Predictor of progression in gastric carcinoma: 17p(p53) loss of heterozygosity displays an increased risk for cancer progression

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Abstract

Background/aims:: To study the frequency of 17p(p53) loss of heterozygosity (LOH) and increased 4N and /or aneuploidy in gastric adenocarcinoma and chronic atrophic gastritis(CAG). We reported 17p(p53)LOH, may be an important prognostic factor in early gastric carcinoma.

Methods: We have used the endoscopic biopsies from patients for DNA extraction and for flow cytometric abnormalities. The PCR was carried out by using a set of primers containing codon 72 of exon 4 of the p53 gene. Amplified fragments were subjected to restriction enzyme BstUI for detecting 17p(p53) LOH. Biopsy specimens were processed for flow cytometry. Ki-67/DNA content was used to see increased 4N or aneuploid fractions.

Results: 17p(p53)LOH was observed in (21 of 41) 51.21% of gastric adenocarcinomas and in (13 of 43) 30.23% chronic atrophic gastritis(CAG). Similarly, increased 4N and/or aneuploidy was detected in (18 of 41) 43.9% of adenocarcinomas and in (11 of 43) 25.58% of CAG.

Conclusions: 17p(p53) LOH may be a predictor of progression in gastric carcinoma and role of neoplasm processing that can be combined with a panel of other validated biomarkers for risk assessment such as family history, biochemical and histopathological markers.

INTRODUCTION

A high incidence of gastric adenocarcinoma has been regularly seen in the eastern region of Turkey for years([1]). The development of as gastric cancer in humans has been shown to be a multi step process, ranging from chronic gastritis to atrophy, intestinal metaplasia, dysplasia and finally gastric cancer([2,3,4,5]).

We reported that development of gastric cancer, based on 17p(p53) LOH, could be important prognostic factor in early gastric carcinoma. There is a type of early gastric carcinoma with a poor prognosis, mainly due to early hematogenous recurrence([6,7,8]). It has, therefore, been suggested that early carcinoma of the stomach involves various patterns of growth related to the mode recurrence and prognosis. Mutation or allelic deletion of p53 gene appears to play an important role in the development of human carcinoma([9,10,11]). It has been established that accumulation of wild-type p53 protein results in two pathways; Cell cycle arrest and programmed cell death, which together are involved in tumor suppressor functions([12]). Therefore mutation of p53 leads to disruption of these pathways, a selective growth advantage for tumor cells and loss of function may result in increased proliferation activity and tumor development([13,14]). However, multiple somatic genetic lesions develop during the multiple step progression to cancer and it is likely that a single biomarker will assess only one or a limited number of stages of progression. Therefore, a panel of biomarkers will probably be needed for risk stratification for endoscopic surveillance and intervention strategies tailored to stage of progression ([15]).

p53 gene is a cell cycle control gene that prevents cells with DNA breaks from entering DNA synthesis where the breaks could be replicated cause chromosome damage and lead to progressive genetic instability and cancer ([16,17]). The p53

gene is located at chromosome 17p (p53 locus). Most people inherit two normal alleles of p53, one from the mother and one from the father. Inactivation of the p53 gene involves a two-step mechanism consisting of a point mutation on one allele, consistent with Knudson's two-hit hypothesis. One copy is typically inactivated by mutation, whereas the second copy is lost by a mechanism called 17p loss of heterozygosity (LOH). p53+/+ cells respond to genotoxic injury by undergoing cell cycle arrest or programmed cell arrest or programmed cell death. However, p53-/- cells do not arrest in the presence of DNA damage and continue to proliferate, accumulating genetic lesions that lead to cancer ([12,13,15,18]).

The p53 gene has been strongly implicated as the target for 17p(p53) LOH, since p53 mutations have been frequently reported in gastric carcinoma ([19,20,21,22,23,24]). Inactivated p53 by 17p (p53) LOH and mutation seems to be early event in neoplastic progression in gastric carcinomas, because it develops in diploid cells before aneuploidy and other LOH events involving choromosomes 1, 5, 6,7,10, 11 and 12 ([25,26,27,28,29,30]).

Thus, 17p(p53) LOH is a promising biomarker for assessing risk of neoplastic progression in gastric carcinoma. In this study, we demonstrated that LOH involving p53 occurs in 51.21% of gastric cancers and 30.23% CAG. We aimed to evaluate biopsies of gastric cancer and CAG related to p53-LOH and aneuploidies.

MATERIALS AND METHODS

PATIENTS

A hundred patients who had gastric complains, no history of gastric malignancy and an endoscopic biopsy evaluation were involved in the study in University hospital of Erzurum that is eastern part of Turkey between January 2004 and July 2006.

A total of 100 patients, including 68 men and 32 women underwent basic drug treatment to modulate stomach acidity. 84 of these have had endoscopies. Biopsies were pathologically assessed and 41 of then were diagnosed as gastric carcinoma and 43 of them were diagnosed as chronic atrophic gastritis (9 negative for dysplasia , 5 indefinite ,10 low-grade dysplasia and 19 high-grade dysplasia).

ENDOSCOPY AND BIOPSY

Multiple biopsies were taken and endoscopically visible abnormalities samples were sent to pathology. Biopsies were classified according to the extent of abnormality [negative for dysplasia, indefinite, low-grade dysplasia (LGD), high-grade dysplasia (HGD) in chronic atrophic gastritis (CAG), and gastric cancer] interpretations were made without knowledge of 17p(p53)LOH or flow cytometric status.

PCR ANALYSIS

The PCR was performed by using one thermal cycle (PE 9700) with 50 ng of genomic DNA extracted from biopsy samples, 20 pmol of each primer, all four deoxynucleotide triphosphates (dNTPs), reaction buffer and 1µl (5 units) of fermentase Taq polymerase in a reaction volume of 100 µl. The PCR reaction consisted of an initial cycle of at 95°C 10 min, at 57°C 1 min and at 72°C 10 sec, followed by 34 cycle of at 95°C 1min, at 57 °C 30 sec and 72 °C 10 sec.

The primer used were

Sens: 5'- CAG ATG AAG CTC CCA GAA-3'(upstream)

Anti-sens: 5'- GTG TAG GAG CTG CTG GTG-3'(downstream)

The amplicon of 66bp was produced which was cleaved in to fragments of 29 and 37 bp with the enzyme BstU1, if its recognition site (CGCG) was present. The primer pairs amplified a region containing codon 72 in exon 4 of p53 (27,28).

FLOW CYTOMETRY

All collected biopsy samples were frozen in DMSO and kept at – 60 °C. These samples were processed by Ki-67/DNA content flow cytometry as described in Reid B. J. et al ([29]). Ki-67 recognize an antigen expressed in G1, S and G2, but not G0 phases of cell cycle ([30]). Evaluation and classification were made according to Reid B.J. et al., study ([29]). The content of Ki-67/DNA for flow cytometry represented, Ki-67/positive G, (2N) and increased 4N or aneuploid. Interpretations of flow cytometric results were evaluated without knowledge of histological status of biopsies

STATISTICAL ANALYSIS

Statistical analysis were performed using Pearson χ^2 test and Fisher's exact test. The data were computerised and statistical tests were performed with the program, Statistical Package for Social Sciences (SPSS version 10.05). The tests were considered significant when their overall p values were below 0.05.

RESULTS

We showed that PCR digestion results obtained from 41 of the 84 biopsy samples which were diagnosed as gastric carcinoma, (all adenocancer) and 43 samples CAG. Primer anneal on either side of a BstU1 polymorphism were demonstrated 51.21% 17p(p53) LOH for 21 of 41 adenocancer and 30.23% 17p(p53) LOH for 13 of 43 gastritis. Amplicon of 66 bp is produced (Figure 1). Amplicons from cancer and non-cancer samples (CAG) are then digested with BstUI and run on the PAGE (Figure 2). 17p(p53) LOH was common in well differentiated biopsies of gastric carcinomas.

Figure 1

Figure 1: Analysis of the exon 4 region of the p53 gene in gastric cancer and chronic atrophic gastritis. Amplification of the within exon 4 produces a segment 66 bp long. Lane 1, DNA marker(puc 18 Hae III Digest 587-11bp, sigma). Lanes 2 and 3, wellÂ–differentiated adenocarcinoma ; Lanes 4 and 5, high-grade dysplasia.

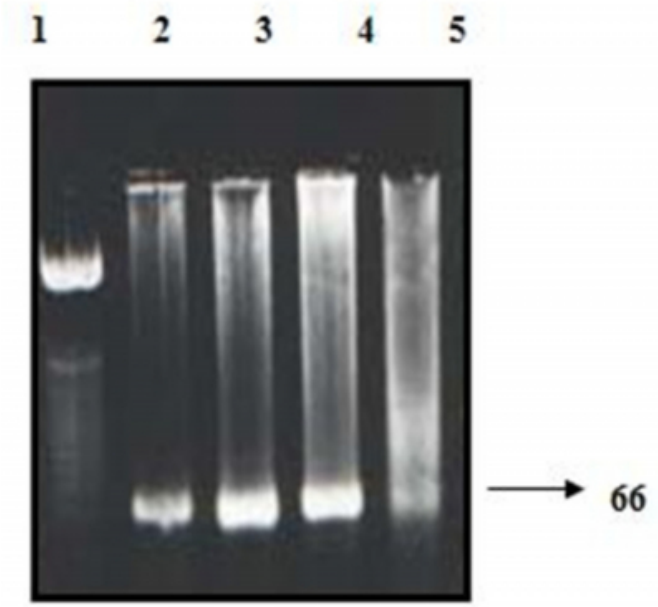
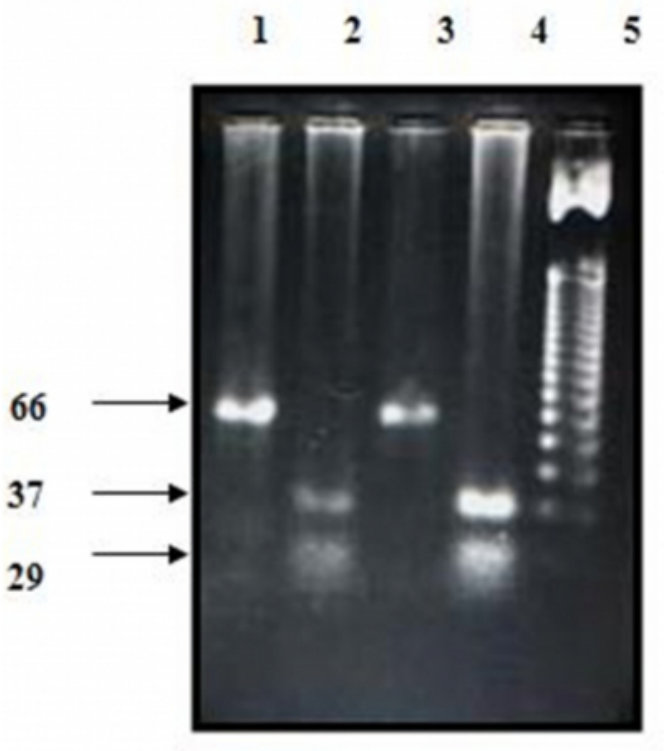


Figure 2

Figure 2: Amplification of the BstU1 within exon 4 produces a segment 66 bp long; cleavage results in fragments 37 and 29 bp long. Lanes 1 and 2, wellÂ–differentiated adenocarcinoma, Lanes 3 and 4, high-grade dysplasia. Lane 5, DNA marker. Lanes 2 and 4, cases with LOH at p53 loci. Lanes 1 and 3, there is no loss of heterozygosity.



PREVALENCE OF FLOW CYTOMETRIC ABNORMALITIES (INCREASED 4N, AND ANEUPLOIDY) AND 17(P53) LOH OF THE GASTRIC CARCINOMAS SAMPLES

Flow cytometric analysis of the gastric cancer samples showed increased 4N and/or aneuploid cell populations (18 of 41 flow cytometric content of 43.9%). Prevalence of 17p(p53) LOH, increased 4N fractions and aneuploidy were observed in different grades of malignancies at the baseline endoscopy for patients, which are represented in Figure 3. For flow cytometric analysis, the prevalence of elevated 4N and/or aneuploidy increased from (3 of 8) 38% in patients who were poorly differentiated to (4 of 12) 33% in patients who had moderately differentiated and (11 of 21) 52% who were well differentiated (Table 1). 17p(p53) LOH increased from (2 of 8) 25% in patients whose biopsy samples were poorly differentiated to (5 of 12) 42% in patients who had moderately differentiated and (14 of 21) 67% in patients who had well differentiated (Table 1).

Figure 3

Figure 3: percentage distribution of patient with LOH and flow cytometric abnormalities (elevated 4N, aneuploidy) in gastric carcinoma. The Y axis presents percentage of patients, X axis gives histological statues. PDAC: Poorly differentiated adenocarcinoma; MDAC: Moderatelly differentiated adenocarcinoma; WDAC: well-differentiated adenocarcinoma.

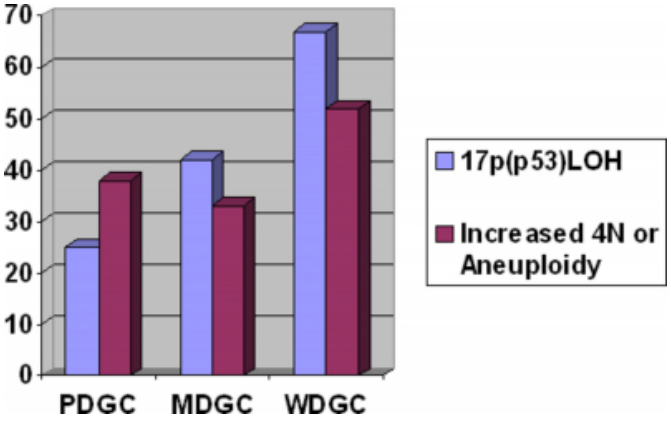


Figure 4

Table 1: 17p(p53) LOH and Increased 4N or Aneuploidy with Clinicopathologic Correlations in Gastric Adenocarcinomas

Clinico-pathological Features	Number 17(p53)LOH			Number Increased 4N or Aneuploidy		
	Positive	Negative	Total	Positive	Negative	Total
Age						
60≤	11(55%)	9(45%)	20(100%)	9(45%)	11(55%)	20(100%)
60> (mean=60yr,Range=47-76)	10(48%)	11(52%)	21(100%)	9(43%)	12(57%)	21(100%)
	P=0.6; Pearson χ^2 test			P=0.9; Pearson χ^2 test		
Gender						
Male	14(50%)	14(50%)	28(100%)	14(50%)	14(50%)	28(100%)
Female	7(54%)	6(46%)	13 (100%)	4(31%)	9(69%)	13(100%)
	P=0.8; Pearson χ^2 test			P= 0.3; Fisher's test		
Localization of tumor						
Corpus	11(79%)	3(21%)	14(100%)	7(50%)	7(50%)	14(100%)
Antrum	9(64%)	5(36%)	14(100%)	7(50%)	7(50%)	14(100%)
Cardia	1(9%)	10(91%)	11(100%)	4(36%)	7(64%)	11(100%)
Fundus	0(0%)	2(100)	2(100%)	0(0%)	2(100%)	2(100%)
	P=0.002; Pearson χ^2 test			P=0.5; Pearson χ^2 test		
Histologic type						
Tubular	11(52%)	10(48%)	21(100%)	7(33%)	14(67%)	21(100%)
Mucinous	7(58%)	5(42%)	12(100%)	8(67%)	4(33%)	12(100%)
Papillary	1(20%)	4(80%)	5(100%)	2(40%)	3(60%)	5(100%)
Signetring cell	2(67%)	1(33%)	3(100%)	1(33%)	2(67%)	3(100%)
	P=0.5; Pearson χ^2 test			P=0.3; Pearson χ^2 test		
Tumor differantiation*						
Well	14(67%)	7(33%)	21(100%)	11(52%)	10(48%)	21(100%)
Moderately	5(42%)	7(58%)	12(100%)	4(33%)	8(67%)	12(100%)
Poorly	2(25%)	6(75%)	8(100%)	3(38%)	5 (62%)	8(100%)
	P=0.1; Pearson χ^2 test			P=0.5; Pearson χ^2 test		

*Tumors were classified according to the procedure of Lauren (38).

There was no significant variation in p53 LOH frequency among different histological types and the degree of histological tumor differentiation. We found that there was positive correlation between prevalence of 17p(p53)LOH in localization (p=0.002) of gastric cancers. No significant correlation was found between incidence of 17p(p53)LOH and other clinicopathologic factors. Similarly, the increased 4N or aneuploidy was not related to tumor differantiation and other clinicopathologic factors in gastric cancers.

Prevalence of Flow Cytometric Abnormalities (Increased 4N, and Aneuploidy) and 17(p53) LOH of the Chronic Atrophic Gastritis Samples

Flow cytometric analysis of the chronic atrophic gastritis samples showed increased 4N or aneuploid cell populations (11 of 43 flow cytometric content of 25.58%). Prevalence of 17p(p53) LOH, increased 4N fractions and aneuploidy were observed in differend histological grades of chronic atrophic gastritis, which are shown in Figure 4. For flow cytometric analysis, the prevalence of elevated 4N and/or aneuploidy increased from (8 of 19) 42% in patients who were HGD to (2 of 10) 20% in patients who had LGD and (1 of 9) 11% negative for dysplasia (Table 2). 17p(p53) LOH decreased from (9 of 19) 47 % in patients whose biopsy samples were HGD to (3 of 10) 30% in patients who had LGD and (1 of 5) 20 % in patients who had indefinite for dysplasia (Table 2).

There was correlation between prevalence of 17p(p53) LOH in histological type(p=0.04) of CAG. The prevalence 17p(p53)LOH was not related to patients' age, sex, localization and histological grade of CAG. Similarly, no relationship was detected between increased 4N or aneuploidy and patients' age, sex, localization , histological grade and histological type of CAG (Table 2).

Figure 5

Figure 4: percentage distribution of patient with LOH and flow cytometric abnormalities (elevated 4N, aneuploidy) as a function of histological type in gastric precancerous lesions. The Y axis presents percentage of patients, X axis gives histological statues. CAGND: chronic atrophic gastritis negative for dysplasia; IND: indefinite for dysplasia, LGD: low-grade dysplasia; HGD:high-grade dysplasia

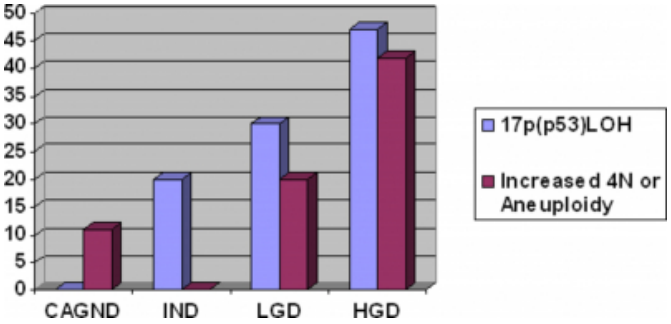


Figure 6

Table 2: 17p(p53) LOH and Increased 4N or Aneuploidy with Clinicopathologic Correlations in Chronic Atrophic Gastritis

Clinico-pathological Features	Number 17p(53) LOH			Number Increased 4N or Aneuploidy		
	Positive	Negative	Total	Positive	Negative	Total
Age						
51≤	7(35%)	13(65%)	20(100%)	6(30%)	14(70%)	20(100%)
51> (mean=51yr,Range=32-70)	6(26%)	17(74%)	23(100%)	5(22%)	18(78%)	23(100%)
	P=0.5; Pearson χ^2 Test			P=0.5; Pearson χ^2 test		
Gender						
Male	12(31%)	22(69%)	34(100%)	9(26%)	25(74%)	34(100%)
Female	1(11%)	8(89%)	9 (100%)	2(22%)	7(78%)	9(100%)
	P=0.2; Fisher's test			P= 1; Fisher's test		
Localization						
Corpus	4(29%)	10(71%)	14(100%)	6(43%)	8(57%)	14(100%)
Antrum	4(31%)	9(69%)	13(100%)	2(15%)	11(85%)	13(100%)
Cardia	3(43%)	4(57%)	7(100%)	2(29%)	5(71%)	7(100%)
Fundus	2(22%)	7(78%)	9(100%)	1(11%)	8(89%)	9(100%)
	P=0.84; Pearson χ^2 test			P=0.27; Pearson χ^2 test		
Histologic type						
Displasia	13(38%)	21(62%)		10(29%)		24(71%)
CAG+	0(0%)	9(100%)		1(11%)		8(89%)
	P=0.04; Fisher's test			P=0.4; Fisher's test		
Histologic Grade						
HGD+ +	9(47%)	10(53%)	19(100%)	8(42%)	11(58%)	19(100%)
LGD +++	3(30%)	7(70%)	10(100%)	2(20%)	8(80%)	10(100%)
Displasia Negative	0(0%)	9(100%)	9(100%)	1(11%)	8(89%)	9(100%)
Indefinite	1(20%)	4(80%)	5(100%)	0(0%)	5(100%)	5(100%)
	P=0.08; Pearson χ^2 test			P=0.13; Pearson χ^2 test		

+CAG: chronic atrophic gastritis; ++HGD:high-grade dysplasia; +++LGD:low-gradedysplasia,

This pattern of results show that more frequent 17p(p53) LOH arise rapidly as a quite late event during gastric cancer.

CORRELATION BETWEEN 17P(P53) LOH AND INCREASED 4N AND/OR ANEUPLOIDY

Of 21 17p(p53)LOH positive tumors, 13 (62%) showed 4N increased or aneuploidy. The incidence of 17p(p53)LOH was higher in the 4N increased or aneuploidy positive tumors than in the other tumors (p=0.017) (Table 3). Of 13 17p(p53)LOH positive benign biopsies, 8 (61%) showed 4N increased or aneuploidy. We found association between the presence of LOH and 4N increased or aneuploidy of CAG in our study(p = 0.001) (Table4).

Figure 7

Table 3: Correlation Between 17p(p53) LOH and 4N Increased Aneuploidy in Gastric Adenocarcinoma

	4N increased or Aneuploidy positive	4N increased or Aneuploidy negative	Total
17p(p53) LOH positive	13(62%)	8(38%)	21(100%)
17p(p53) LOH negative	5(25%)	15(75%)	20(100%)
	P=0.017; Pearson χ^2 test		

Figure 8

Table 4: Correlation Between 17p(p53) LOH and 4N Increased Aneuploidy in Chronic Atrophic Gastritis

	4N increased or Aneuploidy positive	4N increased or Aneuploidy negative	Total
17p(p53) LOH positive	8(61%)	5(39%)	13(100%)
17p(p53) LOH negative	3(10%)	27(90%)	30(100%)
	P=0.001 ; Fisher's test		

Our results support use of 17p(p53) LOH and flow cytometry with histological type of biopsies in evaluation of risk for gastric cancer.

DISCUSSION

The development of gastric cancer in humans has been shown to be a multistep process, ranging from chronic gastritis to atrophy, intestinal metaplasia, dysplasia and finally gastric cancer ([2,3,4,5]).

Multiple genetic and epigenetic alterations in oncogenes, tumor suppressor genes, cell-cycle regulators, cell adhesion molecules, DNA repair genes and genetic instability as well as telomerase activation are implicated in the multistep process of gastric carcinogenesis. p53, a tumor suppressor gene is thought to play a critical role in the multistep process of gastric carcinogenesis ([19,24,31,32,33]).

Functional inactivation of the p53 gene throung mutation or allelelic deletion might play through an important role in the development of variety human tumours. Several studies have shown that many tumours with allelic deletion of chromosome 17p had point mutations of the p53 gene in the

remaining allele and that the presence of p53 LOH might be an important factor involved in the association between p53 gene abnormality and development of cancer ([6,8,11,18,28,34]).

Numerous studies have found evidence to suggest that p53 inactivation contributes to the development of gastric adenocarcinoma. High rates of 17p allelic loss have been detected in gastric adenocarcinoma ([7,8,25,26]). Over expression of the p53 protein and p53 gene mutations have also been identified in these tumors. Additional studies have indicated that p53 inactivation may occur at an early stage in gastric tumorigenesis. Allelic loss of 17p has been detected in aneuploid cell populations from gastric adenocarcinoma ([24,35]).

Until now, several results regarding the alterations in the p53 gene of CAG have been reported. Testino et al. described p53 antibody in %74 of HGD and in 8.5% LGD ([36]). Roa et al. detected LOH-p53 in 83% of gastric carcinomas and in 54% of intestinal metaplasia ([37]).

In the present study, 17p(p53) LOH was found in 21 of 41 adenocarcinomas (51.21%), and 13 of 43 CAG (30.23%). Similarly, increased 4N and aneuploidies was detected in 18 of 41 adenocarcinomas (43.9%), and 11 of 43 gastritis (25.58%). These results suggest that LOH at 17p and increased 4N and aneuploidies are frequently found in gastric adenocarcinoma and may contribute to the progression of gastric carcinoma.

In our study, the prevalence of 17p(p53)LOH increased from 0% in patients whose biopsy specimens were negative for dysplasia to 47% in patients who had HGD ($p = 0.042$). Thus, inactivation of p53 may be a feature of neoplastic transformation in CAG.

The correlation was found between 17p(p53)LOH and increased 4N or aneuploidy of gastric cancer. Similarly, there was correlation between prevalence of 17p(p53) and differentiation increased 4N or aneuploidy of CAG. Our results indicate that 17p (p53) LOH, increased 4N fractions or aneuploidy are all predictors of progression to gastric adenocarcinoma in CAG, providing a panel of objective biomarkers that can be measured in a single endoscopic biopsy and used for risk assessment.

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