Co-mutation of p53, K-ras genes and accumulation of p53 protein and its correlation to clinicopathological features in rectal cancer

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Abstract

AIM: To determine the accuracy of p53 gene mutations predicted by overexpression of p53 protein immunohistochemically, and to investigate the co-mutation of p53 and K-ras genes in rectal cancer and its effect on promoting malignant biologic behaviors of tumors.

METHODS: Ninety-seven specimens of rectal cancer were surgically resected in our hospital from August 1996 to October 1997. The hot mutation areas of p53 gene (in exons 5-8) and K-ras gene (in codon 5/12 and 13) were detected with polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP), and overexpression of p53 protein was detected with immunohistochemistry (IHC) in the 97 specimens of rectal cancer. Correlation between gene mutations and tumor clinicopathologic factors was studied, and survival analysis was performed as well.

RESULTS: There were 36 cases of p53 gene mutations in 61 p53 protein positive cases, and 21 cases of p53 gene non-mutation in 36 p53 protein negative cases respectively. The coincidence rate of p53 gene mutation by IHC method with PCR-SSCP method was 58.8% (57/97). The mutation rate of p53 gene was 52.6% (51/97), while K-ras gene mutation was observed in codons 12 and 13 in 61 cases with a mutation rate of 62.9% (61/97). Single gene mutation of p53 or K-ras was found in 32 cases. Both p53 and K-ras gene mutation were found in 48 cases. Statistical analysis showed that p53 and K-ras gene mutations were not related to the clinicopathologic factors, including tumor size, gross tumor type, histological classification, differentiation, invasion to intestinal veins, lymphatics and nerves, invasive depth to wall, lymph node metastasis, and Dukes' stages (P>0.05). The survival in patients with no gene mutation, single gene mutation and both gene mutations were similar (P>0.05).

CONCLUSION: IHC has a certain false positive and false negative rate in detecting p53 gene mutations. Malignant biological behaviours of rectal cancer are not enhanced by p53 and K-ras gene mutations. Co-mutation of p53 and K-ras gene has neither synergic carcinogenesis-promoting effect, nor prognostic effect on rectal cancer.

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Introduction

Carcinogenesis and development of colorectal cancer are a process with multiple steps, in which epithelial cells of colorectum go through small adenoma, large adenoma, and finally become adenocarcinoma[1]. The changes of several genes involve this process, including the activation of K-ras oncogene and the mutation and deletion of p53 anti- oncogene[2]. To date, the clinical significance of the change of these genes is still disputed. Some studies reported that simultaneous mutations of p53 and K-ras could promote the development of colorectal cancer directly or indirectly, and were related to lymphatic metastasis, and a poor prognosis, but others had opposite results[3]. It is still unclear which one is the prognostic factor, the p53 cDNA mutation or the alteration of p53 protein expression or both[4]. Moreover, 90% dot mutations of p53 happen in exons 5-8, the individual incident rate of p53 mutations in these exons is not known[5]. Therefore, to explore the effect of simultaneous mutations of p53, K-ras gene on the promotion of malignant biologic behaviors of rectal cancer and its clinical significance, the mutation patterns of exons 5-8 of p53 gene and codons 12 and 13 of K-ras gene in rectal cancer were detected by PCR-SSCP.

Materials and Methods

Patients

From August 1996 to October 1997, 97 patients with rectal cancer underwent surgical resection in our hospital, including 54 males and 43 females, aged from 21 to 84 years with an average age of 53 years. Gross classification of tumors consisted of 27 cases of protruding type, 63 cases of ulcerating type, and 7 cases of infiltrating type. Histological classification included 3 cases of adenoma with cancer, 17 cases of papillary adenocarcinoma, 69 cases of tubular adenocarcinoma, 1 case of mucinous adenocarcinoma, 7 cases of signet-ring cell carcinoma, with 20 cases of well differentiated, 51 moderately and 26 poorly differentiated. According to Dukes classification, patients were categorized into four stages: stage A (n = 27), stage B (n = 22), stage C (n = 36) and stage D (n = 12).

Methods

Serial paraffin sections were used for HE staining, anti-p53 immunohistological staining, and detection of p53 and K-ras gene mutation by PCR-SSCP.

Immunohistochemistry

The Labeling-streptavidin-biotin peroxidase complex (LSAB) method was used for IHC staining. The colorectal tissue positive for p53 was taken as positive control, normal tissue as negative control, PBS instead of first antibody as blank control. Background of IHC staining was clear.

Positive standards for IHC staining were the same as previously described. Cells with pale brown granules in nuclei were judged as positive. The sections with 10-25% positive cells were defined as +, 26-50% as ++, 51-70% as ++++, more than 70% as +++++, less than 10% as -.

PCR-SSCP

DNA was abstracted from tissues of 5 μm paraffin sections.
with routine method, and then stored at -20 °C. Exons 5,6,7,8 of p53 gene and codons 12, 13 of K-ras gene were amplified by polymerase chain reaction (PCR, PCR amplifier produced by American PE Company) respectively. The primers were synthesized and provided by Genetics Department of Sun Yat-Sen University. The sequences of primers are shown in Table 1. The single DNA chain from denatured PCR products was treated by normal vertical plane electrophoresis and stained with silver. Increased, and decreased numbers and translocation of electrophoresis bands in tumor specimens compared with normal tissues suggested the existence of gene mutations.

Statistical analysis
Data were analyzed with SPSS statistical software (Version 8.0). The relationship between mutations of p53, K-ras genes and clinicopathological factors was analyzed by χ² test. Survival rates were calculated with life table and Wilcoxon-Gehan test.

RESULTS
Expression of p53 protein
p53 protein was detected in 61 cases (62.9%, 61/97) according to IHC staining, strong positive (+++) and moderately positive (++), weak positive (+) were found in 26/61, 22/61, 10/61, and 30/61, respectively.

Sensitivity of p53 IHC method to p53 gene mutation detection
p53 gene mutations (in exons 5-8, hot mutation area) were found in 51 cases of rectal cancer by PCR-SSCP, with a mutation rate of 52.6% (51/97). Among the cases with p53 positive IHC staining, 36 cases were positive for p53 gene mutations by PCR-SSCP in 21 cases negative for p53 IHC staining (Table 2). The accuracy of IHC method to detect p53 gene mutations was 58.8% (57/97), the sensitivity was 70.6% (36/51), and the specificity was 45.7% (21/46).

| Gene | Extron/codon | Sequences of primers | Product size |
|------|--------------|----------------------|-------------|
| p53  | Exons5-6     | 5'TGTTCACCTGCGCCTGACT3' | 489bp |
|      | Exon 7       | 5'GGAGGGGCACACTGACAAAC3' |      |
|      | Exon 8       | 5'GGCTGCACGCAGCAAGGAGG3' | 286bp |
| K-ras| Codon 12-13  | 5'TCAAAGAATGGTGTTTGAGATCTG3' | 320bp |
|      |              | 5'GCCGAGGAAGGTGATAAAAGTGA3' |      |

Correlation of gene mutation and clinicopathological factors
The differences between p53 and K-ras gene mutations and clinicopathological factors had no statistical significance (P>0.05), including tumor size, gross tumor type, histological classification and differentiation, invasion to intestinal veins, lymphatics and nerves, invasive depth to intestinal wall, lymph node metastasis, and Dukes’ stages.

Correlation of gene mutation and prognosis
All cases were followed up to December 2002, ranging from 14 d to 2058 d with an average of 1107 d. One case died of cardiopulmonary disease 14 d after operation, 28 cases died of tumor metastasis or recurrence, 3 cases were lost for follow-up, 2 cases died of other diseases rather than tumor. Life table was used to assess survival rates. Results indicated that p53 and K-ras gene mutations had no effects on prognosis (P>0.05, Table 3).

| Survival (year) | p53 mutation | K-ras mutation | p53 and K-ras mutation |
|----------------|--------------|----------------|------------------------|
|                | (-)          | (+)            | (-)                     | (+)         | Neither | Either | Both |
| 1 -            | 86.52        | 95.83          | 91.04                  | 91.53       | 81.25   | 93.48  | 93.44|
| 2 -            | 77.04        | 82.30          | 87.79                  | 75.37       | 71.48   | 86.55  | 72.29|
| 3 -            | 67.25        | 73.16          | 74.79                  | 67.93       | 54.17   | 79.44  | 65.06|
| 4 -            | 64.33        | 62.32          | 66.91                  | 61.36       | 54.17   | 71.08  | 56.38|
| 5 -            | 64.33        | 62.32          | 66.91                  | 61.36       | 54.17   | 71.08  | 56.38|
| P              | 0.5672       | 0.4536         | 0.2030                 |
DISCUSSION

The methods normally used to detect p53 gene mutations include immunohistochemistry (IHC), PCR-SSCP, denaturing gradient gel electrophoresis (DGGE), and sequencing. According to literatures, the sensitivity of IHC was 75%, and its predictive value for detecting p53 gene mutations was 63%. More than 85% p53 gene mutations in exons 5-8 could be detected by PCR-SSCP with a sensitivity of 80-90%.[6,7] In the present study, p53 gene mutations in rectal cancer were detected by IHC and PCR-SSCP respectively. The positive rate of IHC was 62.9% (61/97), while that of PCR-SSCP was 52.6% (51/97). The accuracy of IHC was 58.8%, and the sensitivity was 70.6%. However, its specificity was only 45.7%, implying that IHC had a rather high false positive and false negative rate in detecting p53 gene mutations.

It was reported that wild type p53 proteins could combine with viral oncoproteins or cellular oncoproteins to enhance their stability and prolong their half-life, leading to p53 protein accumulation in cells. In such cells, IHC staining was still positive even without p53 gene mutations, furthermore, about 10% p53 gene mutations could take place out side of exons 5-8. Therefore the positive rate of PCR-SSCP targeting only exons 5-8 was usually lower than that of IHC.[8-11] The present study also had similar results. The variance in p53 gene mutations and p53 protein accumulation indicated that dysfunction of p53 gene might be caused by mechanisms other than mutations.

It is known that the oncogenesis and development of most colorectal cancers abide by the rule from normal epithelia to adenoma then to adenocarcinoma, and finally to metastasis. This complicated process has been found to involve several oncogene changes in a certain order, that is, from APC to K-ras to p53 to DCC[12]. p53 and ras gene mutations could be observed in both adenoma and carcinoma of large intestine[31]. Extent of p53 expression varies during different phases of tumor oncogenesis, p53 expression can be found extensively in villous adenoma, early stage of adenocarcinoma and well differentiated adenocarcinoma. The above lines of evidence support that p53 and K-ras gene mutation might happen before canceration and may be an early event in colorectal carcinogenesis and development. Participation in the process from adenoma to adenocarcinoma, p53 and ras gene mutations may be used as the markers for early detection and diagnosis of colorectal cancer arising from adenoma.

Many studies indicated that the point mutation of p53 gene was related to lymph node metastasis in rectal cancer ($P<0.001$), while the cross deletion was related to distal metastasis ($P=0.0001$). The 5-year survival of colorectal cancer patients with chromosome 17p deletion was much poorer. p53 gene mutation was an important prognostic factor for colorectal cancer[44]. On the contrary, some studies showed that intensity, distribution area and positive rate of p53 IHC staining were independent of the biological factors, such as tumor grade, histological type, tumor size, invasion depth to wall, invasion of local lymphatics and veins, lymph node metastasis, peritoneal seeding, and liver metastasis[33]. Therefore, the clinical significance of p53 is still controversial.

In the present study, detected by PCR-SSCP, the p53 gene mutation rate in exons 5-8 was 52.6%. Most mutations (in 50 cases) took place in exon 7, 3 in exons 5-6, 2 in exon 7. No mutation was found in exon 8. The mutation rate of codons 12 and 13 of K-ras gene was 62.9%. Statistically p53 and K-ras gene mutations were independent of clinicopathological factors of rectal cancer, including tumor size, gross classification, histological type, differentiation, invasion of veins, lymphatics and nerves, invasive depth to wall, lymph node metastasis and Dukes' stages. Co-mutation of K-ras and p53 gene had no correlation to clinicopathological factors or prognosis.

In conclusion, p53 and K-ras gene mutations have no effect on biological behaviours of tumor cells. The co-mutation of both genes has neither carcinogenesis-promoting effect, nor any effect on prognosis. Carcinogenesis and development of colorectal cancer involve changes of several genes, such as APC, myc, K-ras, MMC and p53. The accumulative effects of such genes may play a more important role during carcinogenesis than the order of change in these genes[12,16]. So, genetic therapy for colorectal cancer should target multiple genes involved in the process of carcinogenesis.

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