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Differential p53 protein expression in stomach adenomas of gastric and intestinal phenotypes: possible sequences of p53 alteration in stomach carcinogenesis

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Abstract In a comparative study, the expression of p53 protein was investigated in intestinal- and gastric-type adenomas of the stomach. The former is a conventional type, which is well known to be a premalignant lesion of the stomach, but the latter is a rare, more recently noted entity. Of 28 intestinal-type adenomas, 17 (60.7%) contained more than 5% of p53 immunoreactive cells. In these adenomas, the extent of positivity for p53 protein was significantly higher in high-grade dysplasia than in low-grade dysplasia ($P<0.05$), suggesting that p53 alteration plays a part in the dysplastic progression of intestinal-type adenomas. Among 18 gastric-type adenomas in which most of the tumour cells displayed gastric-type mucin, substantial expression of p53 protein was found only in the 3 tumours with high-grade dysplasia. Thus, the incidence of p53 expression was significantly higher in intestinal-type adenomas than in gastric-type adenomas ($P<0.01$). These results suggest that p53 gene alteration is an earlier event in the gastric carcinogenetic sequence with the intestinal phenotype than in that with the gastric phenotype.

Key words Gastric-type adenoma · Intestinal-type adenoma · p53 Expression · Mucin expression · Carcinogenesis

Introduction

Stomach carcinomas have been classified into two main subtypes, so-called intestinal and diffuse types, based on their tendency to gland formation [23]. These types almost correspond to the differentiated and undifferentiated types, respectively [26, 32]. They have different clinicopathological characteristics, and it has also been suggested that they derive from different genetic pathways [7, 14, 34]. One conclusion of the studies cited is that on the pathway of so-called intestinal-type carcinoma, stomach adenomas are situated between intestinal metaplasia and differentiated adenocarcinoma. This conclusion is based on the hypothesis that the development of stomach adenomas and differentiated adenocarcinomas is closely associated with intestinal metaplasia (most show exclusively intestinal differentiation).

Recently developed mucin histochemical methods and immunohistochemistry for detecting gastric-type mucin have led to the detection of gastric-type differentiation in the stomach neoplasm [3, 4, 10, 11, 13, 20, 35]. We and other authors have recently noted stomach adenomas or dysplastic lesions with gastric differentiation, namely gastric-type adenoma or dysplasia, although this lesion is rare in the stomach [4, 13, 43]. There have been few studies on genotypic abnormalities in this type of gastric neoplasm [20, 22].

We have investigated the expression of p53 protein in intestinal-type adenomas of the conventional type and in gastric-type adenomas. We also discuss the development and progression of gastric carcinomas from the viewpoint of p53 alteration and phenotypic expression.

Materials and methods

Twenty-eight endoscopically resected intestinal-type adenomas examined at the Institute of Pathology of the Heinrich Heine University of Düsseldorf from 1988 to 1993 were used. Eighteen gastric-type adenomas resected endoscopically or surgically were collected by F.B. (Düsseldorf) and M.S. (Bayreuth). All materials had been fixed in 10% formalin and embedded in paraffin. The adenomas were divided according to the presence of low-grade and high-grade dysplasia [5]. Low-grade dysplasia is characterized by cigar-shaped hyperchromatic nuclei in a palisading arrangement with only slight stratification. High-grade dysplasia is recognized by the presence of swollen nuclei with prominent nucleoli and

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Table 1 p53 Immunoreactivity in intestinal- and gastric-type adenomas of the stomach according to grade of dysplasia

Subtype of adenoma and grade of dysplasia	p53 Immunoreactivity[a]			
	Negative, no. (%)	Positive		
		<5%, no. (%)	5–10% no. (%)	10% <, no. (%)
Intestinal-type adenoma (n=28)**				
Low grade* (n=16)	3 (18.8)	7 (43.8)	2 (12.5)	4 (25.0)
High grade* (n=12)	1 (8.3)	0	1 (8.3)	10 (83.3)
Total	4 (14.3)	7 (25.0)	3 (10.7)	14 (50.0)
Gastric-type adenoma (n=18)**				
Low grade (n=10)	10 (100)	0	0	0
High grade (n=8)	4 (50.0)	1 (12.5)	2 (25.0)	1 (12.5)
Total	14 (77.7)	1 (5.5)	2 (11.1)	1 (5.5)

[a] The incidence of tumours with more than 5% of p53 immunoreactive cells was significantly higher in high-grade dysplasias than in low-grade dysplasias of intestinal type (* P<0.05), and it was also higher in intestinal-type adenomas than in gastric-type adenomas (** P<0.01)

stratification extending to the apical surface. The intestinal-type adenoma consists of columnar cells with acidophilic cytoplasm similar to intestinal absorptive cells. It sometimes contains goblet and/or Paneth cells. The surface coat mucin and goblet cells are demonstrated on alcian blue and periodic-acid Schiff staining to be mostly alcianophilic. In contrast, the gastric-type adenoma consists of columnar to cuboidal cells resembling foveolar cells or pyloric gland cells of the stomach. In most tumour cells (more than 90% cells), foveolar-type mucin (M1) and/or deep gastric mucin (M2) can be demonstrated immunohistochemically. We used monoclonal antibodies (MAbs) against the mucin moieties M1 (MAb R3C4, dilution 1:5) and M2 (MAb 2B5, dilution 1:5) as classified by Bara et al.[2], which were produced in our laboratories. M1 recognizes foveolar mucin and no other type of mucin in the mature gastrointestinal mucosa [6]. M2 stains (pseudo)pyloric mucin of deeper gastric glands and of (pseudo)pyloric metaplasia in different parts of the gastrointestinal tract; it recognizes the sequence truncated *O*-glycan, GalNAc [12]. MAb DO-7 (Dianova, Hamburg, Germany, dilution 1:100) was used to demonstrate p53 protein expression. For the immunohistochemical study, we used avidin-biotin complex methods. The specimens were treated by microwave (in citrate buffer pH 6.0, three times for 5 min in 750 W) before the reaction with MAb DO-7, since this was demonstrated to be the most specific and sensitive procedure by Baas et al. [1] when they compared six different antibodies. The percentage of p53-immunoreactive tumour cells was estimated in relation to all tumour cells present in the stained specimen. The adenomas were divided into four groups according to the result of p53 staining: (1) p53-negative adenomas; (2) tumours with under 5% adenoma cells positive; (3) tumours with 5–10% adenoma cells positive; (4) tumours with more than 10% adenoma cells positive.

Results

Immunohistochemical results for p53 protein are summarized in Table 1. The 28 intestinal-type adenomas included 24 (85.7%) that showed nuclear positivity for p53 protein, which was detected in less than 5% of tumour cells in 7 cases (25.0%), in 5–10% in 3 (10.7%), and in more than 10% in 14 (50.0%). More than 5% of tumour cells were positive for p53 protein in 11 of 12 intestinal-type adenomas with high-grade dysplasia, but only in 6 of 16 adenomas with low-grade dysplasia; this difference was significant (P<0.05, Chi-square test).

Among the gastric-type adenomas, only 4 of 18 tumours with high-grade dysplasia (22.2%) were positive for p53 protein, 3 of these containing more than 5% of p53 positive cells. Thus, the incidence of tumours containing more than 5% of p53-positive cells was significantly higher in intestinal-type adenomas than in gastric-type adenomas (P<0.01, Chi-square test). Representative examples of findings recorded in the immunohistochemical study are shown in Fig. 1.

Discussion

Mutation of the p53 gene is the most common abnormality in various human tumours, including those of the stomach [15, 16, 22, 24, 25, 28–31, 37, 39, 40, 45, 46]. A close relationship between expression of p53 protein and mutations of the p53 gene has been demonstrated in tumours of various organs [8, 9, 17, 41], but some tumours with p53 immunoreactivity lack evidence of p53 gene mutation [28, 44]. Further, some tumours negative for p53 protein carry non-sense mutations [38, 42]. It has recently been reported in studies on colorectal neoplasia that tumours containing more than 5% of p53-immunoreactive cells mostly carry p53 gene mutations [8] and that p53 protein expressed sporadically in tumours is probably overexpressed p53 wild-type protein [18].

Recently, there have been several studies on p53 protein expression in stomach adenomas in which 15–60% of adenomas have been found to be positive for p53 protein [19, 24, 29, 30]. The present study also demonstrated a high incidence of nuclear expression of p53 protein in intestinal-type adenomas, even though tumours in which immunohistochemical testing revealed under 5% positive cells were regarded as negative. The present findings also indicate that p53 alteration may play a part in the dysplastic progression of adenomas of the intestinal type.

The incidence of p53 protein expression is also high in differentiated adenocarcinomas (reported to be

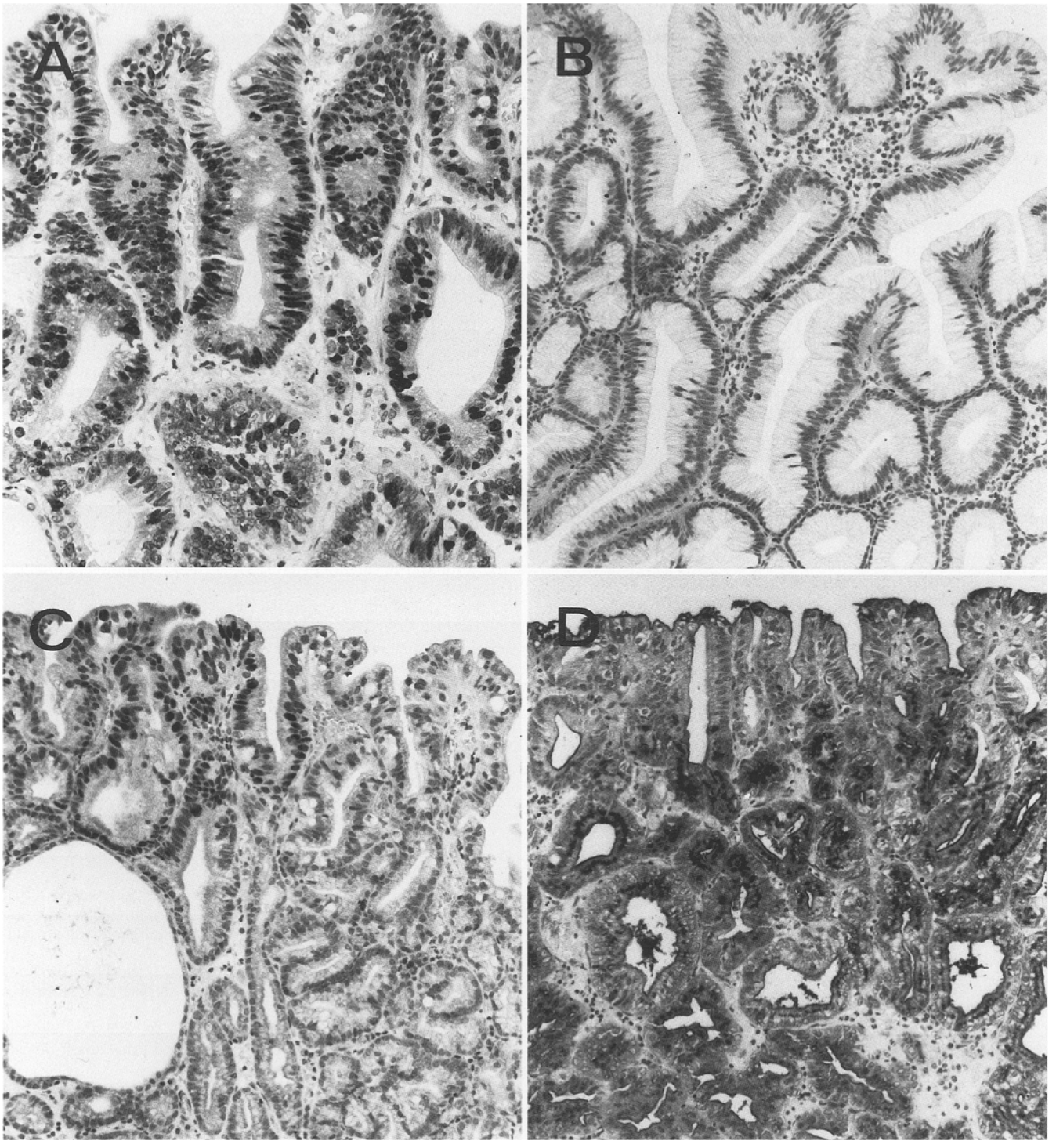

Fig. 1A–D Immunohistochemical findings in stomach adenomas. **A** Intestinal-type adenoma, positive for p53 protein. ×72. **B**. Gastric-type adenoma composed of tall columnar cells similar to foveolar cells, negative for p53 protein. ×45. **C** Gastric-type adenoma composed of cuboidal to low columnar cells similar to pyloric gland cells, focally (5–10%) positive for p53 protein. ×45. **D** Same case as in **C**: most tumour cells contain deep gastric mucin (M2). ×45

30–70% [16, 22, 28, 39, 46]). Furthermore, p53 protein expression has also occasionally been found in cells of the generative cell zone of intestinal metaplasia [22, 27, 31], some of which have been demonstrated to carry p53 gene mutations [27]. Taken together, p53 alteration is an early event in the sequence of intestinal metaplasia, adenoma and differentiated adenocarcinoma in the stomach.

Few previously reported studies have focused on the relation between the phenotypic expression of adenoma or differentiated adenocarcinoma and p53 protein ex-

pression [22], however, since such gland-forming neoplasms of the stomach as adenoma or differentiated adenocarcinoma have been widely considered to be exclusively intestinal in type. Recently, most stomach carcinomas have been demonstrated to be mixed in phenotype [3, 10, 11, 35], but they could still be divided into gastric or intestinal phenotypes according to the predominance of expression of markers for gastric or intestinal differentiation [21, 35]. The incidence of p53 protein expression in gastric-type adenocarcinomas did not differ from that of intestinal-type adenocarcinomas, in a study on intramucosal carcinomas [22], which might maintain the primary characteristics of their initial stages of transformation [36]. However, the present study showed a lower incidence in gastric-type adenomas, and in the carcinogenetic sequence of gastric-type adenocarcinoma from gastric mucosa proper to gastric-type adenocarcinoma through foveolar hyperplasia and gastric-type adenoma or dysplasia [13, 20] p53 alteration appears to be a later event than in the sequence leading to intestinal-type adenocarcinoma.

In diffuse-type carcinoma, which has been considered to originate in the gastric mucosa proper [26, 32] and has been reported to have an exclusively gastric phenotype (at least in the intramucosal stage [21, 33, 36]), while p53 protein expression is rarely observed in the early stages, its incidence later becomes as high as that in differentiated adenocarcinoma. This suggests that p53 alteration plays an important part in the progression of diffuse-type carcinomas [16, 22, 28, 39, 46].

In conclusion, it is suggested that p53 alteration is an early event in the carcinogenetic sequence of differentiated adenocarcinoma, especially in the intestinal phenotype. It seems to be more important in the later stages of diffuse-type carcinoma.

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