb) and mutations of individual genes**

The relationship between the postoperative survival in only advanced gastric cancer(Ⅲa, Ⅲb) and the mutations of p16, p21 and p53 genes was analyzed in table 5.

Table 5. The relationship between survival of the postoperation and the individual mutation of p16, p21 and p53 genes in advanced gastric cancer(Ⅲa, Ⅲb) ($n$=23)

| Genes | Diagnosis | Mutation | Survival· number | | Total |
|---|---|---|---|---|---|
| | | | Lower 5 years | Upper 5 years | |
| p21 | Advanced cancer(Ⅲa, Ⅲb) | Neg | 5(35.7) | 3(33.3) | 8(34.8) |
| | | Pos | 9(64.3) | 6(66.7) | 15(65.2) |
| p16 | Advanced cancer(Ⅲa, Ⅲb) | Neg | 3(21.4) | 7△△(77.8) | 10(43.5) |
| | | Pos | 11**(78.6) | 2(22.2) | 13(56.5) |
| p53 | Advanced cancer(Ⅲa, Ⅲb) | Neg | 1(7.1) | 4(44.4) | 5(21.7) |
| | | Pos | 13**(92.9) | 5(55.6) | 18(78.3) |

* $p$<0.05, ** $p$ <0.01(comparative to negative), △△ (comparative to positive)

As shown in table 5, the presence of p21 gene mutation in advanced gastric cancer was not associated with 5 years survival, but the frequency of p16 gene mutation at the period of the lower 5 years survival was high, but low at the period of the upper 5 years survival. And the frequency of p53 gene mutation in advanced gastric cancer was significantly high at the period of the lower 5 years survival.

**Conclusion**

We identified the mutation rate of p16, p21 and p53 genes were associated with pTNM stage and postoperative survival in gastric cancer.

The positive rates of p16 and p53 genes mutation in gastric cancer was significantly higher in high pTNM stage than low pTNM stage.

The postoperative survival of patients with gastric cancer was significantly shorter in

positive mutation of p16 and p53 genes than that in no mutation.

The postoperative survival of the upper 5 years is significantly and independently associated with negative mutation of p16 gene and the postoperative survival of lower 5 years is significantly associated with positive mutation of p16, p53 genes in advanced gastric cancer.(Ⅲa, Ⅲb)

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