# Tumor Suppresser Gene p53 Expression in Premalignant Lesions and Gastric Carcinoma - Prognostic Value

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## Abstract

The aim of the study was to verify the presence of mutated tumor suppresser gene p53 in intestinal mucosa with histologically confirmed premalignant lesions and gastric carcinoma, and assess its prognostic value. The paper presents prospective study that included 50 patients with gastric adeno-carcinoma of intestinal type that were treated at Gastroenterohepatology Clinic, and 50 patients with histologically confirmed chronic atrophic *H. pylori* positive gastritis. In the mucosa biopsy samples, we analyzed presence, frequency and severity of inflammatory-regenerative, metaplastic and dysplastic changes. We typed intestinal metaplasia immunohistochemically and confirmed the presence of p53 onco-protein in antigen positive gastric carcinoma cells, and evaluated its prognostic value. Our results suggest that *H. pylori* acts as an initiator of inflammatory processes in gastric mucosa, which are followed by emergence of precancerous lesions. p53 is expressed late in carcinogenesis (14%) and as such, may be considered as an indicator of transformation of premalignant into malignant lesion.

KEY WORDS: intestinal metaplasia (IM), epithelial dysplasia, *Helicobacter pylori (H. pylori)*, p53.

## Introduction

Molecular dissection of gastric carcinoma development revealed involvement of numerous genetic alterations (1). Genetic instability of tumor suppresser genes inactivation and telomerase reactivation are responsible in the early stages of gastric carcinogenesis, while oncologene activation enhances growth factors and cytokine expression thus leading towards gastric carcinoma. Genetic instability stimulates gastric carcinogenesis in several ways(2). Somatic mutations in microsatellite sequences caused by DNA replication errors may present the source of genetic instability. Chromosome instability is caused by the replication of errors in telomere reductions coupled with telomerase reactivation. Normally, in the absence of telomerase activity, telomeres are progressively reduced with each somatic cell division and aging. The reduction may function as a mitotic clock used by cells to calculate divisions leading towards replicative cell maturity. Telomerase activity strongly correlates with the progression of malignancy (3,4). Inactivation of multiple tumor suppresser genes caused by either mutation or deletion is the most frequently observed genetic abnormality in gastric carcinoma. Anomalies in p53, APC, MCC, DCC genes were found in 40-60% tumors. Mutations in p53 are the most frequent genetic abnormalities in human carcinoma. p53 acts as a DNA linking factor and transcription regulator thus supporting genetic control over initiation of S phase of cell division. It also has an important role in apoptosis as well as final cell differentiation. In 1970 it was discovered as a cell protein, labeled viral oncoprotein, large tumor antigen (T-ag) in monkey cells transformed with SV40 - Simian virus. It was not identified in normal cells, while its levels significantly increase in transformed tumor cell lines (8). Accumulation of mutations at p53 locus occurs in the loss of alleles - loss of heterozygocity (LOH). The loss was recorded in the later events and the progression into carcinoma. In well differentiated gastric carcinoma coupled with chronic atrophic gastritis, mutations most frequently occur in A-T pairs with high incidence of transmission. p53 also correlates with aneuploidia, depth of tumor invasion and uncertain clinical outcome (9).

## Subjects and Methods

The paper presents prospective study of the potential for gastric adenocarcinoma development, which included three viewpoints: clinical, patho- histological and microbiological. The study included 50 patients with gastric carcinoma that were treated at the Gastroenterohepatology Clinic. All the patients were subjected to endoscopic examination and biopsy was performed in antropyloric region, lesser curvature and corpus. Carcinoma was histologically verified. We also analyzed sample of mucosa 1-2 cm removed from the border of tumor lesion. Biopsy samples were conserved in 10 % buffered neutral formalin, paraffin embedded and sliced with microtome into 5 μm thick sections. The sections were stained with Heamatoxylin and Eosin (HE staining), immunohistochemically for p53 antibodies. Based on well-defined histological criteria we graded inflammatory-regenerative changes as chronic superficial gastritis or chronic atrophic gastritis of the degree I, II and III. The degree of activity was defined either as active or dormant phase based on leukocyte infiltration. Using the standing criteria for simple separation of inflammatory-regenerative from dysplastic changes we defined epithelial dysplasia as slight, moderately severe and severe. Classification into one of the groups was conducted based on the analysis of 19 criteria that are visually graded 1-4. Following the assessment the slides were prepared for immunohistochemical analysis if IM and assigned into one of the three possible categories. The categorization was based on detailed analysis of mucines and morphological changes in epithelia. The analysis included degree of expansion and type of IM. Antibody monoclonal mouse anti human p53 protein (code No M7001, clone Do-7, lot 056 by Dako) was used in p53 visualization. p53 was targeted in inflammatory-regenerative changes in gastric carcinoma, various stages of dysplastic changes, in IM, and in carcinoma. Immunohistochemical status of p53 protein recognizes grade: 0 - no antigen positive cells, 1 - antigen present in less than 5 % cells, 2 - 5-25 % of antigen positive cells, and 3 - 25-50 % positive cells. Intensity of p53 coloration was graded as: 1 - pale yellow, 2 - darker-yellow, 3 - brown. According to the location of p53 positive cells we marked that they take up 1/3, 2/3 or 3/3 (the total) of the crypt length. Our intent was to determine correlation between the expression of the studied antigen and the degree of morphological change, and to explore prognostic value of p53 in pre-cancerous lesions in gastric mucosa as well as in gastric carcinoma.

## Results

Immunohistochemical staining confirmed presence of p53 tumor suppresser proteina in 7 subjects with gastric carcinoma. p53 was found in more than 25 % cells in 4 subjects while in 3 subjects it was present in less than 5%

| | | DEGREE OF CELLS POSITIVE FOR p53 ANTIGEN | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 |
| localization of p53 positive crypt cells | 1/3 | 0 | 0 | 0 | 0 | 0 |
| | 2/3 | 0 | 0 | 0 | 0 | 0 |
| | 3/3 | 0 | 3 | 4 | 0 | 0 |

TABLE 1.Degree of p53 oncoprotein expression with regards to the localisation in crypts as detected in the group of subjects with gastric carcinoma

cells. In all subjects p53 was found in cells along the total crypt length.

ONCO-PROTEIN P53 EXPRESSION

In our control group of subjects diagnosed with *H. pylori* positive chronic atrophic gastritis p53 was detected in one subject in more than 25 % cells taking up 2/3 of a crypt.

## Discussion

Tumor suppresser gene p53 is located in short hand of chromosome 17 (17p 13.1). It codes for nuclear phosphoprotein of 53 kD, which acts in cell cycle control. Normal protein coded by this gene labeled wild-type supports normal cell phenotype. A part of its function pertains to the control of DNA damage and unplanned DNA synthesis, while the ultimate effect of p53 (initiation of apoptosis) would depend on cell type and the extent of the detected DNA damage (12). Protein p53 acts as growth suppresser protein. It acts at G1/S restriction point of cell cycle by controlling the damages on DNA. In untransformed cells p53 protein level is very low with half-life of 20-40 minutes. It appears that the levels of p53 mRNA expression correlate with cells proliferation degree. Just before S phase p53 mRNA level increases 10-20 times, before it reverts to its normal low level in between cell divisions. It reaches its peak in organogenesis. In apoptosis p53 levels are also quite elevated (13). Intracellular levels of p53 increase 10-20 times as the cell cycle progresses in order to reach its maximum in the late G1 phase, just before S phase. Thus, the progression of cell cycle towards the succeeding S phase is blocked, and the time necessary for DNA repair provided. Hence, p53 protein modulates the genes' transcription process, monitors DNA synthesis and, when needed, induces apoptosis in G1 phase during the arrested cell growth (14,15). Mutations in p53 gene result from various environmental factors. When one allele is lost and the other sustains point mutations, the gene loses suppressers role which results in genetic instability in cells. In this way, tumor suppresser gene loses its role and, in certain cases gains ability to promote cell proliferation (16). This ability extends life time of inadequately controlled cells and they may become susceptible to additional genetic alteration, which lead them into malignant transformation (17). Semi-quantitative method of p53 antigen positive cells detection showed p53 presence in 7 (14%) subjects with gastric carcinoma. 4 out of 7 (57,14%) subjects were classified grade II, which means antigen presence in more than 25% cells. Coloration intensity was pale yellow and was found in cells along the total crypt length. In 3 out of 7 (42,86%) subjects it was classified grade I and was found in less than 5% cells. Coloration was pale yellow and was found in cells along the total crypt length. In the control group, antigen was positive in only one (2%) subject. That patient was diagnosed with atrophic gastritis grade II in active phase associated with moderately severe epithelial dysplasia as well as intestinal metaplasia. More than 25% cells were antigen positive and the case was classified as grade II. Coloration was dark yellow (grade III) and was found in the cells along the total crypt length. Our research shows alteration of p53 in 14% subjects with intestinal type gastric carcinoma. Among the cases of pre-malignant lesions, we identified p53 alteration in only one case of moderately severe dysplasia and chronic atrophic gastritis of grade II. Sasaki et al. states that p53 expression closely correlates with the progression of changes into carcinoma, and its levels are lower in early carcinogenesis and pre-cancerous lesions. Considering concurrence of the results we may accept this statement. Our results, which show p53 alteration in 14% patients with gastric carcinoma, concur with previous findings.

## Conclusion

Our results show expression of p53 protein as a late event in gastric carcinoma as well as chronic atrophic gastritis grade II in active phase with moderately severe dysplasia. This event is correlated with moderately severe dysplasia and gastric carcinoma and indicates progression of dysplasia as a pre-cancerous lesion into malignancy. Therefore, p53 protein cannot be used as an initial screening marker. However, it may be useful in the assessment of moderately severe and severe epithelial dysplasia reaction and its evolvement from pre-malignant lesion into gastric carcinoma.

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