

OVEREXPRESSION OF P53 IN GASTRIC CARCINOMAS AND IT'S CORRELATION WITH LAUREN AND GOSEKI CLASSIFICATION

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Key words: gastric cancer, P53

Abstract. Despite their importance, prognostic factors in gastric cancer are not yet well known. The study included surgical specimens from 40 patients with gastric carcinoma. All specimens were fixed in 10% buffered neutral pH formaldehyde and paraffin embedded. Histological sections were stained using current techniques: haematoxylin-eosin, tricromic van Gieson, and Alcian blue. We used Laurén histological classification with two main types of gastric carcinoma: intestinal and diffuse and Goseki classification with four main types of gastric carcinoma. We assessed p53 immunoreactivity. (DO 7 Biogenex). Using Lauren classification, immunoreactivity was positive for p53 in 62.5% of the cases: 7 cases of well differentiated intestinal low index gastric carcinoma, 1 case of poorly differentiated intestinal low to moderate index gastric carcinoma, 15 cases of diffuse moderate to high index gastric carcinoma. Using Goseki classification, immunoreactivity was positive for p53 in 62.5% of the cases: 3 cases with low index and 1 case with moderate index in type I, 3 cases with low index in type II, 9 cases with low to high index in type III and 6 cases with low to high index in type IV. The positivity for p53 was expressed in 66% of the gastric carcinomas with a coloring intensity growth pattern from the well differentiated types to the poorly differentiated types. The percentage of tumors positive for p53 did not correlate with their differentiation degree.

INTRODUCTION

Gastric adenocarcinomas make up 90-95% of all malignant stomach tumors (1). Due to endoscopic investigations correlated with histopathological examinations, early adenocarcinoma has been having increasingly good prognosis, contrary to advanced gastric carcinoma. Epidemiological, immunohistochemical (IHC) and progressive data also confirm the two types of most common histological variants, that is intestinal and diffuse gastric carcinomas (2).

Just as the WHO classification, Goseki classification (1992) relies on histological evidence. Tubular differentiation assessment and intracytoplasmic mucus amount determination enable us to distinguish 4 categories (3):

- Type I – well differentiated tubes and scarce mucus.
- Type II – well differentiated tubes and abundant mucus.
- Type III – poorly differentiated tubes and scarce mucus.
- Type IV – poorly differentiated tubes and abundant mucus.

Numerous studies dwelt on IHC used for prognosis determination, together with protein gene products.

Normal P₅₃ protein has an extremely short life and can be found in very low amounts inside the cells. It cannot be detected in normal cells using IHC techniques. P₅₃ protein produced by the abnormal *p53* gene has a much longer life than normal P₅₃, it gathers inside malignant cells and may be IHC detected by means of Ac anti P₅₃. Theoretically, P₅₃ overexpression as a surrogate marker of gene *p53* mutations is an IHC “test” employed to determine neoplasia – dysplasia. In practice however, we cannot say the same since a correlation between stain and gene *p53* abnormalities is not precise.

Therefore, the purpose of this study is to assess p53 immunoreactivity in gastric carcinoma and its correlation with Lauren and Goseki classification.

MATERIAL AND METHODS

The study material consisted of surgical specimens from 40 patients diagnosed with gastric neoplasm. All specimens were fixed in 10% buffered neutral pH formaldehyde and paraffin embedded. Histological sections were stained using current techniques: haematoxylin-eosin, tricromic van Gieson, Alcian blue, and PAS, which enabled us to classify injuries and determine their differentiation. We employed Lauren and Goseki classification to assess the histological types. (4)

Immunohistochemical stain using the Avidin-Biotin Complex method was performed by means of the Optimax autostainer manufactured by Biogenex. Pretreatment consisted of 12-15min. boiling in citrate solution followed by 2 hours incubation with p53 antigen (DO7 Biogenex – Ready to use). DAB viewing followed by a slight haematoxylin stain (1min) revealed p53 under the form of a brown reaction color with nuclear location (5,6,7).

Statistical processing was performed using the Spearman Rank R Correlation Test, Chi-Square Anova tests.

RESULTS AND DISCUSSIONS

P₅₃ expression in the 40 cases under survey was graded according to a scale based on the percentage of tumor cells with positive nucleus (8)

- 0 negative stain;
- + positive reaction in less than 10% of the tumor cells nuclei;
- ++ positive reaction in less than 10% of the tumor cells nuclei, however less than 33%;
- +++ positive reaction in less than 33% of the tumor cells nuclei.

We interpreted a positive p53 reaction, with distinct homogenous or granular nuclear stain. The reaction product was brown (9).

We also considered that tumors overexpress p₅₃ protein only when we obtained an intense nuclear stain in any of the 3 patterns. We considered negative the cases with an equivocal or poor nuclear stain. Likewise for the cases with extremely scarce or isolated positive nuclei.

We found no positive p₅₃ cells on the normal gastric epithelium neighboring the tumor and the negative witness slides. We detected an overexpression of the P₅₃ protein in 25 (63 %) of the 40 gastric tumors under survey, with a stain intensity and stain pattern heterogeneity (**Table 1**).

Table no.1 *p₅₃ overexpression in the 40 cases under survey*

P ₅₃	No. cases	%
0	15	37.5%
+	9	22.5%
++	6	15%
+++	10	25%
Total	40	

By correlating p₅₃ overexpression with Lauren histological classification we identified the following:

- 8 of the **12 intestinal ADK** (66.6%) have p₅₃ overexpression. This overexpression was marked + in 7 of the cases and ++ in 1 of the cases (this case being moderately differentiated, (**Fig. 1, 2**);
- 15 of the **26 diffuse gastric carcinomas** (57.6%) have overexpression p₅₃ overexpression. This overexpression was marked ++ in 5 of the cases and +++ in 10 of the cases (**Fig. 3**)
- the 2 mixed forms both had P53 protein overexpression, one being marked + and the other ++.

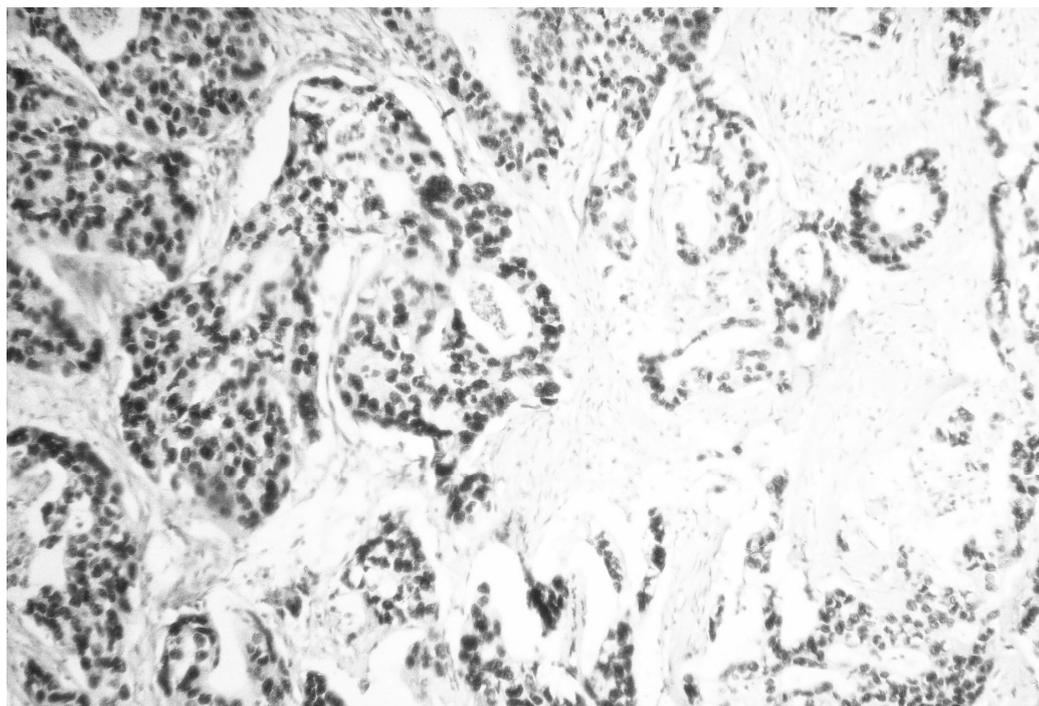


Fig. 1. *Distinct homogeneous or granular nuclear stain revealing p₅₃ (++) in a moderately differentiated ADK, IHC x40*

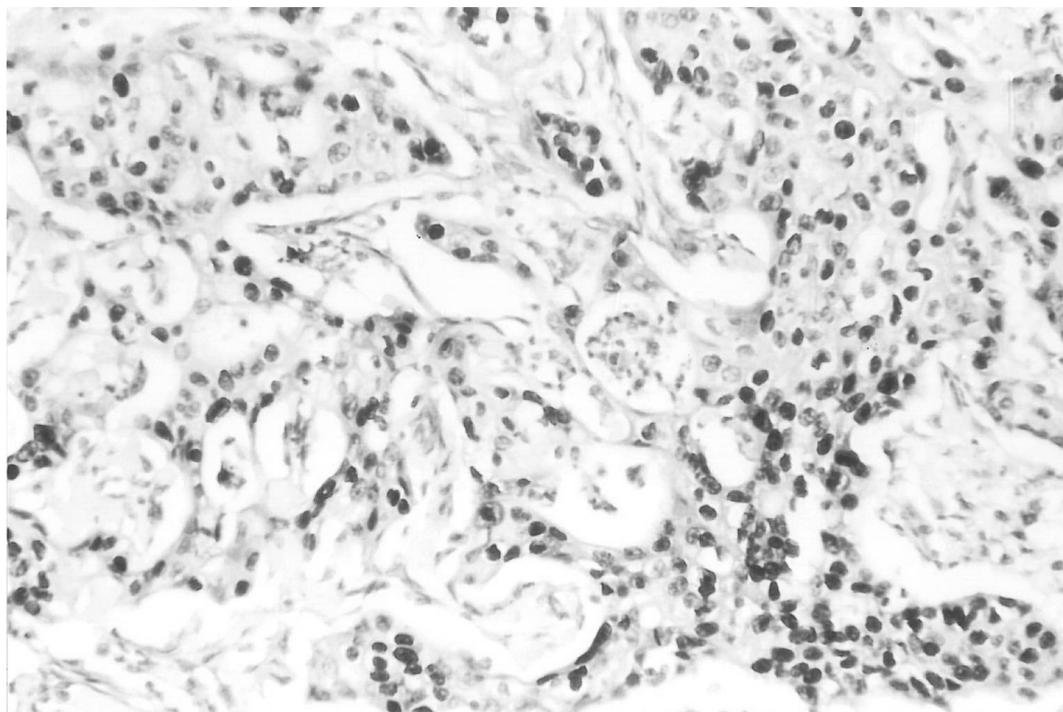


Fig. 2. *Distinct homogeneous or granular nuclear stain revealing p₅₃ (++) in a moderately differentiated ADK, IHC x40*

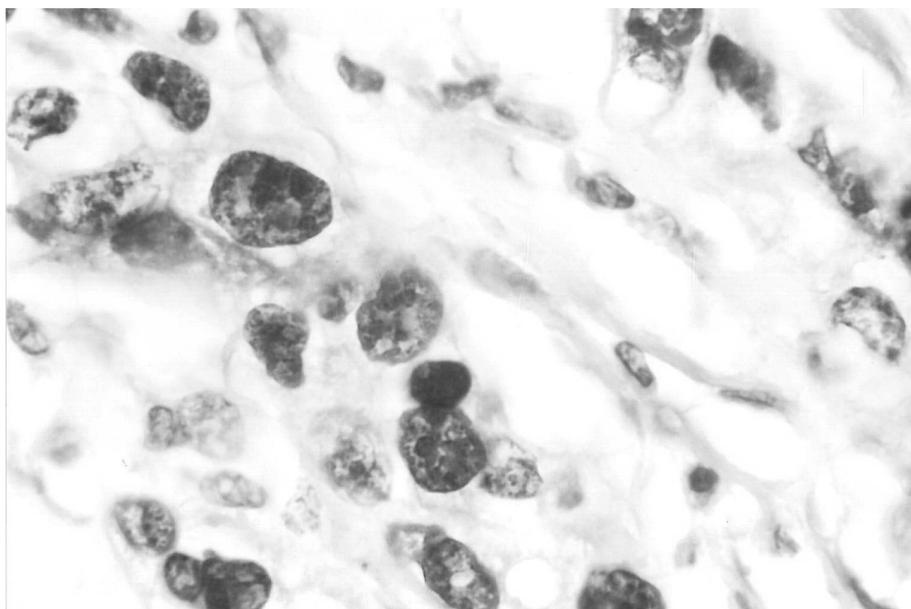


Fig. 3. Distinct homogeneous or granular nuclear stain revealing p₅₃ (+++) in a diffuse gastric carcinoma, IHC x100

Both the parametric Chi-square test and the nonparametric correlation coefficient reveal the existence of a significant correlation between p53 overexpression and Lauren classification ($\chi^2=28.44$, $p=0.00476$, $r=0.6319$, 95%CI) (Table 2, 3).

Table no. 2 Parameters considered for the p53 association vs. Lauren classification testing

	Chi-Square χ^2	df	p 95% confidence interval
Chi-Square - χ^2	28.44874	df=12	p=0.00476
Yates Chi-Square	29.71917	df=12	p=0.00308
Correlation coefficient (Spearman Rank R)	0.630572		p=0.01088

By correlating p₅₃ overexpression with Goseki classification we identified the following:

- Type I – 4 (50%) of 8 cases experienced p₅₃ overexpression. This overexpression was marked + in 3 of the cases and ++ in 1 of the cases (whose correspondent was the moderately differentiated form of the Lauren classification) (Fig. 4).

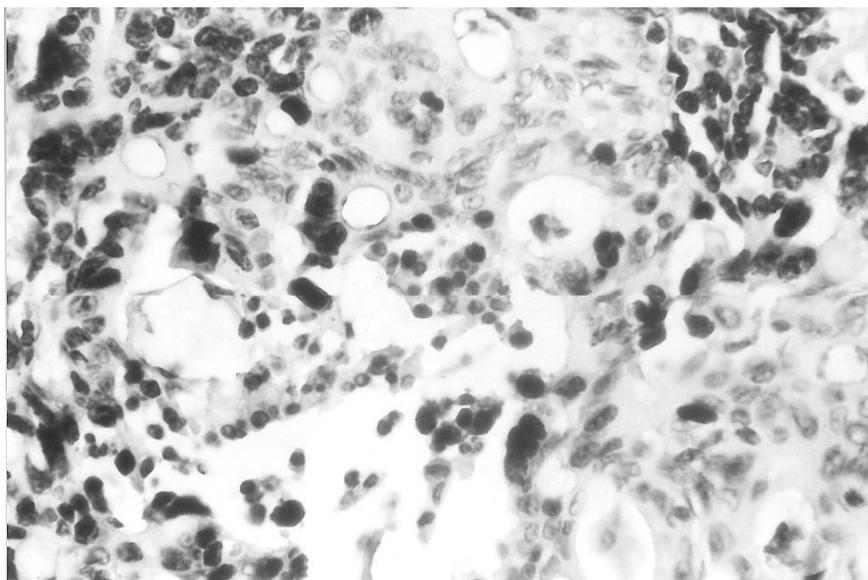


Fig. 4. *Distinct homogeneous or granular nuclear stain revealing p₅₃ (++) in a moderately differentiated ADK, IHC x40*

- Type II – 3 (75%) of 4 cases experienced p₅₃ overexpression marked +.
- Type III – 11 (68.8%) of 16 cases experienced p₅₃ overexpression marked + in 2 cases, ++ in 2 cases and +++ in 7 cases
- Type IV – 7 (58.3%) of 12 cases experienced p₅₃ overexpression marked + in 1 case, ++ in 3 cases and +++ in 3 cases (**Fig. 5**)

The 2 mixed cases in the Lauren classification were, in the Goseki classification, a mixture of the I and III forms, both with p₅₃, + overexpression.

The study of the p53 association vs. Goseki classification reveals a significant correlation between p53 overexpression and Goseki classification ($\chi^2=46.47$, $p=0.009$, 95%CI), accounted for by a high number of cases that have no p53 and belong to class I, as well as by a high percentage of cases with p53 (++, +++) and belonging to class IV. (**Table 3**)

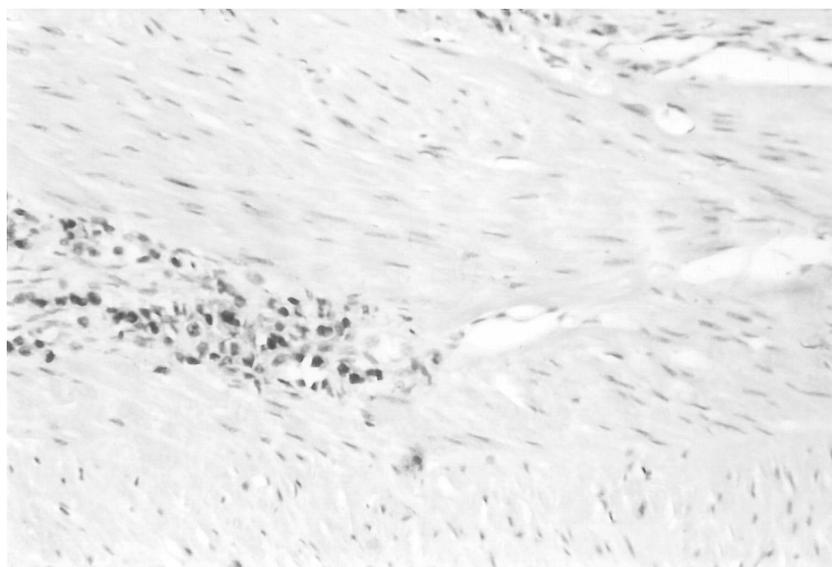


Fig. 5. Distinct homogeneous or granular nuclear stain revealing p_{53} (+++) in a diffuse gastric carcinoma. Muscle invasion, IHC x20 stain

Table no. 3 Parameters considered for the p_{53} association vs. Goseki classification testing

	Chi-square χ^2	df	p 95% confidence interval
Chi-square - χ^2	44.95370	df=9	0.009223
Yates Chi-square	46.47770	df=9	0.005757
Correlation coefficient (Spearman Rank R)	0.5450		0.025434

P_{53} suppressor gene (“wild” type) induces apoptosis, while the mutant protein blocks the function of the “wild” form, inhibiting apoptosis induction. Mutant p_{53} protein accumulation may behave as an immunogenic agent, generating anti- p_{53} antibodies. P_{53} positive immunoreaction is correlated with the amount of mutant p_{53} gene, being able to reveal cell proliferation and apoptosis inhibition; it is mainly observed in the nucleus, as a brown deposit.

In normal circumstances, p_{53} is considered the “genome guardian” (p_{53} protein is a transcription factor monitoring genome stability) or a “dual gene”, since in its normal state it works as antioncogene that blocks cell cycle allowing modified DNA repair, and in its modified state it becomes oncogenic.

DNA alteration triggers p_{53} protein accumulation, which stimulates the *cip1* gene, which in its turn encodes a protein called p_{21} (21 KDa) and inhibits cyclic dependent kinases (p_{21} is also called cip-cdk inhibitor protein). Cell proliferation is thus blocked. When mutation repair is insufficient by cell cycle blocking in G1, then p_{53} induces cell death by apoptosis, designed to prevent daughter cell flaw perpetuation. (10).

Gene p_{53} mutation (tumor suppressor gene) occurs in 50% of the breast cancer patients. P_{53} protein loss and/or alteration further to gene rearrangement may result into cell growth derangement, by replication flaws and genetic accumulations.

Replication is not blocked in cells where p₅₃ is inactive, which leads to genetic instability. This instability is expressed during cell division, favoring a quick aneuploid clones or rearranged cells selection.

Systemic gene p₅₃ mutation is the most common genetic event occurring in human cancers, as it leads to the inactivation of the specific protein p₅₃-to-DNA bonding function.

Allelic TP₅₃ loss occurs in over 60% of the gastric carcinomas, and mutations are found in about 30-50% of the cases. However, these percentages also depend on the mutation screening method and on the size of the specimen. TP₅₃ mutations are identified in some cases of intestinal metaplasia, most of the alterations being characteristic of advanced tumors (11).

TP₅₃ mutations in gastric injuries are similar to those found in other mainly base transition cancers, especially at CpG nucleotide level.

Systemic mutations determine spatial p₅₃ protein configuration by altering the amino acids sequence. Conformation alterations triggered by these mutations (considered critical malignant cell transformation stages) are associated with gene p₅₃ product stabilization (12) and mutant protein accumulation in the nuclei of the tumor cells in sufficient quantities to enable their IHC detection. (13).

In order to study the prognostic meaning of protein P₅₃ overexpression and its prognostic involvement, we analyzed its expression using the monoclonal anti-p₅₃ antibody (Biogenex DO₇-AM).

- Positive p₅₃ immunoreaction found in 62.5% of the cases allowed the identification of nuclear protein accumulation tumors, as well as the subclassification of p₅₃ positive tumors according to the immunocoloring pattern and the percentage of positive cells in each injury.
- Lauren and Goseki classifications together with p₅₃ overexpression enabled us to notice that well differentiated forms (type I in Lauren classification, types I and II in Goseki classification) experienced a predominant stain intensity marked + or ++. The percentage of positive p₅₃ tumors belonging to this category varied, ranging from 66% for the type I of the Lauren classification and 50, that is 75%, for the types I and II of the Goseki classification.
- In the diffuse forms (type II in Lauren classification and III and IV, respectively, in Goseki classification), we noticed a stain intensity marked ++ and +++, respectively. The percentage of positive tumors varied from 57.6% (type II in Lauren classification and 64.2, that is 50%, in types III and IV of the Goseki classification).
- If from the viewpoint of the coloring pattern intensity we may state that it grew from well differentiated forms marked + to poorly differentiated forms marked +++ anticipating an aggressive behavior from the standpoint of the percent of positive tumors, we noticed a higher number of p₅₃ positive tumors in well differentiated forms (type I in Lauren classification 62.5%, type I – II in Goseki classification, 50-75%) as compared to poorly differentiated forms (type II in Lauren classification 57.6%, type III, IV in Goseki classification 64.2% and 50%, respectively). This enables us to conclude that p₅₃ as sole prognostic indicator is not sufficient, which is accounted for by that codon 72 polymorphism, that is by the fact that this is more frequently encoded by a proline then by an arginine. Or, it is well known that the latter is very common in antral gastric cancers.

- Another conclusion drawn from here would be that these facts could explain why a histologically well differentiated tumor, which we could expect to have a good prognosis, may have an aggressive behavior, thus contradicting its histopathological description.

CONCLUSIONS

Positive p₅₃ occurrence in the 40 cases under survey was expressed in 66% of the gastric carcinomas, with an increased coloring pattern from well differentiated forms marked + to poorly differentiated forms marked ++++. This evolution foresees the tumors' aggressive behavior.

As for the percentage of p₅₃ positive tumors, there is no relation between the percent of positive tumors and their differentiation, which makes us conclude that p₅₃ as sole prognostic indicator is not relevant.

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