

Genetic alterations in gastric cancer

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Summary. - The aim of this paper is to review the most frequent genetic alterations found in gastric carcinoma, and to focus on the main differences between intestinal and diffuse cancer types. Particular emphasis will be given to tumor suppressor genes, above all to *p53*. The role of normal and mutated *p53* proteins has been studied in depth in a variety of cell types, and *p53* alterations have been extensively analysed in many human tumors, including gastric cancer. Accordingly, the paper will report *p53* data on this neoplasia, and consider these data in the light of various more general notions.

Key words: gastric carcinoma, genetic alterations.

Riassunto (*Alterazioni genetiche nel carcinoma gastrico*). - Questo lavoro si propone di passare in rassegna le alterazioni genetiche più frequenti nel carcinoma gastrico, sottolineando le principali differenze riscontrate fra cancro di tipo intestinale e cancro di tipo diffuso. Sono trattate più estesamente le alterazioni relative ai geni oncosoppressori ed in particolare a *p53*. Il ruolo della proteina *p53*, normale e mutata, è stato oggetto di studi approfonditi in numerosi tipi cellulari diversi e le alterazioni del gene sono state analizzate nella maggior parte dei tumori umani, incluso il cancro gastrico. Di conseguenza, i dati relativi a *p53* nel carcinoma dello stomaco vengono qui discussi nell'ambito di conoscenze più generali.

Parole chiave: carcinoma gastrico, alterazioni genetiche.

Introduction

The development of cancer is believed to result from a series of genetic alterations that lead to the progressive disorder of the normal growth control mechanisms. These genetic alterations underlie the multifactorial etiology and multistage progression of the tumors, but in most cases their precise sequence and biological relevance remain unclear.

Colorectal carcinoma lends itself admirably to investigation into the accumulation of genetic lesions, since most carcinomas arise from adenomas, and it is possible to obtain such tumors at various stages of development for analysis. Accordingly, a genetic model for tumor progression has been extensively developed for this neoplasia, in which the transformation process involves the inactivation of at least three tumor suppressor genes (*APC*, *p53* and *DCC*) and the activation of a dominant oncogene (*c-K-ras*).

The development of all gastrointestinal cancer types involves multiple oncogene and tumor suppressor gene alterations. Some of these alterations are shared by colorectal, esophageal and gastric carcinomas. However, these latter tumors also show distinct genetic changes, and very likely progress through different genetic pathways. Moreover, recent observations on gastric cancer demonstrate that some genetic lesions in this neoplasia depend on the subtype, namely intestinal or diffuse.

Gastric carcinoma of the intestinal type (also called glandular or well differentiated) is composed of cells that resemble intestinal columnar epithelial cells, while the

diffuse type carcinoma (also called undifferentiated) is characterized by poorly cohesive cells that show wide and diffuse infiltration into the gastric wall [1]. Epidemiological, histopathological and clinical studies indicate the existence of two distinct gastric carcinogenetic pathways: both starting with *Helicobacter pylori* gastritis, one passes through chronic atrophic gastritis, intestinal metaplasia, and dysplasia, and leads to intestinal cancer type, while the other is unrelated to this sequence of histological changes and ends in diffuse cancer (see the model for gastric carcinogenesis proposed by P. Correa in [2]). These differences in pathway between intestinal and diffuse cancers very likely reflect distinctions as to etiology and mechanisms of development.

Proto-oncogenes and growth factors

Activating point mutations in *ras* gene family members are frequent genetic lesions in most human carcinomas. In particular, *K-ras* mutations are quite common in pancreatic (about 80%) and colorectal (about 50%) tumors, and represent an early event in intestinal tumorigenesis (as has been well demonstrated by the analysis of pre-neoplastic lesions, i.e. small, intermediate and late adenomas).

In contrast, *ras* gene alterations, which mainly involve the *K-ras* gene, have been found less frequently in gastric carcinomas. The reported incidence of *K-ras* mutations in this type of cancer ranges from 0 to 21%, rising to 20-50% in some high-risk areas of Japan (see results and

data quoted in [3]). While this variation may depend on the population studied, other possible influencing factors include the sensitivity of the method utilized for the analysis, and idiosyncrasies in the tumor cases pooled for the given screening. It is not coincidental that, if we consider intestinal and diffuse type cancers separately, *K-ras* gene mutations seem to be limited to carcinomas of the intestinal type [4].

The *c-met* gene, which encodes for the hepatocyte growth factor receptor, is frequently amplified in both types of gastric tumors (19.2% in intestinal and 20% in diffuse), and particularly in scirrhous tumors (38.5%), a type of poorly differentiated (diffuse) cancer [5]. Gene amplification is significantly associated with advanced tumor stages and poorer prognosis [5]. A *tpo-met* oncogenic rearrangement was reported to be expressed in gastric carcinoma and precursor lesions [6]. An aberrant expression of *c-met* mRNA characterizes both intestinal and diffuse tumors: a 6.0-kb transcript of the gene was found to be expressed at high levels in 52% of tumor samples, whereas it was expressed only slightly in a small number of cases of normal mucosa [7].

A member of the fibroblast growth factor receptor family, the *K-sam* gene is frequently amplified in diffuse cancers, preferentially of the scirrhous type (33%) [8, 9].

The *c-erbB2* gene encodes for a protein that is closely related to the epidermal growth factor receptor. The gene is amplified in about 10% of gastric carcinomas and its protein product is overexpressed in 10-25% of cases; these alterations are more frequently detectable in intestinal than in diffuse cancers [10-13]. *c-erbB2* gene amplification and protein overexpression have been found in several types of human tumors. Numerous studies have clearly demonstrated that these combined genetic alterations correlate with poor clinical outcome in breast tumors, whereas a series of contradictory results have been obtained for gastric carcinoma. A recent immunohistochemical study seems to exclude the hypothesis that *c-erbB2* overexpression represents a useful prognostic indicator in this neoplasia [13]. On the other hand, *c-erbB2* amplification and/or overexpression have been reported to correlate with the presence of liver metastases and with invasion and nodal involvement in well differentiated gastric carcinomas [14].

Some other amplified oncogenes have been found in stomach cancer. However, these genetic alterations are uncommon; appearing to be associated with advanced tumor stages and the presence of metastases, they likely represent late and aspecific events in the temporal development of this cancer [15, 16].

Gastric cancer cells express a broad spectrum of growth factors, gut hormones and cytokines that, acting as autocrine or paracrine modulators, play a role in cancer development [17].

Transforming growth factor α (TGF- α) and epidermal growth factor (EGF) act as autocrine growth factors for gastric cells and overexpression of EGF, TGF- α and EGF-receptor contributes to malignant transformation [17,

18]. *cripto*, a gene of the EGF family, is expressed in most intestinal cancer tissues at higher levels than is the case in normal mucosa [19]. In addition, *cripto* protein is overexpressed in 44% of early gastric cancers, and in gastric intestinal metaplasia where expression is correlated to metaplasia diffusion [9].

Transforming growth factor β (TGF- β), which acts as a negative growth regulator, platelet-derived growth factor (PDGF), insulin-like growth factor II (IGF-II), and basic fibroblast growth factor (FGF) are frequently overexpressed in diffuse type gastric carcinomas [9].

Alterations in TGF- β receptors are also frequent in gastric cancer. TGF- β receptors are classified into three types, I, II and III; type I and II receptors are transmembrane proteins that contain serine/threonine kinase domains and that act as signal-transduction molecules, whereas type III is thought to act as modulator of TGF- β access to its signalling receptors. More than 80% of gastric cancers display a reduced level of TGF- β I receptor [20]. The observation that this reduction is related to the depth of tumor invasion has led to the idea that most advanced tumors escape from growth inhibition by TGF- β through the reduction in type I receptor [18, 20]. More recently, alterations in type II receptor gene or in type II receptor mRNA and protein expression have been observed in gastric cancer cell lines that are able to resist the TGF- β growth inhibitory effect [21]. These findings imply that type II receptor is required for the growth inhibitory effect, and demonstrate that its alterations are important events in gastric carcinogenesis [21].

Tumor suppressor genes and cell adhesion molecules

The analysis of restriction fragment length polymorphisms has led to the identification of several chromosomal regions that show loss of heterozygosity (LOH) in primary gastric cancer. Significant allelic losses have been found at the *bcl-2* and *c-met* loci [9], and at some polymorphic markers that map on 1p, 1q, 5q, 11p, 12q, 13q, 17p and 18q chromosome arms [18, 22-29]. Some specific regions, such as the 11p15 chromosome band, show high LOH frequencies in gastric cancer [27] as well as in other carcinomas, and are thought to contain candidate tumor suppressor genes. Regions 5q21, 17p13 and 18q21, which very frequently display LOH in gastric cancer, harbor well known tumor suppressor genes, namely, and respectively *APC-MCC* (adenomatous polyposis coli-mutated in colorectal cancer), *p53* and *DCC* (deleted in colorectal cancer). These genes are quite frequently inactivated by loss of coding sequence and/or by point mutation in colorectal cancer, where they play a crucial role in the carcinogenetic process. In gastric cancer, mutation analysis of *APC* and, more extensively, of *p53* genes, has served attempts to better assess the role of these genes in stomach tumorigenesis.

The *APC* gene codes for a huge protein of 2843 aminoacids: its cellular localisation, its predicted structure and its interaction with β -catenin support the hypothesis that the protein plays a role in the cell adhesion process [30, 31]. Catenin proteins are known to be associated with E-cadherin, a cell adhesion molecule, and are thought to link the E-cadherin/catenin complex to the cytoskeleton.

APC germline mutations are responsible for inherited predisposition to colon cancer (FAP: familial adenomatous polyposis) [32], whereas somatic mutations are present in the great majority of sporadic colon tumors [33, 34]. In colon cancer, over 95% of mutations result in premature truncation of the gene product (reviewed in [35]). A recent analysis has demonstrated that the central region of the protein, which is typically deleted or truncated in tumor cells, is responsible for a down-regulation of the β -catenin; this suggests that the tumor suppressor activity of mutant *APC* protein may be compromised due to its inability to regulate catenin [36].

APC gene mutations in gastric cancer are limited to particular histotypes [37, 38], and show a high frequency (41%) in very well differentiated adenocarcinomas [38]; in contrast, mutation incidence seems to be very low in cancers of the major histopathological types [39]. The mutation spectrum differs from that of colorectal cancers, with a high prevalence of missense mutations [18]. *APC* alterations have also been observed in gastric adenomas and in a small fraction of gastric hyperplastic polyps [18], suggesting that, at least in some tumor subtypes, *APC* may play an early role in tumorigenesis. The observation that *APC* mutations are rare, whereas 5q21 allelic losses are frequent in common gastric cancer types, raises the question of whether the *MCC* gene, which is closely linked to *APC*, plays some role in gastric carcinogenesis. LOH analysis of polymorphic markers within *APC* and *MCC* genes indicates that LOH at the *APC* locus is accompanied by allelic losses at the *MCC* locus [28]; the direct mutation analysis of *MCC* will clarify the possible role of this gene in stomach tumorigenesis.

The *p53* tumor suppressor gene encodes for a nuclear phosphoprotein, and acts as a transcription factor (reviewed in [40]). The protein is involved in the control of cell cycle, and helps to prevent cancer development by blocking the proliferation of cells that have sustained DNA damage [41] and by triggering programmed cell death (apoptosis) [42].

p53 alterations are the most frequent genetic changes known to occur in human cancer, and both point mutations and allelic losses have been detected in a wide variety of tumors. In carcinomas, 75-80% of mutations are missense mutations that give rise to a faulty protein product; the "second" allele is frequently lost, and the mutant allele is reduced to homozygosity. Most *p53* gene mutations are confined to exons 5-8, which correspond to the highly conserved domains of the protein. In addition, some mutations at specific codons ("hot spots": codons 175, 245, 248, 249, 273 and 282) occur very frequently (see [43-45] for reviews on mutation data).

The half-life of the *p53* protein is very short in normal tissues, but very prolonged in tumor cells. Accordingly, the protein is normally undetectable by immunohistological analysis, whereas *p53* positive immunostaining, typically confined to the nucleus, is detectable in most transformed cells. The accumulation of the protein is a consequence of its stabilisation, which usually results from point mutations, that modify protein conformation and stability [44]. The high correlation that exists between protein accumulation and gene mutation has prompted intensive investigations into protein expression by immunohistochemical methods. Thanks to the rapidity and simplicity of the immunohistochemical assay, thousands of tumors and many pre-neoplastic lesions have been analyzed with the aim of correlating *p53* overexpression to tumor types, to stages of progression and to clinico-pathological parameters.

In gastric cancer, various molecular analyses have demonstrated a high incidence of *p53* gene mutations: this ranges from 30 to 65%, depending on the population studied and, possibly, on the method utilized for the screening (reviewed in [3, 43-45]). High mutation frequencies characterize advanced cancers both of the intestinal and the diffuse types, although *p53* mutations have been found to be more frequent in intestinal than in diffuse tumors [46, 47]. These results agree well with those obtained by immunohistochemical methods [48, 49]. Figs 1 and 2 show the DGGE (denaturing gradient

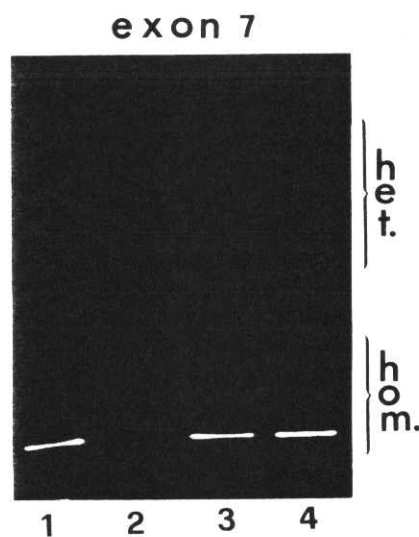


Fig. 1. - Denaturing gradient gel electrophoresis showing patterns from different tumor DNAs. DNA samples were amplified with primers specific for exon 7 of the *p53* gene, and the PCR products were loaded onto an appropriate gradient of denaturants. *Sample 1*, normal sequence; *sample 2*, GGC→AGC (Gly→Ser) substitution at codon 245 (*sample n. 5* of Table 1); *samples 3 and 4*, control samples carrying different mutations. *het.*: heteroduplex molecules; *hom.*: homoduplex molecules.

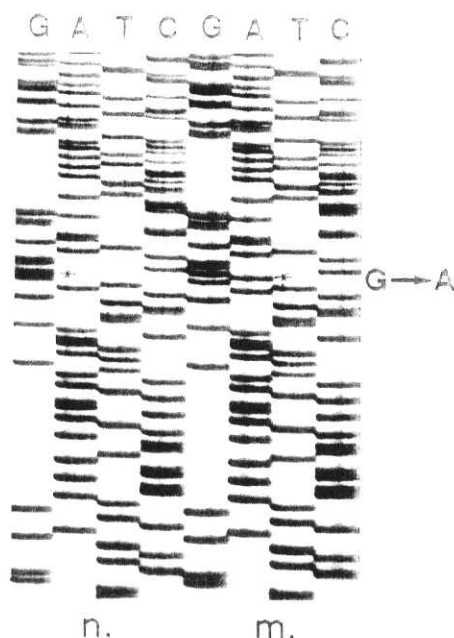


Fig. 2. - *p53*-exon 7 sequence of a normal (*n.*) and a mutated (*m.*) DNA sample. The mutated sample (tumor n. 13 in Table 1) harbors a GGC→AGC (Gly→Ser) base change at codon 244.

gel electrophoresis) patterns and the DNA sequence of gastric tumor samples that carry *p53* mutations. Fig. 3 shows gastric tumor samples that display *p53* immunopositive staining.

In some types of tumors, the presence of *p53* alterations in pre-neoplastic tissue areas suggests that this genetic lesion plays a direct role in carcinogenesis; in other tumors, the preferential involvement of high grade and/or invasive cases supports the hypothesis that *p53* alterations are involved in the progression rather than in the initiation of tumors. Molecular and immunological studies carried out on early and advanced gastric cancers of varying histotypes indicate that *p53* alterations are early events in intestinal type cancer, but that they are late events, mainly associated with tumor progression, in the diffuse type [46, 47]. Moreover, *p53* alterations have been reported to appear specifically at the stage of dysplasia [47, 50-52] during tumorigenesis of the intestinal gastric cancer, and, more recently, they have also been reported to occur in intestinal metaplasia samples [53]. No data are available on *p53* alteration timing in diffuse gastric cancer, since no clear pre-cancerous lesions have yet been identified in this type of tumor.

Although human tumors generally show a good correlation between *p53* immunohistochemical protein detection and presence of gene mutations, over-expression without mutation has also been observed

[44]. The degree of concordance is variable, and depends, at least partially, on the tumor analyzed. The factors that cause immunoreactivity of apparently non-mutated *p53* protein are complex and only partially understood. Concordance between *p53* gene mutations and protein overexpression has important clinical applications; immunohistochemical methods rapidly detect *p53* alterations in large retrospective and prospective series of archival tumors, and provide prognostic information.

Recently, we made parallel use of molecular and immunohistochemical methods (PCR-based DGGE and sequencing methods, and immunostaining with DO7 and CM1 *p53*-specific antibodies) to analyze a panel of gastric tumors, composed of 71 early and 18 advanced cancers [47]. The results obtained are summarized in Table 1 and are shown there as an example of *p53* alteration screening in gastric cancer (compare the results obtained with the two antibodies, and notice the concordance between mutation and immunoreactivity). Overall, of the tumors without detectable *p53* mutations, about 50% showed some positive immunostaining. This value decreases to about 30% if one removes tumors that display less than 5% immunoreactive cells (see [47] for a critical evaluation of molecular and immuno-histo-chemical data in gastric cancer).

The literature reports immunohistological studies that evaluated the prognostic significance of *p53* protein accumulation in gastric cancer: different analyses indicate that *p53* overexpression is of some importance [47, 48, 54], and that it represents an independent marker for shortened survival in gastric cancer patients [54].

In human cancers, the incidence, distribution, and nature of *p53* mutations show tumor type and tissue specificity. The *p53* mutation analysis carried out on thousands of different human tumors provided the first extensive set of mutations at a single locus. These mutations arise *in vivo* in the various cell types from which cancers originate. Since exogenous carcinogens and endogenous biological processes damage the genome in characteristic ways, *p53* mutations can yield inferences about the prevalent sources of DNA damage in a given tumor. Base transitions at CpG dinucleotides represent about 1/4 of all *p53* mutations in human cancers, and probably arise from "spontaneous" deamination of 5-methylcytosine, which yields thymine and thus C→T substitution.

In gastric cancer, a high incidence of G:C→A:T transitions has been found in patients of European origin (about 90%). In Oriental patients, the incidence of G:C→A:T changes is lower (48%), whereas A:T→G:C transitions, as well as transversions, are more frequent

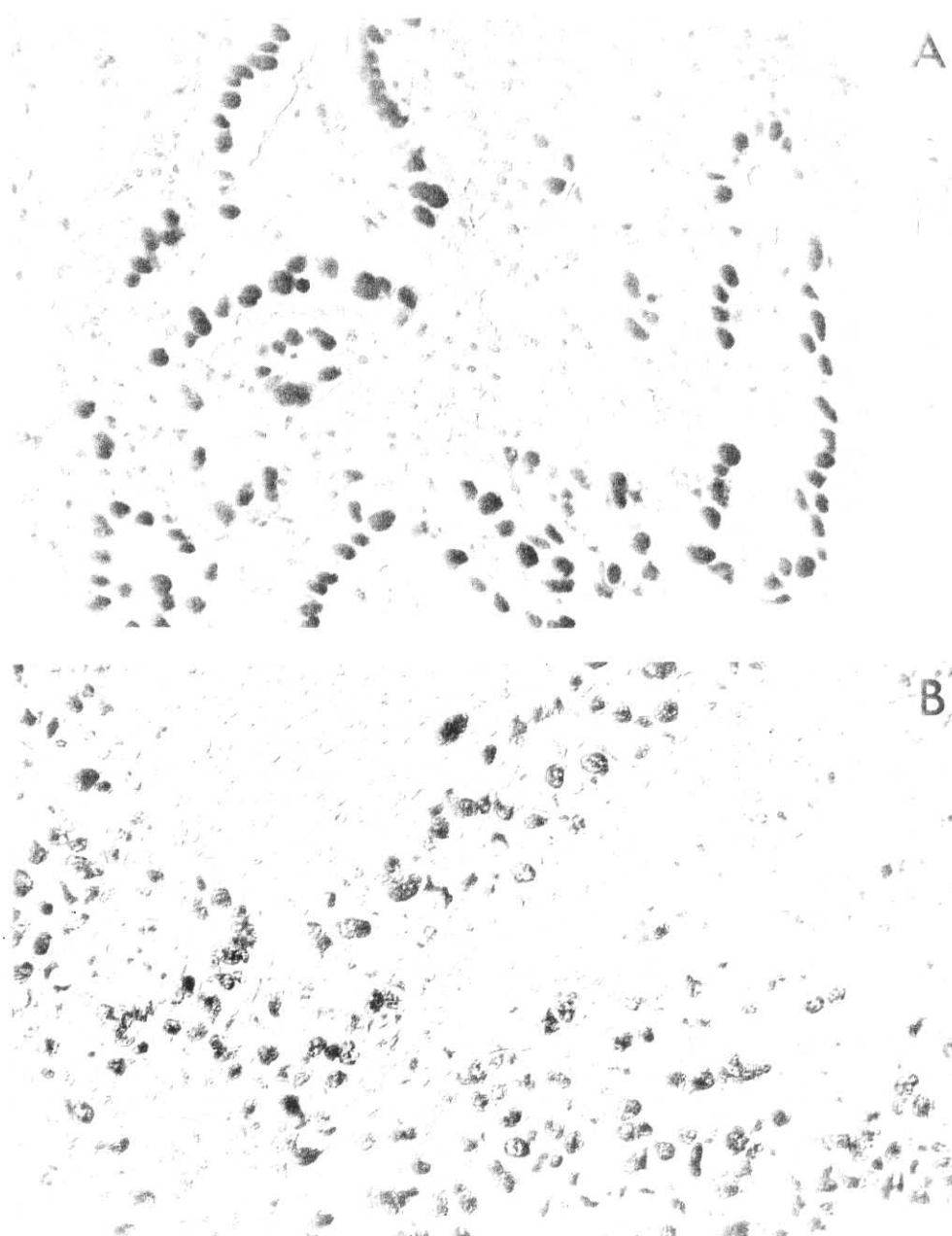


Fig. 3. - Immunohistochemical staining of p53 in gastric cancer. A) Intramucosal, intestinal type gastric carcinoma. Nuclei of gland-forming cells are strongly immunoreactive with p53 antibodies. Note an unreactive, non-neoplastic gland on the right side. Immunoperoxidase with DO7 monoclonal antibodies, hematoxylin counterstained. B) Advanced diffuse-type gastric carcinoma. Non-cohesive neoplastic cells, diffusely infiltrate the muscular wall and stain with p53 antibodies. Immunoperoxidase with DO7 monoclonal antibodies, hematoxylin counterstained.

(Courtesy of R. Fiocca, IRCCS-S. Matteo and University of Pavia).

than in European cases. Frequencies for transitions at CpG sites also vary, even between populations from neighbouring regions ([55]; see results and data quoted in [3]). Differences in genetic and/or environmental backgrounds may explain geographic differences in the pattern of *p53* genetic changes. In any case, data pooled from differing population samples show that G:C→A:T transitions at CpG sites are the most common *p53*

mutations in gastric cancer. This is evident from Fig. 4, which shows the *p53* mutation spectrum in gastric cancer compared to the spectra of other tumors of the digestive tract[56]. It is interesting to note that G:C→A:T transitions are specifically induced by nitroso compounds, which are considered to be carcinogens involved in the pathogenesis of gastric cancer. On the other hand, as already mentioned, “spontaneously” occurring

Table 1. - *p53* gene mutations and protein nuclear accumulation in early (egc) and advanced (agc) gastric cancers of different histotypes

Case	Histology	p53 gene mutations				p53 protein histochemistry			
		exon	codon	base change	amino acid substitution	DO7		CM1	
	intestinal /diffuse					positive cell %	staining intensity	positive cell %	staining intensity
egc									
1	int	5	175	CGC->CAC	Arg->His	100	+++	80	+++
2	int	5	175	CGC->CAC	Arg->His	50	++	20	+
3	int	6	216/7/8	GTG deletion	Val deletion	80	+++	70	++
4	int	5	178	C insertion	His-His-Glu->Pro-Pro-stop	1	+	0	
5	mix	7	245	GGC->AGC	Gly->Ser	50	++	20	+
6	int	5	157	GTC->TTC	Val->Phe	60	++	50	++
7	int	8	273	CGT->CTT	Arg->Leu	50	++	50	++
8	int	5	162	ATC->AAC	Ile->Asn	40	++	30	+
9	int	7	248	CGG->TGG	Arg->Trp	5	+	1	+
10	int	6	213	CGA->TGA	Arg->stop	0		0	
11	int	8	280	AGA->ACA	Arg->Thr	80	+++	70	++
12	int	6	202	C insertion	6 aa changes - stop	0		0	
13	int	7	238	TGT->TAT	Cys->Tyr	50	+++	25	++
"	"	7	244	GGC->AGC	Gly->Ser				
14	int	8	273	CGT->TGT	Arg->Cys	100	+++	100	+++
15	int	7	248	CGG->TGG	Arg->Trp	80	+++	80	+++
16	int	5	175	CGC->CAC	Arg->His	80	++	70	+++
17	int	5	175	CGC->CAC	Arg->His	80	+++	80	+++
18	dif	7	248	CGG->TGG	Arg->Trp	5	++	2	++
19	mix					20	+	60	++
20	int					80	++	80	++
21	mix					20	+	10	+
22	int					0		5	+
23	int					80	+++	80	+++
24	int					5	+++	5	++
25	int					5	+	2	++
26	int					90	+++	0	
27	dif					50	+++	50	++
28	dif					1	+	0	
29	dif					2	++	2	+
30	int					80	+++	60	+++
31	int					60	++	60	++
32	int					1	++	0	
33	int					50	+++	80	++
34	int					3	++	1	+
35	dif					1	+	0	
36	dif					5	+	0	
37	dif					2	++	0	
38	dif					1	+	1	+
39	dif					2	+	5	++
40	dif					1	+	0	
agc									
41	dif	7	243/4	G deletion	Gly-Gly-Met->Ala-Ala-stop	50	++	0	
42	dif	8	273	CGT->CAT	Arg->His	90	+++	90	++
43	dif	6	189	GCC->GGC	Ala->Gly	20	+	0	
44	dif	7	248	CGG->TGG	Arg->Trp	90	++	80	++
45	dif	8	273	CGT->CAT	Arg->His	90	+++	80	++
46	dif	6	192	CAG->TAG	Gln->stop	0		0	
47	dif					5	+	5	+
48	dif					40	++	20	++
49	dif					1	+	1	+
50	dif					8	+	5	+
51	dif					80	++	70	++
52	dif					1	+	1	+
53	dif					3	+	3	+

Modified from Ranzani *et al.* 1995. See ref. [47] for more details.
 int: intestinal; dif: diffuse; mix: mixed.

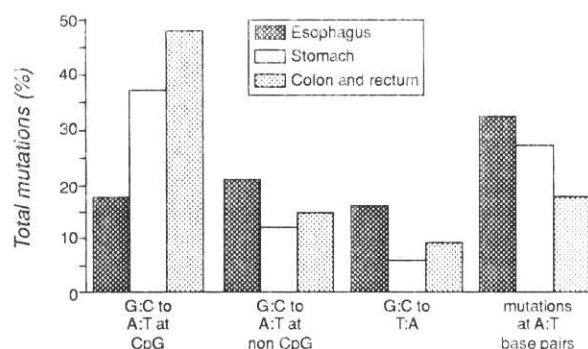


Fig. 4. - *p53* mutation spectra in gastrointestinal cancers. (Modified from Hollstein *et al.* [56]).

deamination of 5'-methylcytosine at CpG dinucleotides has been proposed as a possible explanation for G:C->A:T replacements.

Recent research has revealed a relationship between *p53* status and cancer cell sensitivity to chemotherapeutic agents. This revelation has substantial clinical implications. It has been shown that wild-type *p53* is required for the efficient execution of programmed death of some types of cells in response to the DNA-damaging action of anti-cancer agents [57]. Experimental data on gastric cancer cell lines indicate that the mutational status of *p53* predicts the chemosensitivity of gastric adenocarcinomas and suggest a mechanism in which *p53* protein assists cellular response to chemotherapy [58].

An interesting recent development is the idea that some cell adhesion molecules may act as tumor suppressor genes. Of these molecules, E-cadherin plays a major role in the organization and integrity of epithelial tissues; its reduced expression is considered a crucial event in the dysfunction of the cell adhesion system, which in turn triggers cancer invasion and metastases [59,60]. In some studies, E-cadherin immunoreactivity has been shown to be inversely correlated with tumor differentiation [61]. E-cadherin gene mutations have been found in human gastric carcinoma cell lines [62] and in a substantial (50%) proportion of diffuse type cancers [63]; these tumors are also characterized by the absence of specific E-cadherin immunoreactivity [64]. All these observations are of particular interest, since E-cadherin gene alterations can give rise to the growing pattern and the scattered-cell phenotype which characterise diffuse type tumors.

Finally, *CD44*, which codes for a cell surface molecule that plays an important role in cell-to-cell interaction, and *nm23*, a tumor metastasis gene, have been implicated in the development of metastasis and disease progression in some tumors, including gastric carcinoma [18].

Genetic instability

Cells from colorectal tumors and carcinomas that develop in affected members of HNPCC (hereditary nonpolyposis colorectal cancer) families are characterized by size alterations in the short tandem repeat sequences that are scattered throughout the genome. This microsatellite instability (RER+: replication error phenotype) reflects defective function of mismatch repair proteins encoded by the genes that are responsible for HNPCC. Mismatch repair genes play a crucial role in DNA replication fidelity, a role that includes the stabilisation of microsatellite sequences. The mechanism by which mutations in these genes causes susceptibility to cancer development is not clearly understood. Mutations are believed to inactivate the repair system, and thus to yield a mutator phenotype that could alter critical genes and lead to tumor formation (reviewed in ref. [65, 66]).

The RER+ phenotype has been found not only in HNPCC tumors, but also in a fraction of sporadic colorectal cancers [67, 68], as well as in a subset of other sporadic tumors, including endometrial, pancreatic, breast and gastric carcinomas [69].

In stomach cancer, genetic instability is of some importance, since RER+ phenotype frequency is about 30%, and ranges in the literature from 15 to 38% [69, 70-75]. Discordant results have been obtained on RER+ phenotype frequencies in intestinal and diffuse types [70, 74, 75]. Microsatellite instability seems to occur as a late genetic event in gastric cancer, in that its incidence is significantly higher in tumors of advanced stages than is in those of early stages [72]. On the other hand, it has also been shown that the RER+ phenotype mostly precedes tumor growth, and thus possibly contributes to malignant transformation [73].

Whether somatic mutations in already cloned HNPCC genes account for genetic instability in gastric cancer remains to be investigated.

Conclusions

All the above mentioned results clearly demonstrate that gastric cancer develops through the accumulation of multiple genetic lesions that involve oncogenes, tumor suppressor genes and DNA mismatch repair genes. Moreover, intestinal and diffuse type cancers, not only represent two distinct histological and clinico-pathological entities, but also progress through differing genetic pathways.

Tahara [18] has recently proposed a molecular model for the tumor progression in gastric cancer. The model includes the main genotypic events that occur in the gastric cancer and pre-cancerous lesions which have been reviewed here. Although further analyses are needed to confirm various observations, and to ascertain the timing and the importance of specific genetic lesions, the

construction of such a model is evidence of the striking advances in the molecular analysis of gastric cancer. Current research is attempting to deepen our understanding of the role of the different genes, and of their interactions in the transformation process of gastric epithelial cells. A more complete understanding of this process will make it possible to improve cancer diagnosis, to foresee patient prognosis and, most importantly, to provide specific therapeutic approaches.

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