16-bp insertion polymorphism of p53 gene in gastritis lesion

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ABSTRACT

Aim: The purpose of this study was to assess the incidence of 16bp insertion of intron 3 p53 in gastritis lesion and its correlation with clinicopathological aspects.

Background: p53 alterations have been implicated in the development of gastric malignancies.

Patients and methods: 97 gastritis and normal adjacent tissues were investigated for p53 gene analysis using PCR—sequencing of intron3 and immunohistochetry technique.

Results: All of samples express p53 protein. In addition 64.9% of patients had no insertion and 7.2% had homozygous insertion while the others were heterozygous. This alteration has no association with clinicopathological findings.

Conclusion: Most of our patients had no insertion but an insertion frequency obtained here was found to be higher than those reports in a previous work on colorectal and esophageal cancer. It is possible that the polymorphism has correlation with gastritis and maybe with gastric cancer development. If so, this alteration associates with as yet unknown molecular and /or clinical factors which are involved in the (dys) regulation of p53 expression and function, and exerts its effects during gastritis development through them.

Keywords: p53 gene, Polymorphism, Gastritis lesion.

INTRODUCTION

As gastric cancer is the second cause of cancer among men and the forth in women in Iran (1) with a 5 year survival rate of 23.6% and the median life expectancy of 19.9 months (2), therefore it seems necessary to evaluate and determine molecular events which originate from non-cancerous lesions and lead to malignancy. Sequential changes in the gastric mucosa (3) may occur over a period of years as a result of exposure to a variety of exogenous and/or endogenous factors which cause genetic alterations such as p53 mutations and or select an allele at polymorphic sites. Therefore early detection of p53 alterations in precancerous gastric lesions such as gastritis may provide information, and can be useful for either the detection of patients prone to gastric cancer or the prevention from gastric cancer.

The human p53 gene is located on the short arm of chromosome 17 and spans 16–20 kb DNA (4). The tumor suppressor gene p53 encodes a key cellular component in maintaining genomic stability by either arresting cell cycle to allow DNA repair or by inducing apoptosis (5, 6) and therefore is essential for preventing aberrant cell proliferation following various intra and extracellular stimuli, such as DNA damages or hypoxia. After activation p53 emerges as a pivotal regulatory protein which triggers G1/S arrest through induction of p21cip1kip1 protein which per
se binds to and inhibits CDK2 from association with cyclin E. By the way p53 induces programmed cell death (apoptosis) in some cell types (7).

With regarding the importance of p53 as an essential brake in cell cycle progression it is clear that disruption of its function has a salient effect on the integrity of cell and confers a selective advantage for the tumor cells.

P53 gene alterations appear to be key factors in the development of gastric cancer (8) but as carcinogenesis in gastric tissue like other tissues occurs step by step, therefore it is necessary to evaluate molecular events in other histological level in addition to cancer.

P53 abnormalities occur frequently in tumors arising from gastric tissue and can be observed at relatively early stages of gastric carcinogenesis (8, 9), such as gastritis, dysplasia or intestinal metaplasia and subsequent genetic abnormalities enable these precancerous lesions to grow into neoplasms and ultimately gastric cancer.

This model of gastric carcinogenesis in which the mucosa evolves through a series of sequential changes, is extremely useful to study molecular alterations, because these alteration may occur at different steps of tumor development and also can serve as intermediate biomarkers. Several genetic events such as mutations or amplification of proto-oncogene as well as allelic deletions of TSG have been described in this sequence of premalignant changes (10) although the exact level/ order in which they work is still unclear.

Introns are integral elements of eukaryotic genomes that perform various important functions and actively participate in gene evolution. Several important functions of introns have been uncovered so far such as its role in alternative and trans-splicing and are active participants in gene regulation (11, 12). Therefore, it is valuable to assess introns to obtain new insight about its role during cancer development.

The 16 bp insertion in intron 3 of p53 (p53IN3) found to be associated with increased risk of several cancers such as colorectal (13), esophageal (14) lung (15, 16), breast (17, 18) and ovary cancer (19, 20).

In the esophagus cancer about 11% of patients had this duplication (14) and in colorectal cancer duplication was associated with reduced mRNA levels (13). According to study on breast cancer it was suggested that inheritance of an intronic polymorphism in the p53 gene increases breast cancer risk appreciably in women by the age of 50 years with a family history of breast cancer (18).

As so far little work was done on gastritis lesion in our country and with respect to the importance of p53 function for cell integrity and its intronic insertion during gastric cancer it deserved to evaluate p53IN3 variants in Iranian patients and to elucidate its correlation with clinicopathological aspects. Also so far there is no report about this insertion in gastric cancer and also this issue stimulated us more to do this study.

**PATIENTS and METHODS**

This study was approved by the ethics and scientific committee of our Institution. Gastritis lesions with matched normal tissue were taken from consenting patients who had undergone endoscopic evaluation of upper gastrointestinal tract in the Taleghani hospital from 2008-2009 (Tehran-Iran). Biopsies were removed with standard gastric biopsy forceps. Half the biopsy sample was sent for histological examination, while the other was stored at -20°C. The histological grading was performed by two pathologists according to the update Sydney classification. Patients with present or previous neoplastic disease, previous gastric surgery, and gastric or duodenal ulcer were excluded. In total 97 pair of gastritis and normal adjacent samples were included in this study. Also demographic data such as age and gender was gathered.
The DNA from gastric biopsy was extracted using DNeasy kit and QIAamp DNA Blood Mini Kit (QIAGEN) according to the manufacturer’s instructions.

Alteration of intron 3 of p53 gene was evaluated using primers spanning intronic splicing site in 97 cases of gastritis lesion together with normal adjacent tissue using PCR-sequencing. The primers used for PCR amplification were as follow:
Forward: 5' TCTCAGACACTGGCATGGTG 3'
Reverse: 5' GGCAAGGGGGACTGTAGATG 3'
PCR was carried out in 25 µl of reaction containing 1x PCR buffer, 37 mM MgCl₂, 10 pmol of each primer, 200 mM of each dNTP and 0.5 U Taq polymerase. PCR program was as follow: An initial cycle of 5 min at 94°C and then 30 cycles of 94°C for 30s, 62.1°C for 30 s, 72°C for 45 s that was concluded by 10 min at 72°C for final extension. Sequencing was done using ABI 3130X Genetic Analyzer.

Paraffin tissue sections of 4 μm thick were placed on a polyleysine-coated glass slide. Paraffin was removed from tissue sections by incubating at 60°C for 2 h and then washed 3 times in xylene for complete dewaxing. The sections were gradually rehydrated using alcohol and distilled water. Slides were then incubated for 10 min in 0.3% hydrogen peroxide in methanol to quench endogenous peroxidase activity. Antigen retrieval was achieved by boiling sections in 0.01 micromolar citrate buffer (PH= 6.0) in microwave oven (4 cycle, 10 min each, 300 w). Sections were then incubated with the primary monoclonal antibodies anti-p53 (clone DO-7, DAKO A/S, Denmark; dilution 1:50) which detected both the wild and mutant type. The reaction products were visualized by using a streptavidin biotin immunoperoxidase complex (DAKO, Denmark) as secondary antibody complex with diaminobenzidine (DAB). Sections were then counterstained with hematoxylin and mounted with permanent mountant DPX.

SPSS13 software was used to evaluate the association of p53 nucleotide alterations and clinicopathological findings (histological subtype, gender and age) by chi-square test and ANOVA. For all tests the significance level was set at 5%.

RESULTS
This study was done on samples from 41 (42.3%, mean age: 44.5±17.7) male and 56 (57.7%, mean age: 42.6±15.6) female in the age range of 15-83 years. Gastritis tissues were evaluated by our pathologists and were as follow: 33 moderate active chronic gastritis, 39 moderate chronic gastritis, 21 sever active chronic gastritis and 4 sever chronic gastritis. According to their declaration, they have neither alcohol consumption nor cigarette smoking history.

According to our study 64.9% of patients (63 patients) had no insertion (figure 1) and 7.2% (7 patients) had homozygous insertion (figure 2a) while the others (27 patients, 27.8%) were heterozygous (figure 2b). The allelic frequency for the polymorphism was as follow: 21.2% for duplicated allele and 78.8% for normal allele. This insertion and its types (homo or hetero) have no association with age (p 0.514) and gender (p= 0.23) (table 1), H.pylori infection detected using RUT (p= 0.273) and specific staining (p= 0.272), pathological subtypes (p= 0.578) and activity (p= 0.242).

Table 1. The correlation of age and gender with p53IN3 variations

<table>
<thead>
<tr>
<th>Insertion</th>
<th>Mean age</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Homozygous insertion</td>
<td>43.6±20.4</td>
<td>5(12.2)</td>
</tr>
<tr>
<td>No insertion</td>
<td>42.1±16.2</td>
<td>24(58.5)</td>
</tr>
<tr>
<td>Heterozygous insertion</td>
<td>46.5±16.3</td>
<td>12(29.3)</td>
</tr>
<tr>
<td>Total</td>
<td>43.4±16.5</td>
<td>41</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation; † Figures in parenthesis are in percent.
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Figure 1. Sequencing data of the 16 bp insertion. The red line depicts wild-type p53 sequence.

No nuclear staining for p53 protein was seen in gastritis and normal adjacent tissues.

Figure 2. Sequencing data showing the 16 bp insertion. a: Homozygous insertion, b: Heterozygous insertion. Black line represents the insert sequence and the starting nucleotide was shown by arrow.

DISCUSSION

As gastric cancer is one of the most prevalent cancers in Iran (1), therefore understanding the events involved in gastric cancer development seems necessary. If we know detours during cell proliferation and development processes especially in the beginning stages toward malignancy, it would be possible to manipulate and dominate over these aberrant processes to suppress the disease or lessen its progress. Therefore we explored p53 gene polymorphism at the gastritis level. P53 has a salient role in the genome stability and normal proliferation of cells and therefore its alteration lead to aberrant proliferation and maybe development of cancer (21).

According to our study most of patients have no insertion (ACCTGGAGGGCTGGGG, rs17878362) while the other was prominently heterozygout. The obtained frequency was higher than that of previously reported on colorectal and esophageal cancer (13, 14). Therefore, it can be inferred that this polymorphism has a correlation with gastritis and probably with gastric cancer development. However, this alteration was not associated with clinicopathological findings of current study. Maybe this polymorphism has some correlation and association with as yet unknown molecular and /or clinical factors which are involved in the (dys) regulation of p53 expression and function, and this insertion exert its effects during gastritis and most probably may lead to gastric cancer development through these processes.

Our samples have no nuclear staining for p53 protein although some of them have the insertion. This issue implies that at least the insertion has no effect on the translation of the protein. Generally there is discordance between IHC and p53 alteration. Some types of p53 gene alterations probably, don't result in the accumulation of p53 protein. For example no association was observed between the polymorphic genotypes and different alleles' frequency of p53 codon such as codon 72 and protein expression in gastric cancer (22).

Intron 3 of p53 is only 112 bp and it is possible that an increase of 15% (16bp) in its length could alter mRNA splicing or its expression and probably p53 functions (13). Therefore, detailed analysis would be required to evaluate the consequence of these sequence variants on RNA splicing, translation and the stability of the various transcripts. Also, it is worthwhile to evaluate its correlation with other genetic alterations of p53 gene such as codon 72 polymorphism. However we shouldn't exclude the effects of geographical and/or racial differences on the genomic alterations and susceptibility towards exo and endogenous carcinogens.

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