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*p53 REVIEW ARTICLE*

# TP53 and Gastric Carcinoma: A Review

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*For the p53 Special Issue*

**In this article, we survey the major *p53* (*TP53*) alterations identified in gastric carcinomas and their precursors. These include p53 expression, mutations, and loss of heterozygosity (LOH). Not only are the various abnormalities summarized, but in addition there is a survey of the literature with respect to the impact of these changes on patient prognosis and treatment response. The majority of published studies involve the immunohistochemical detection of the protein. These use different antibodies, different detection techniques, and different methods of interpretation. Therefore not surprisingly, the results of many of the studies are contradictory with one another. Overall, however, it appears that p53 alterations occur early in the development of gastric carcinoma, being present even in the nonneoplastic mucosa and they increase in frequency as one progresses along the pathway of gastric carcinoma development. p53 immunoreactivity is seen in 17%–90.7% of invasive gastric carcinomas. p53 alterations occur much more commonly in proximal lesions than in distal ones, suggesting that the molecular events leading to the development of gastric carcinoma may be very different in proximal vs. distal tumors. p53 mutations occur in 0%–77% of gastric carcinomas. The mutations are distributed widely across the gene from exons 4–11 with hot spots of mutation at codons 175, 248, 273, 282, 245, and 213. G:C>A:T transitions at CpG sites are the commonest type of mutation. At least 60% of carcinomas with mutations also exhibit p53 LOH. Hum Mutat 21:258–270, 2003.** © **2003 Wiley-Liss, Inc.**

KEY WORDS: p53; TP53; expression; prognostic value; treatment response; loss of heterozygosity; cancer; tumor

DATABASES:
**TP53** – OMIM: 191170; GenBank: NM_000546 (mRNA)
http://p53.curie.fr/ (p53 Web Site at Institut Curie)
www.iarc.fr/p53 (IARC p53 Mutation Database)

## INTRODUCTION

The p53 tumor suppressor gene (TP53; MIM# 191170) is the most commonly mutated gene in human tumors [Hollstein et al., 1991]. It acts as a tumor suppressor gene, negatively regulates the cell cycle, and requires loss of function mutations for tumor formation [Levine, 1997]. The gene spans 20 Kb of genomic DNA located at 17p13, contains 11 exons, and encodes a 53 kD phosphoprotein that is a transcription factor for genes that induce cell cycle arrest or apoptosis [Levine, 1997]. p53 is also a genomic stabilizer and an inhibitor of angiogenesis [Dameron et al., 1994]. TP53 mutations are predominantly inactivating and can induce changes in protein conformation. Loss of p53 function may result in defective DNA replication and malignant transformation [Kastan et al., 1991], increased genetic instability, changes in ploidy, and survival of cells with an increased mutational load [Levine, 1997]. Loss of p53 function could result from 1) mutations; 2) bi-allelic gene deletions that result in the loss of the p53 protein; and potentially from 3) genetic polymorphisms that may result in different encoded proteins.

Although nuclear localization of the p53 protein is essential for its activity [Shaulsky et al., 1991], nuclear accumulation is usually not detectable due to the short half-life (5–20 min) of the wild-type protein [Giaccia and Kastan, 1998]. In contrast, p53 mutations result in the production of p53 proteins with a prolonged half-life leading to nuclear protein accumulation [Bodmer et al., 1992]. Thus, many erroneously equate the immunohistochemical detection of nuclear p53 with the presence of missense mutations. However, most antibodies used in immunohistochemical studies detect both the wild type as well as the mutant form of the protein [Bosari and

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Viale, 1995] and thus physiological accumulations of the wild-type protein will also be detectable.

Nuclear accumulations of the p53 protein can result from upregulated expression of the wild-type p53 protein or decreased protein degradation in response to various cellular stresses, including DNA damage. Overexpression of the wild-type protein is a normal physiological response to slow down the cell cycle at the G1 phase to allow repair of damaged DNA. In addition, during DNA damage, Mdm-2-dependent p53 degradation is inhibited [Ashcroft et al., 1999]. Therefore, low levels of wild-type p53 can be detected in the nucleus, especially if sensitive immunohistochemical detection techniques, such as antigen retrieval, are used [McKee et al., 1993]. In addition, gene abnormalities other than missense mutations do not lead to nuclear protein accumulations and therefore escape detection by immunohistochemical techniques. Some missense mutations result in a stop codon, and therefore may result in transcription of a truncated protein that is not detectable by immunohistochemistry. In other circumstances, a point mutation does not stabilize the protein sufficiently for it to be detected immunohistochemically.

We have studied the relationship between the immunohistochemical detection of nuclear p53 protein and gene mutation and have found that the likelihood of finding a correlation between the two in gastric cancer (GC) is poor, especially when the tumor is p53 positive. The correlation is much better in immunohistochemically negative cases. Furthermore, the likelihood of finding a correlation between the presence of a gene mutation and a positive immunohistochemical reaction differs depending on where in the gene the mutation is located (unpublished observations). The rate of p53 immunoreactivity may also reflect the presence or absence of p53 loss of heterozygosity (LOH).

In this review, we survey the published literature with respect to p53 alterations in GC. Most studies addressing alterations in p53 have used immunohistochemical techniques to detect nuclear protein accumulation. A smaller number of studies have actually sequenced the gene. After a brief discussion of the pathogenesis of GC, we will survey immunohistochemical and genetic studies that have been published in GC and its precursor lesions. These studies have generated significant confusion with respect to both the frequency of p53 alterations and their implications.

## GASTRIC CARCINOMA: A BRIEF OVERVIEW

The designation of GC into two main histopathological patterns (intestinal and diffuse) has value in understanding the epidemiology, demography, progression, and survival of GC patients [Lauren, 1965]. The commonest histological variant present in high-risk populations is intestinal type GC [Fenoglio-Preiser et al., 2000]. It results from exposure to various environmental factors including *H. pylori* infection and it evolves via a series of sequential events that include chronic gastritis, atrophy, intestinal metaplasia (IM), dysplasia, early carcinoma, invasion, and metastases [Correa, 1988]. In low risk populations, the diffuse type of GC is more common. Diffuse tumors associate with the same superficial gastritis as intestinal tumors. They demonstrate high *H. pylori* antibody levels as well.

## TECHNICAL CONSIDERATIONS

Numerous studies have been published to determine the frequency of p53 staining in GC and to relate the presence or absence of p53 nuclear staining to patient outcome (Table 1) and/or treatment results (Table 2) with conflicting results. Different antibodies have been used, tissue preparation and immunohistochemical detection techniques have varied, some investigators use antigen retrieval methods, while others do not, and the methods used for antigen retrieval vary. Finally, the criteria for judging a reaction to be positive or negative vary (Table 1). False negative staining reactions can occur when the tissues are improperly fixed or embedded, or when the staining is performed on slides that have been previously cut and stored for long periods of time. Positive staining reactions not related to mutations may result from failures of the normal degradative p53 pathways so that wild-type protein accumulates in the nucleus or it accumulates when there is upregulation of the gene in response to cellular environmental stresses. The antibody CM-1 recognizes the entire p53 protein (amino acids 1–393). The antibodies DO7 (recognizes amino acids 19–26) and DO1 (recognizes amino acids 37–45) bind shorter segments of the protein [Vjtesek et al., 1992]. The antibody PAb 1801 recognizes a longer protein segment between amino acids 32 and 79 [Banks et al., 1994]. A higher degree of correlation between p53 immunoreactivity and gene mutations has been reported for the monoclonal antibodies Pab1801 and DO7 than for the polyclonal antibody CM-2 [Baas et al., 1994]. Tolbert et al. [1999b] performed a comparative analysis using multiple different antibodies and found that the immunohistochemical results in GC were comparable with all of the antibodies tested.

As is the case for immunohistochemical studies, different techniques are used to find p53 mutations and different regions of the gene are examined, resulting in significant differences in mutation frequency in different studies (Table 3). Some advocate the use of single strand conformation polymorphism (SSCP) analysis to detect p53 mutations and polymorphisms since SSCP has the ability to identify

TABLE 1. Selected Immunohistochemical Studies of p53 in Gastric Carcinoma*

| Reference | Antibody | % of cells+to be called+ | % of patients+ | Relationship of nuclear staining to survival | Comment |
|---|---|---|---|---|---|
| Danesi et al. [2000] | DO7 | Any cells+ | 137 pts; 48.9%+ | ns | No correlation with clinicopathological variables |
| Gabbert et al. [1995] | DO1 | Any cells+ | 418 pts; 57.5%+ | No | All T stages. No assoc w/ depth tumor invasion, LN mets; more common IT than DT; no relation to tumor size |
| Ichiyoshi et al. [1997] | PAb 1801 | Any cells+ | 196 pts; 48%+ | Yes (mv) | Advanced GC; no assoc w/ stage |
| Ikeguchi et al. [1997] | BP53-12 | ≥ 10 of cells+ | 156 pts; 60.2%+ | Yes (mv) | Advanced GC |
| Joypaul et al. [1994] | CM-1 | Any cells+ | 206 pts; 46%+ | Yes worse/mv | Tumors all stages |
| Kaye et al. [2000] | ? | Any cells+ | 100pts; 40%+ | No | Assoc w/ LN mets |
| Kim et al. [1997] | DO7 | Any cells+ | 129 pts; 42%+ | ns | No assoc w/ T stage or tumor location; relationship w/ LN mets |
| Kim et al. [1994] | DO7 | ≥ 10% of cells+ | 152 pts; 46%+ | No | Tended to increase w/ stage but not statistically significant |
| Kim et al. [1995] | DO7 | Any cells+ | 101 pts; 36.6%+ | ns | Assoc w/ depth of tumor invasion, LN+ and mets |
| Lee et al. [1998] | ? | Any cells+ | 168 pts; 20.2%+ | Yes in DGC (uv) no (mv) | No assoc w/ clinicopathological variables; no relation to survival in IT |
| Lim et al. [1996] | DO7 | ≥ 5% of cells+ | 116 pts; 23%+ | Yes (uv) No (mv) | Assoc w/ nodal mets |
| Liu et al. [2001a] | DO7 | Any cells+ | 140 pts; 43.6% | No (m) | Prognostic if combined w/ p27 and p21(waf1) |
| Liu et al. [2001b] | DO7 | Any cells+ | 178 pts; 50% IT 30% DT | ns | Positivity tended to occur early in IT and late in DT |
| Liu et al. [2001c] | DO7 | Any cells+ | 190 pts; 50%+IGC; 34.6% +DT | ns | In IT-no assoc w/ pathological variables; in DT, assoc w/LN mets |
| Maeda et al. [1998] | DO7 | Any cells+ | 120 pts; 42%+ | Yes | tumors all stages; P53+ tumors tended to express VEGF |
| Martin et al. [1992] | CM-1 | Any cells+ | 125 pts; 57%+ | Yes (mv) | %+varied w/ fixation and w/ depth of invasion |
| Matturi et al. [1998] | Pab1801 | Any cells+ | 126 pts; 17%+ | No (uv; mv) | Preoperative bx study; more common in WDGC |
| McCulloch et al. [1997] | DO7 | Any cells+ | 88 British; 89 Japanese ?+ | ns | No relation to stage in either patient population |
| Monig et al. [1997] | DO7 | ≥ 20% of cells+ | 133 pts; 26.3% + | Yes; >20% p53+ cells vs. 0-19%+cells (P = < 0.01) | No assoc w/depth tumor invasion; significant assoc w/LN mets & peritoneal dissemination; more common PGC |
| Motojima et al. [1994] | PAb1801 (m) | Any cells+ | 135 pts; 27.4%+ | Yes (uv) No (mv) | Tumors all stages. Correlation w/ depth of tumor invasion and LN status |
| Muller and Borchard [1996] | DO1 | Any cells+ | 120 pts; 43%+ | Marginal relation >35% p53+ | No relation with pathological variables |
| Ogawa et al. [2001] | DO7 | Any cells+ | 164 pts; 50% + | ns | P53– & p21+ tumors displayed less aggressiveness and no recurrences following curative resection |
| Roviello et al. [1999] | DO1 | ≥ 10% of cells+ | 136 pts; 51%+ | Yes IT: no DT | Tumors all stages |
| Sakaguchi et al. [1998] | DO7 | ≥ 5% of cells+ | 116 pts; 50.9%+ | ns | Tumors all stages; correlation with cyclin E |
| Sasaki et al. [1999] | DO7 | Any cells+ | 108 pts; 75% LN +; 46% LN | ns | Early GC. More common in LN+ than LN- tumors. |
| Schneider et al. [1994] | CM-1 PAb1801 | Any cells+ | 131 pts; 43% in Hispanics; 61% in Anglos | No | No relation w/ tumor stage; weak correlation w/ DGC |
| Setala et al. [1998] | DO7 | Any cells+ | 116 pts; 90.9% + | Yes high p53 score or totally neg tumors worse prognosis | Stage I & II tumors. No relation to standard pathological variables; relation to aneuploidy, S phase fraction mitotic activity in tumors that were p53 negative or had the highest p53 scores |
| Shiao et al. [2000] | CM-1 | Any cells+ | 105 pts; ?%+ | No | Tumors that were p53 negative or had the highest P53 scores correlated w/mets. Tumors w/intermediate scores had lowest rates of mets |
| Shun et al. [1997] | ? | Any cells+ | 112 pts; 54.5% + | No | P53+ correlated with advanced, intestinal cardiac tumors |
| Soong et al. [1996] | DO7 | ≥ 5% of cells+ | 116 pts; 23%+ | ns | 73% p53 IHC correlation with mutation |
| Starzynska et al. [1996] | CMI | Any cells+ | 200 pts; 42.5%+ | Yes (mv) | Tumors all stages. P 53 more commonly positive in PGC > DGC; correlation w/depth of invasion |
| Tang et al. [1997] | PAb1801 | ≥ 5% of cells+ | 170 pts; 28.8%+ | No | Much more common PGC than DGC |
| Uchino et al. [1992] | PAb 1801 | Any cells+ | 149 pts; 30%+ | ns | Assoc w/ depth of tumor invasion, stage, BV status |
| Victorzon et al. [1996] | DO7 | ≤ 20% ofcells+ | 242 pts; 39% + | Yes(uv) no(Mv) | Assoc w/ stage; distant mets and IT |

Continued

TABLE 1. (Continued)

| Reference | Antibody | % of cells+to be called+ | % of patients+ | Relationship of nuclear staining to survival | Comment |
|---|---|---|---|---|---|
| Wu et al. [1997] | DO1 | ≥5% of cells+ | 181pts; 46.6%+ | ns | More common in early IT than early DT; assoc w/stage in DT but not IT |
| Wu et al. [1998b] | DO1 | ≥5% of cells+ | 135pts; 42.4% | ns | More common early IT than DT |
| Xiangming et al. [1999] | ? | ≥5% of cells+ | 101 pts; 29.7% | ns | All early GC; assoc w/ LN mets |
| Yasui et al. [1996a] | CM-1 | ≥5% of cells+ | 336 pts; 42.5%+ | ns | Tumors all stages |
| Yasui et al. [1996b] | CM-1 | ≥5% of cells+ | 439 carcinomas 182 adenoma pts; 45% overall | ns | Assoc w/ expression of cyclin E |

*Only covers studies with at least 100 patients; column showing %+ includes only positive cells unless indicated otherwise.
Assoc, association; BV, blood vessel; Bx, biopsy; DGC, distal gastric carcinoma; DT, diffuse type gastric carcinoma; GC, gastric carcinoma; IT, intestinal type carcinoma; LI, labeling index; LN, lymph node; mets, metastasis; mv, multivariate analysis; neg, negative; ns, not studied; PGC, proximal gastric carcinoma; pts, patients; sig, significantly; T, tumor; uv, univariate analysis; VEGF, vasculat endothelial growth factor; w/, with; WDGC, well differentiated gastric carcinoma.

TABLE 2. Relationship of p53 Alterations to Treatment Response or Chemosensitivity Test in Gastric Carcinoma

| Reference | Alteration | No. of Patients Studied | Relationship to treatment effect | Comments |
|---|---|---|---|---|
| Boku et al. [1998] | p53 exp; ? Ab | 39 | p53− tumors more likely to respond than p53+ tumor | Unresectable gastric cancers; rxed w/ 5FU; CDDP |
| Cascinu et al. [1998] | p53 exp; BP 53-12 | 30 | p53− tumors more likely to respond than p53+ tumor | Locally advanced GC ADM,CDDP,5-FU. Leucovorin, glutathione |
| Diez et al. [2000] | p53 exp; ? Ab | 46 | p53−: 82% 4yr surv p53+: 45% 4 yr surv p(0.001) | Stage II & III pts MMC, 5 FU |
| Giatromanolaki et al. [2001] | p53 exp; DO7 | 28 | No | Locally advanced GC Paclitaxel and carboplatin |
| Hosaka et al. [2001] | p53 exp; DO7 | 11 | P53 exp inversely correlated w/ chemosensitivity for 5-FU & MMC but not ADM & CDDP | Advanced tumors 5-Fu, MMC, ADM, CDDP |
| Ikeguchi et al. [1997] | P53 exp; BP 53-12 | 74 | No | Advanced GC; CHHPw/MMC |
| Kikuyama et al. [2001] | P53 exp; DO1 | 28 | P53−: 28% response P53+: 47% response | 5-FU; CDDP, pirarubicin |
| Nakata et al. [1998] | P53 exp; DO7 | 20 | 70% responders p53− 86.4 nonresponders p53+ p = 0.013 | 5-FU, CDDP |
| Yeh et al. [1999] | P53 exp; DO7 | 30 | No | 5 FU, leukovorin |

ADM, doxorubicin; CHHP, continuous hyprthermic peritoneal perfusion; MMC, mitomycin C; CDDP, cisplatin; 5-FU, 5- fluorouracil; w/, with; rxed, treated.

single base pair substitutions. Its main disadvantage is that it does not detect 100% of mutations [Kutach et al., 1999; Tolbert et al., 1999b]. The sensitivity of the SSCP analysis is affected by the length of the PCR fragment being analyzed. The efficiency of detection of single base substitutions is greatest in fragments of 135–200 bp [Sheffield et al., 1993]. We found that SSCP misses 38% of mutations [Tolbert et al., 1999b]. This may be due to the fact that the tissues analyzed were not microdissected prior to SSCP analysis, since mutation detection in GC can be underestimated if the sample is contaminated by normal cells [Hong et al., 1994]. This could be a substantial problem in analyses of diffuse GC because isolated, discohesive cells diffusely infiltrate the gastric wall. Furthermore, the tumor cells may be difficult to distinguish from inflammatory cells. These two features can make it difficult to microdissect the tumor cells from normal cells.

## P53 ALTERATIONS IN NONNEOPLASTIC GASTRIC LESIONS

An increasing frequency of p53 abnormalities occurs as the gastric mucosa progresses from gastritis, through IM, dysplasia, early and to advanced invasive GC. The highest frequency of abnormalities is seen in metastatic lesions [Yamada et al., 1991]. Some suggest that a small percentage of p53 immunoreactive cells are present in the normal gastric mucosa and in patients with chronic gastritis, even before the development of IM or dysplasia [Wang et al., 1994; Feng et al., 2002]. However, it should be noted that most investigators do not find staining in the normal

**TABLE 3.** Selected Studies P53 Mutations in Gastric Carcinomas*

| Reference | Exons examined/ technique used | No. tumors analyzed | Mutations | Comments | Comments/ Correlation with survival |
|---|---|---|---|---|---|
| Flejou et al. [1999] | 5–8<br>DGGE & sequencing | 70 | 42% cardia;<br>25% antrum | Base transitions at CpG sites most frequent change | No relationship w/ histological tumor type; significantly more common in cardia |
| Gleeson et al. [1998] | 5–8<br>SSCP followed by sequencing | 35 | 62% | Predominance of base transitions at CpG sites | All cardiac tumors |
| Hongyo et al. [1995] | 5–8<br>SSCP followed by sequencing | 34 | 65% | 91% base substitutions;<br>90% G:C to A:T | Inverse correlation of mutation with Helicobacter infection |
| Kobayashi et al. [1996] | 5–9 direct sequencing | 46 | 43.5% | Missense mutations | 3 tumors contained 2 mutations |
| Leung et al. [2001] | 5–8<br>PCR, SSCP | 39 | 51.3% | All missense; 90% G:C→A:T | No relation to survival, mets or tumor type; mutated tumors has sig higher levels of cox2 |
| Lim et al. [1996] | 5–8<br>SSCP | 116 | 28% | Majority of mutations in exons 5 & 7 | Correlation with survival in multivariate analysis; correlation with IHC results in 73% of cases |
| Maesawa et al. [1995] | 5–8<br>SSCP followed by sequencing | 30 adenomas<br>72 carcinomas | 35% | Missense; deletions; frameshift | Incidence of mutations similar in intestinal and diffuse types of tumors |
| Poremba et al. [1995] | 2–11<br>SSCP followed by direct sequencing | 56 | 37.5 | Missense mutations<br>Some mutated tumors also had p53 LOH | Mutations profile highly variable 1 tumor to another |
| Renault et al. [1993] | 5–8<br>PCR-based<br>DGGE | 29 | 52% | Missense; nonsense; deletions<br>91% G:C to A:T transitions;<br>54% of mutations found in tumors without allelic losses | No correlation of the frequency of mutation with tumor stage. |
| Ricevuto et al. [1996] | 5–9<br>FISH | 31 | 35% stage III & IV;<br>0% stage I & II | Frameshift; insertions missense; nonsense | Relationship to tumor stage |
| Rugge et al. [2000] | 5–8<br>PCR, SSCP | 105 | 8% | All mutations at CpG sites | No relationship with histological variables.<br>Tend to occur in cardia tumors<br>Uncommon in young pts |
| Seruca et al. [1994] | 4 hot spots for mutations<br>Screened by CDGE followed by sequencing | 56 | 17.8% | Missense | No statistical correlation with pathological features although a tendency to occur in intestinal, aneuploid tumors and in those with a high S phase |
| Shiao et al. [1998] | 5–8<br>PCR, SSCP | 105 | 42.8% intestinal type;<br>50% unclassified tumors;<br>21% diffuse tumors | Majority missense<br>Deletions<br>Silent<br>Intron 5<br>G:C→A:T at P situ | Affected all tumor types<br>No relationship with histology |
| Strickler et al. [1994] | 5–8<br>SSCP followed by sequencing | 40 | 22.5% | Mostly missense; most at C or G sites | Mutated tumors were predominantly PGC |
| Sud et al. [2001] | 2–11<br>PCR, SSCP; heteroduplex analysis | 26 | 31% | Mostly missense<br>1 tumor contained 2 mutations | More common IGC than DGC but not statistically significant |
| Taniere et al. [2001] | 4–9<br>temporal temperature gradient electrophoresis followed by direct sequencing | 26 | 31% | Majority C:T transitions at CpG sites | Only cardia tumors |
| Shepherd et al. [2001] | 4<br>direct sequencing | 217 | 3.2% | Mostly missense; majority of mutated tumors were neg by IHC | 2 polymorphic sites at codons 36 and 72<br>Codon 72 genotype varied sig w/ race |
| Tolbert et al. [1999a] | 5–9<br>direct sequencing | 100 | 35% | Mutations occurred in exons 5-8; none in exon 9 | Mutations sig more frequent PGC; tendency to be more common in IGC than DGC |
| Wang et al. [2001] | 5–8<br>PCR; SSCP<br>Direct sequencing | 36 | | Missense 1 mutation in splice donor site of intron 5 | Gastric cancers all stages |

CDGE, constant denaturing gel electrophoresis; DGGE, denaturing gradient gel electrophoresis; FISH, fluorescent in situ hybridization; PCR, polymerase chain reaction; SSCP, single strand conformation polymorphism; Sig, significant.

glands [Rugge et al., 1992; Starynska et al., 1992; Joypaul et al., 1993; Craanen et al., 1995; Gomyo et al., 1996; Imatani et al., 1996; Wu et al., 1998b; Sasaki et al., 1999] and not all find it in gastritis [Blok et al., 1998]. Surprisingly, Shiao et al. [1994] found p53 mutations in 25% of "normal" appearing mucosa adjacent to invasive carcinomas, even when the mucosa was immunohistochemically p53 negative. Whether the mucosa examined in the study was truly "normal" is unclear. It is more likely that the areas of "normal" mucosa were areas of *H. pylori* gastritis adjacent to the neoplastic lesions. The authors did show that mutations occur before p53 nuclear staining appears.

p53 immunopositive cells can be found in the mucus neck region, the gastric proliferative zone, and in *H. pylori* infection [Hibi et al., 1997; Polat et al., 2002]. In *H. pylori* gastritis, free radicals produced by activated leukocytes cause mucosal DNA damage [Mai et al., 1988; Correa and Shiao, 1994]. Thus, one would expect to see nuclear p53 expression in the proliferative zone reflecting the normal p53 response to DNA damage. Data supporting this is the finding that patients with *H. pylori* infections have significantly more p53 positive cells during the active infection than after eradication of the *H. pylori* [Satoh et al., 2001]. However, p53 mutations have also been demonstrated in areas of *H. pylori* associated gastritis; these mutations tend to affect non-hot spot regions of the gene [Murakami et al., 1999]. *H. pylori* infections and *p53* may act in a synergistic fashion in gastric carcinogenesis. Helicobacter infections in *p53* knockout mice result in the development of gastric dysplasia, whereas these infections in normal mice fail to produce neoplastic changes [Dunn et al., 1995]. The loss of normal *p53* function presumably heightens the genetic instability of the mucosa, thus facilitating the development of GC. Among GC patients, p53 abnormalities are more common in *CagA+* patients than in *Cag* A– patients, also suggesting a link between *H. pylori* infection and p53 alterations [Deguchi et al., 1995; Kubicka et al., 2002; Shibata et al., 2002].

p53 immunopositivity can also be seen in 0%–50% of areas of IM [Tohdo et al., 1993; Correa and Shiao, 1994; Shiao et al., 1994; Gomyo et al., 1996, Hao et al., 1997; Rugge et al., 2000], although it is usually only present as isolated positive cells scattered here and there [Stemmermann et al., 1994]. p53 positive cells tend to localize to the deeper zones of the intestinalized mucosa. The rate of p53 immunoreactivity appears to differ, depending on the type of IM that is present [Gomyo et al., 1996; Ochiai et al., 1996; Wu et al., 1998a] and whether or not the patient has had an *H. pylori* infection [Zhang et al., 2001]. DNA base substitutions occur in up to 50% of areas of IM [Shiao et al., 1994; Hao et al., 1997]. The presence of mutations also correlates with the type of metaplasia that is present. Mutations appear to be restricted to type III (colonic type) metaplasia [Gomyo et al., 1996]. Areas of IM may also show LOH for p53 in 10.3–14% of cases [Tahara et al., 1996; Gomyo et al., 1996].

## GASTRIC DYSPLASIA

p53 abnormalities are common in dysplasia. p53 immunoreactivity can be seen in 15–63.2% of dysplasias in stomachs resected for GC [Joypaul et al., 1993; Brito et al., 1994; Shiao et al., 1994; Miracco et al., 1995; Ranzani et al., 1995; Imatani et al., 1996]. The incidence of positivity varies with the degree of dysplasia that is present, increasing with increasing degrees of dysplasia. Positive cells are present in 19% of cases of mild dysplasia vs. 64–67% of cases of high-grade dysplasia [Rugge et al., 1992; Miracco et al., 1995]. Other studies suggest that p53 overexpression can be found in high-grade dysplasias but not in mild or moderate dysplasia [Joypaul et al., 1993; Miracco et al., 1995]. The high rate of p53 immunoreactivity in high-grade dysplasia has led some to suggest that p53 immunostaining may be useful in distinguishing reactive atypia from areas of true dysplasia [Brito et al., 1994].

Mutations occur in 0–67% of gastric dysplasias, including gastric adenomas [Tohdo et al., 1993; Correa and Shiao, 1994; Strickler et al., 1994; Wang et al., 1994; Maesawa et al., 1995; Ranzani et al., 1995; Sakurai et al., 1995; Gomyo et al., 1996, Imatani et al., 1996; Hao et al., 1997]. In adenomas, the mutations tend to be silent in lesions with only mild or moderate degrees of dysplasia, contrasting with the presence of missense mutations in adenomas containing high-grade dysplasia [Tohdo et al., 1993; Correa and Shiao, 1994]. This has led some to suggest that the presence of missense mutations in adenomas is a key indicator of malignant transformation [Sakurai et al., 1995]. LOH of the 3′ untranslated region of the p53 gene is found in 0–22% of gastric adenomas [Tahara et al., 1996; Sugai et al., 1998; Table 4].

## GASTRIC CARCINOMA

p53 overexpression has been reported in 17–90.7% of invasive tumors (Table 1) p53 nuclear staining can be seen in both intestinal and diffuse type gastric tumors, although it is more common in intestinal than in diffuse type tumors (Table 1). The degree of p53 expression correlates with the proliferative rate of the tumors [Ioachim et al., 1997], perhaps explaining the higher incidence of p53 positivity in intestinal vs. diffuse GC (diffuse GC tends to have low proliferative rates). p53 abnormalities appear to occur earlier in intestinal type cancers than in diffuse types and there is a tendency for p53

TABLE 4. Example of Studies on LOH for p53 in Gastric Carcinoma

| Reference | No. inf tumors / no. tumors | Techniques/ primers used | LOH | Comments |
|---|---|---|---|---|
| Conde et al. [1999] | 13/89 | PCR; D17S513; D17S796 | 41.4% | No relationship to tumor type or tumor location |
| Dockhorn-Dworniczak et al. [1994] | 9/39 | PCR; *MSP1; AccII;*YN222 | 26.1% | Characterization of tumors not described |
| Gleeson et al. [1998] | 29/35 | PCR; MSA | 83% | LOH detected in tumors lacking mutations in exons 5–8 as well as in tumors with mutations |
| Gomyo et al. [1996] | 31/na | FISH | 77% | Also studied areas of intestinal metaplasia. No LOH found in it |
| Kim et al. [1995] | 63/64 | PCR;TP 53; D17S5 | 63% | No relationship with clinicopathological variables; LOH at D17S5 more common than LOH TP53 |
| Kobayashi et al. [1996] | 67 | FISH; southern blot;YnZ22 Significant correlation between the 2 techniques | 39% | Frequent protein overexpression; most LOH occurred in intestinal type tumors not in diffuse ones |
| Rhyu et al. [1994] | 36/52 | PCR; *BstU1* (exon 4) 1 *Alu* (intron 1) | 64% | No allelic losses seen in dysplasia adjacent to carcinoma |
| Saegusa et al. [1996] | 41 | PCR; *BstU1* (exon 4); TP53; 1VNTR intron 1 | 36.6% | Inverse correlation w BCL2 expression |
| Semba et al. [1996] | 9 IM 12 adenomas 24 carcinomas | PCR RFLP analysis TP53 | 14% IM; 22 % gastric adenomas; % carcinomas | LOH present in non-neoplastic mucosa |
| Seruca et al. [1994] | 16/42 | Southern blot PBH p53 | 31.3% | Four of the five cases with LOH had p53 mutations |
| Sugai et al. [1998] | 22/25 | PCR 5′ upstream & downstream of p53 | 42.9% of invasive IGC; 0% of intramucosal IGC; 50% of intramucosal DGC; 0% of invasive DGC | No LOH in early lesions |
| Tamura et al 1996 | 45 carcinomas 20 adenomas; 58% of cases informative | PCR,TP53 | 45% in carcinomas 0% in adenomas | No LOH in early lesions |
| Yustein et al. [1999] | 18 | PCR: D17S974 | 80% | Tumors xenografted into mice |

NA, not applicable; PCR, polymerase chain reaction; IM, intestinal metaplasia.

expression to be more common in poorly differentiated tumors than in well differentiated lesions [Martin et al., 1992; Brito et al., 1994, Sasaki et al., 1999]. There is also a tendency for p53 overexpression to occur in tumors arising in the proximal stomach compared to more distal lesions (Table 1). One study found that all cases with mutant p53 were aneuploid, and no diploid tumor had a p53 mutation [Tamura et al., 1996], possibly supporting the concept that wild-type p53 prevents cells containing damaged DNA from replicating [Kastan et al., 1991].

A comparison of the immunohistochemical reactivity rates in endoscopic biopsies as compared to the rate of positivity in the subsequent resection specimens showed that the positive predictive rate is only about 80% [Jiang et al., 1997]. This undoubtedly reflects the fact that there is often heterogeneity in the p53 staining pattern within a given tumor. In approximately 50% of p53 positive GC, 75% or more of the tumor cells are stained. In approximately 25% of p53 positive GC tumors, less than 25% of the tumor cells are p53 immunoreactive within individual tumors (unpublished observations). Evaluation of the immunohistochemical detection of p53 as a prognostic marker has yielded conflicting results (Table 1). Two interesting studies suggest that it is tumors with intermediate levels of p53 expression that have the lowest risk of metastasis, while tumors that are either negative or strongly positive are more likely to metastasize (Table 1) [Setala et al., 1998; Shiao et al., 2000].

The reported incidence of p53 mutations in invasive carcinomas ranges from a low of 0% to a high of 76.9% [Yamada et al., 1991; Correa and Shiao, 1994; Table 3]. More than one mutation may be present in a single tumor [Flejou et al., 1999] and there can be heterogeneity of the p53 mutational status within a given tumor [Iwamatsu et al., 2001].

As Table 3 shows, there are conflicting results with respect to the prevalence of p53 mutations, their relationship to histological tumor type, and their relationship to tumor stage. While some authors find that mutations tend to affect intestinal type tumors, others find that the incidence of mutation is similar in the two main tumor types (Table 3), suggesting that the p53 gene is a common target in the development of GC in general. Young patients (<age 40) have a lower incidence of p53 mutations than older individuals [Rugge et al., 2000]. Some studies show that advanced GCs tend to have a higher percentage of p53 mutations and that there is a relationship between the presence of p53 mutations and aneuploidy [Tamura et al., 1991], although not all report similar associations [Gleeson et al., 1998]. The one thing that most studies agree on is that p53 mutations are more common in tumors arising in the cardia than in tumors arising in the antrum (Table 3). It has been reported that mutations are more common in metastatic than in primary gastric carcinomas and the percentage of mutations in GC cell lines in general is much higher than that seen in primary GC [Kim et al., 1991; Yamada et al., 1991: Matozaki et al., 1992a]. Furthermore, GC-containing mutations are much more likely to metastasize than those tumors without mutations [Kakeji et al., 1993; Shiao et al., 2000]. The risk of metastasis is further magnified if the mutations are at hot spots (codons 175, 248, and 243) and at non-CpG sites [Shiao et al., 2000]. The presence or absence of mutation combined with the immunohistochemical score may also serve as a prognostic marker. After adjusting for depth of invasion and nodal status, Shiao et al. [2000] found that p53 mutations of any type combined with the lowest or highest level of protein accumulation (scores of 0 or 4, respectively) independently predicted regional metastasis in GC.

Investigators have examined the mutational profile of GC by examining exons 2–11, although most studies restrict their examination to exons 5–8 (Table 3). The mutational spectrum of p53 in GC is wide. However, there are several sites where mutations are more common than others. These include, in a decreasing order of frequency, codons 175, 248, 273, 282, 245, and 213, all of which are CpG sites. G:C→A:T transitions at CpG sites are the most common type of mutation, regardless of the histological type of the tumor. Of interest is the fact that there appears to be a difference in the frequency of G:C to A:T and A:T to G:C transitions in Europeans as compared with Asian populations [Hongyo et al., 1995]. C to T mutations are induced by nitric oxide [Nguyen et al., 1992; Wink et al., 1992], a substance known to be produced during *H. pylori* infections. G:C→A:T transitions are also specifically induced by N-methyl-N′-nitro-N-nitrosoquanidine and N-nitroso compounds found in foods, substances considered to be carcinogens involved in gastric carcinogenesis [Sugimura and Kawaki, 1973]. These foods are commonly consumed in populations with a high risk for developing GC.

In addition to the presence of mutations, p53 contains several polymorphic sites. Only those in exon 4 have been examined in GC. Exon 4 contains two polymorphic sites, one at codon 36 and another at codon 72. Of these, the codon 72 polymorphism is by far more common. The genotype frequency in one study was as follows: arg/arg (54%); arg/pro (33%); and pro/pro (14%). The genotype differed significantly with race ($P = 0.0001$): 64% of whites had the arg/arg genotype compared with 24% of African Americans. There was no statistical significance for tumor location or histological tumor type [Shepherd et al., 2000].

Approximately 50% of all cancer cases involve missense mutations of one p53 allele coupled with a deletion of the second allele [Hollstein et al., 1991]. This is also true of GC. Matozaki et al. [1992b] examined gastric cell lines and found that 6/7 cell lines containing p53 mutations also demonstrated p53 LOH. Sano demonstrated both LOH and mutations in greater than 60% of tumors [Sano et al., 1991]. Overall, p53 LOH has been reported in 26–83% of GC (Table 4). In some cases, there is evidence to suggest deletion of the entire short arm of chromosome 17; the remaining cases show only partial LOH [Kim et al., 2001]. Data suggest that mutational events precede p53 allelic loss in the progression from early to late stage disease.

Certain percentages of GC that display LOH do not contain p53 mutations and vice versa [Kobayashi et al., 1996; Renault et al., 1996]. Some of these cases contain mutations at codons 245, 273, and 282 [Kobayashi et al., 1996]. This leads to the question as to whether any specific mutant allele acts in a dominant negative fashion in GC [Kobayashi et al., 1996]. Mutations in codons 151, 247, and 273 drive wild-type p53 protein into the mutation conformation during translation [Milner and Medcalf, 1991]. Thus mutations at these sites may not require a second event (mutation or LOH) to result in loss of function.

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