Gastric carcinoma: clinical, pathogenic and molecular aspects

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Introduction

It is generally accepted that the incidence of gastric carcinoma is declining,1-6 although some have argued that this decline may have resulted from refinements in diagnosis and classification of abdominal malignancies rather than being a true decline.7 Yet even today, gastric carcinoma still counts as one of most common causes of cancer-related death, with reported overall five-year survival rates rarely in excess of 15% in most Western series, despite continuous efforts to improve diagnostic and therapeutic modalities.8-12 Identification of molecular markers for predicting preneoplastic progression, and thus improving early detection, treatment, and finally prognosis, would have considerable clinical impact. This review focuses on the pathogenesis and molecular biology of gastric carcinoma, since early events bear on the detection of gastric cancer, whereas late events may influence biological behaviour and therefore may influence therapy and overall prognosis. To stress the importance of such insights, several clinical trends are discussed that may negatively influence future survival rates.

Clinical trends

In sharp contrast to the dismal prognosis of patients with advanced gastric carcinoma, the prognosis of patients with surgically-treated early gastric carcinoma (EGC), defined as carcinoma confined to the mucosa or submucosa, with or without lymph-node metastasis, is generally excellent with reported 5-year survival rates of 90-95%.13-17 Numerous studies from Western countries have reported an increase in incidence of early gastric carcinoma over the years. This trend is usually ascribed to improved diagnostic procedures, fiberendoscopy with targeted biopsy in particular.14,18-21 However, many of these studies suffer from confounding factors in that they compare results of a ‘pre-endoscopy’ era with those in a period in which endoscopy was routinely used, or compare time periods in which different radiological techniques were used, sometimes clearly inferior to the preferred biphasic-contrast examination with drug-induced hypotony.22-25 Thus it may well be that the reported increase in incidence of EGC should be ascribed to diagnostic limitations and pitfalls in the earlier study periods rather than being a true increase.26,27

Another disturbing trend with regard to the clinical management of patients with gastric cancer is the observed increase—both relative and absolute—in incidence of proximally-located tumours.28-32 Unfortunately, the prognosis of these proximal tumours is generally poor compared to more distal tumours. Multivariate analysis has shown that this poor prognosis is caused by the majority of these tumours being of advanced T and N stage rather than by tumour sites per se.31,33 These findings appear to correlate well with the observation that proximal tumours have a greater tendency for submucosal invasion, regardless of size or histological type.
than those located in the pyloric region. Although no firm explanation exists, it has been related to the presence of a thinner muscularis mucosae in the fundic region, and the presence of firmly-packed glands, which might be able to block lateral growth.34

Finally, a third major trend has been observed which may affect the clinical management of patients with gastric cancer. As mentioned previously, the incidence of gastric carcinoma has declined. This decline in incidence primarily results from a decrease in incidence of the intestinal-type carcinoma, according to Lauren.35-37 The incidence of the diffuse type, however, varies less over time or may even gradually increase.33 The rising number of patients with diffuse-type tumours may pose some challenging diagnostic and therapeutic problems. In contrast to the intestinal type, no precursor lesion of the diffuse type has been identified, implying that endoscopic-biopsic surveillance of patients at risk for developing this tumour is not feasible.38,39 Moreover, standard biopsies may be too superficial for a correct diagnosis necessitating additional endoscopic-biopptic measures.40-42 From a therapeutic viewpoint, two striking characteristics of the diffuse type are important. First, the diffuse type has a higher propensity for intramural spread beyond the macroscopic tumour margin. This often leads to total gastrectomy in resectable cases. Second, the diffuse type has a greater tendency to spread transmurally via the serosa, which may partly explain the worse prognosis of advanced diffuse-type tumours.43-50

In conclusion, it is unlikely that the detection rate of EGC will drastically improve in the (near) future. Moreover, the increase in the proportion of patients with proximal and diffuse-type tumours—both with a higher propensity for invasive growth—will in all probability have a negative effect on future overall 5-year survival rates.

**Pathogenesis**

Lauren divided gastric carcinoma into two main histological types, namely intestinal- and diffuse-type carcinoma. These two types differ not only in morphology, but also in clinical and epidemiological characteristics. Moreover, it is hypothesized that the diffuse type arises from the normal gastric mucosa, whereas the intestinal type is thought to be the end result of a long and sequential process leading from normal gastric mucosa via various precursor stages to overt carcinoma. One of the first clues that led investigators to hypothesize that chronic atrophic gastritis (CAG) and intestinal metaplasia (IM) were closely linked to intestinal-type gastric carcinoma was the observation that, at least in high-risk areas, a striking correlation existed between the incidence of intestinal-type carcinoma and the overall prevalence of CAG and IM. Moreover, migrants from high-risk areas to low-risk areas not only experienced a reduction of 15% in gastric cancer risk, but also a reduction in the prevalence of IM.51-53 Additional evidence suggesting a link between CAG/IM and intestinal-type gastric carcinoma came from long-term follow-up studies. CAG and IM preceded intestinal-type carcinoma in up to 85% of these patients, the gastric cancer risk increasing up to 18-fold depending on the severity of atrophy.54,55 Further insight into the dynamic link between CAG/IM and intestinal-type gastric carcinoma was provided by Sipponen et al. They showed that in patients with intestinal-type carcinoma—but not in patients with diffuse-type carcinoma—the prevalence and severity of atrophic gastritis and IM in the area affected by the tumour was higher than in controls, regardless of the age group studied. Interestingly, in the tumour-free area, the progression of gastritis and IM was similar to that in controls.56,57 These data together with data from histo-topographical and microscopy studies strongly suggest that CAG/IM and intestinal-type gastric carcinomas are closely linked.39,58-60

So far, however, no study has been published showing conclusively that IM evolves into intestinal-type carcinoma. On the contrary, IM is very common in the mucosa surrounding benign gastric disorders without ever progressing to carcinoma.61,62 Moreover, it has even been suggested that IM and intestinal-type gastric carcinoma may arise coincidentally.63 Interestingly, however, IM is not a single entity but a rather heterogeneous lesion. Although various classifications exist, the most widely used classification of IM subtypes has been the (modified) classification of Jass and Filipie. Based on histochemical staining profiles, three major IM subtypes have been identified, namely types I, II and III IM.54-66 Type III IM appears to carry the highest preneoplastic potential. First, subtype III IM, in contrast to subtypes I and II, is significantly associated with both early and advanced intestinal-type carcinomas, but not with diffuse-type carcinomas or with benign gastric ulcers.61,64,67-69 Second, the mucin profiles of advanced intestinal-type carcinoma and type III IM are similar.70 Third, type III IM can be found in the mucosa surrounding adenomatous gastric polyps in contrast to the gastric mucosa surrounding hyperplastic gastric polyps.71,72 Fourth, in contrast to age-matched controls, a higher prevalence of type III IM has been reported in patients with pernicious anaemia and in first-degree relatives of patients with gastric cancer, which are often considered to be premalignant states.73 Whereas the aforementioned studies are all cross-sectional in design, long-term prospective follow-up studies have reported contrasting data on the value of type III IM as a marker for...
for endoscopic surveillance. Whereas the presence of type III IM in endoscopic biopsies has been reported to have strong predictive significance with regard to the development of (early) gastric carcinoma during follow-up, two other studies fail to confirm this finding. Although one cannot infer causality from these Time course the causation of gastric carcinoma, the so-called Bradford-Hill guidelines for multifactorial determined diseases should be applied. In essence, these guidelines take into account the following items: time course, strength, dose response, consistency, reversibility and biological plausibility.

**H. pylori and gastric carcinogenesis**

Koch's original postulates for causality are useless in assessing the possibly causative role of *H. pylori* in this process, since gastric carcinoma may develop long after *H. pylori* has been cleared from the stomach. Therefore, to assess the role of *H. pylori* in the causation of gastric carcinoma, the so-called Bradford-Hill guidelines for multifactorial determined diseases should be applied. In essence, these guidelines take into account the following items: time course, strength, dose response, consistency, reversibility and biological plausibility.

**Time course**

In cross-sectional studies, intestinal metaplasia can be found more often in *H. pylori*-positive patients, regardless of the age group studied. Moreover, most histological studies have shown that *H. pylori*-associated chronic gastritis is the mucosal background lesion in the majority of gastric carcinoma cases, irrespective of Lauren type or tumour stage. Although one cannot infer causality from these studies, long-term follow-up studies indicate that *H. pylori* infection is an important determinant of atrophy and intestinal metaplasia development and progression. Similarly, serological studies strongly suggest that *H. pylori* and gastric carcinoma are closely associated. Moreover, the risk of developing gastric carcinoma was reported to be significantly higher in subjects previously infected with *H. pylori* than in their non-infected counterparts.

**Strength**

If there is a causal relationship, the question is: how large is the risk of developing gastric carcinoma in infected subjects? The relative risk estimates are usually between 2 and 6, with a pooled estimate of 3.8. Surprisingly, although statistically significant, the increase in risk is not that very high compared to, e.g. the increase in lung cancer risk due to smoking (reported relative risk estimate around 18). However, the relative risk estimates may have been hampered by flaws in study design. When correcting for the time between blood sample collection and diagnosis, the relative risk estimate rose to 8.7 (95% CI 2.7–44.7) when seropositivity was assessed 15 years or more before diagnosis. This correction has its impact on the magnitude of the attributable risk. Assuming a 35% prevalence of *H. pylori*, a relative risk of 3.8 would give an attributable risk of 50%, whereas a relative risk of 8.7 would give an attributable risk of 73%. However, in developing countries with a prevalence of *H. pylori* in excess of 80–85%, the figures would be 70% and 86%, respectively.

**Dose response**

In itself, this item is impossible to assess since no data are available on the impact of *H. pylori* density with regard to subsequent gastric cancer risk. However, one might consider the length of infection as a substitute. It is well known that in high-risk areas for gastric carcinoma, *H. pylori* is acquired during early childhood. This finding is in all probability related to poor socio-economic conditions, which holds true for gastric carcinoma as well. Yet there are also areas with a high prevalence of *H. pylori* infection and low gastric cancer risk, or vice versa. These latter observations once more stress the multifactorial cause of gastric carcinoma.

**Consistency**

Although contradictory results have been reported, it may be concluded from the available data that there is almost unanimous support for a causal association between *H. pylori* and gastric carcinoma. In particular, all cohort studies have shown a positive association.

**Reversibility**

At present, no data are available to show a decrease in gastric carcinoma rates after successful eradication of *H. pylori*. However, since the incidence of gastric carcinoma has been decreasing, it is possible that this decrease may be due to a decrease in the prevalence of *H. pylori* infection during the last decades. Although we do not know what these prevalence rates were some decades ago, it is intriguing that the observed increase in prevalence of *H. pylori* with age could be interpreted as a decreased prevalence in successive birth cohorts. Another explanation might be to assume a constant rate of infection in different age groups. However, in a serological follow-up study (*n* = 115) with a mean interval of 11.5 years, an annual seroconversion rate of 0.3% was found, whereas antibody concentrations did not increase with age. Another study reported similar findings with an annual seroconversion rate of 0.49% during 7.5 years of follow-up. Therefore, these data favour the previous assumption that the observed increase in prevalence
rates of \textit{H. pylori} with age is due to a birth cohort phenomenon. Finally, within the realm of reversibility, data from Finland are also suggestive that \textit{H. pylori} may be causally associated with gastric carcinoma. It was found that the prevalence of chronic gastritis (both atrophic and non-atrophic) had dropped by 18\% on average between 1977 and 1992, showed a birth cohort phenomenon and closely followed the epidemiological downward trend of gastric carcinoma in this study period.\textsuperscript{111, 112} Since \textit{H. pylori} is the principal cause of chronic gastritis, it can be inferred from these findings that the common factor, underlying both time trends, is most likely a decrease in prevalence rates of \textit{H. pylori}.

\textbf{Biological plausibility}

If we consider the biological plausibility of \textit{H. pylori} being causally involved in gastric carcinogenesis, it should be noticed that \textit{H. pylori} infection induces an increase in cell proliferation rates, which normalize after successful eradication.\textsuperscript{113, 114} Moreover, infection with \textit{H. pylori} usually leads to a decrease in the gastric juice concentration of ascorbic acid, a potent anti-oxidant and protective factor against gastric cancer development. Possible mechanisms of action include the scavenging of nitrite, thus preventing the formation of N-nitroso compounds; inhibition of the mutagenicity of the preformed N-nitroso compounds; and the scavenging of free oxygen radicals, which are overproduced in individuals infected with \textit{H. pylori}. In cases of low gastric juice ascorbic acid concentration, nitrite and nitrosamine concentrations may rise. Since N-nitroso compounds constitute some of the most potent carcinogens known to induce gastric cancer, further evidence is provided for a role of \textit{H. pylori} in gastric carcinogenesis.\textsuperscript{115-122} Finally, \textit{H. pylori} induces an influx of inflammatory cells, especially macrophages and polymorphonuclear neutrophils, leading to the accumulation of toxic oxygen radicals, with the risk of inducing mutations. In view of the above, it may be envisioned that the DNA-damaging effects of potential carcinogenic factors may act on the gastric epithelium in a state of \textit{H. pylori}-induced hyperproliferation with less scavenging capacity. As a result, the cumulative chance of somatic mutations in oncologically important genes increases, since each cell division has a finite chance of DNA synthetic error. In view of these data, it is puzzling that some areas, despite a high prevalence of \textit{H. pylori} in early childhood, have a low incidence of gastric carcinoma. A possible explanation might be to consider \textit{H. pylori} as a promoting factor in gastric carcinogenesis, hypothesizing that \textit{H. pylori} alone is insufficient to initiate carcinoma development.\textsuperscript{124, 125}

In summary, whereas the pathogenesis of diffuse-type carcinoma remains poorly understood, the process leading to intestinal-type carcinoma is a multi-step, multifactorially-determined sequence of histopathological changes. It appears that intestinal metaplasia in this model should be replaced by type III intestinal metaplasia. Moreover, although unsolved problems remain, there is a growing body of evidence supporting a causative role for \textit{H. pylori} in gastric carcinogenesis. Undoubtedly, the different outcomes of \textit{H. pylori} infection result from an intricate interplay between host and bacterial factors modified by the inherent genetic susceptibility of the host and by environmental exposure to various other carcinogens such as dietary factors.\textsuperscript{126-128} However, elucidation of the exact underlying mechanisms in gastric carcinogenesis will require exploration of the molecular abnormalities that almost certainly govern the complex phenotypical aspects of this process.

\textbf{Molecular biology of gastric carcinogenesis}

\textbf{Abnormalities in DNA content}

Flow cytometry can be used to detect DNA aneuploidy, defined as a DNA content equivalent to a chromosome number of less than 42 or greater than 50. Yet, when interpreting flow cytometric studies on gastric carcinoma, several potential confounding factors should be taken into account when comparing their results. First, gastric carcinoma may show a marked heterogeneity of DNA content. As a result different parts of the tumour may differ in cytokinetic profiles.\textsuperscript{129, 130} Second, analysis of freshly-obtained tumour tissue may yield discordant results compared to paraffin-embedded tissues, mainly due to a greater amount of debris in the latter materials.\textsuperscript{131, 132} Third, even with freshly-obtained tissue samples, results may be influenced by the techniques used to disaggregate tumour cells (e.g. mechanical, enzymic or chemical) since they may yield different preparations. Finally, other sources of variability may include different selections of cells to be used as the diploid reference group, different definitions of ploidy based on DNA index, and variability in the precision of the instruments used for measurements.\textsuperscript{133} However, most flow cytometric studies on gastric carcinoma report a DNA aneuploidy rate of 50–75\%.\textsuperscript{134, 135} Interestingly, cardia carcinomas appear to have the highest DNA aneuploidy rate of nearly 100\%, compared to more distal tumours, and have the worst prognosis.\textsuperscript{136} In contrast, despite a worse overall prognosis, diffuse-type carcinomas tend to be less aneuploid than intestinal-type carcinoma.\textsuperscript{137-139} Unfortunately, additional data on the
Abnormalities in chromosomal structure

Aneuploidy is usually caused by abnormalities in chromosomal structure. In view of the above, it is not surprising that several karyotypic abnormalities have been reported in gastric carcinomas. Chromosomes 1, 3, 7, 8, 9, 11, 12, X and Y have so far been found to be abnormal. However, none of the abnormalities (translocations, deletions, trisomies) has yet been found to be a hallmark of gastric carcinoma or to have clinical implications in terms of prognostic significance.186-187

Loss of heterozygosity

Allelic loss has been described for chromosomes 1q, 1p, 5q, 7p, 11p, 11q, 12p, 12q, 13q, and 17p. Unfortunately, however, at present it remains unclear what the implications for the carcinogenic process are.148-152

Alterations in oncogenes

ras

The human ras proto-oncogene family includes the homologous H-, K- and N-ras genes, which code for closely related 21 kDa proteins (p21ras). Although considered to represent a rare event in tumorigenesis, activation of the ras genes by gene amplification with enhanced (over)expression of p21ras proteins has been described. With regard to gastric carcinomas, immunohistochemical studies on p21ras overexpression have reported increased expression of p21ras proteins, especially in intestinal-type carcinomas compared to diffuse-type carcinomas. Moreover, the normal mucosa surrounding diffuse-type tumours, but not surrounding intestinal-type tumours, showed increased expression of p21 ras proteins. Interestingly, intestinal metaplasia and dysplasia, surrounding intestinal-type carcinomas, also showed increased expression of p21ras proteins.153-156

It was suggested that these findings supported a role for the ras genes in gastric carcinogenesis and supported the concept of a different carcinogenic pathway of both Lauren types. However, increased p21ras protein expression in tumours does not necessarily indicate that the levels of the p21 ras protein(s) have transforming capability. Moreover, increased p21ras expression is not necessarily synonymous with malignant phenotype, since it has also been observed in regenerating rat liver and in regenerating epithelium adjacent to peptic ulceration.154,157 Presumably, a moderate increase in ras gene expression is therefore related to active cell proliferation rather than to malignant phenotype. Finally, inherent pitfalls of most p21 ras antibodies hamper a correct interpretation of most studies. As such, immunohistochemical studies on ras gene expression do not yield reliable results.158

The second (common) mechanism of ras gene activation is by point mutations at codons 12, 13 or 61 of the ras genes. Unfortunately, conflicting data on the frequency and type of ras gene mutations in human gastric carcinomas have been reported, varying from 0 to 35% of cases.159-166 These differences may relate to e.g. differences in techniques, study design and/or ethnic background of the patients. In view of marked differences in the ras gene mutational spectrum between Caucasian and Asian non-small-cell lung cancers,167,168 it is questionable whether the results on Asian carcinomas can be readily extrapolated to Caucasian gastric carcinomas. Moreover, differences may relate to whether early or advanced gastric carcinomas were included in the analysis, and may relate to differences in histology of the tumours studied. In one study on 45 Caucasian early gastric carcinomas, mutations were not found, irrespective of Lauren type or growth pattern. Apparently, ras gene mutations do not play a role in the development of Caucasian early gastric carcinomas and do not contribute to a different pathogenesis of both Lauren types.169

c-erbB-2

The c-erbB-2 (HER-2/neu) proto-oncogene, located on chromosome 17q21, encodes a tyrosine kinase transmembrane cell receptor glycoprotein of 185 kDa (p185) which is homologous to epidermal growth factor receptor.170-172 Several lines of evidence support the role of this proto-oncogene in tumorigenesis. When over-expressed, c-erbB-2 is capable of transforming NIH3T3 cells. Moreover, over-expression of c-erbB-2 leads to a novel tumorigenic phenotype in MTSV1.7 cells, an immortalized human mammary epithelial cell line. In addition, transfection experiments have shown over-expression of c-erbB-2 to enhance the metastatic potential of human lung cancer cells by inducing metastasis-associated properties. Finally, c-erbB-2 transgenic mice develop a spectrum of tumours, including tumours of the mammary glands.173-178

Immunohistochemical studies on c-erbB-2 overexpression in gastric carcinomas have reported positive c-erbB-2 membrane staining in 9-38% of cases. Moreover, conflicting data exist on the prognostic significance of c-erbB-2 over-expression and the association between c-erbB-2 over-expression and the histological type according to Lauren.179-183
However, the results are difficult to compare, since there is wide variation in the ability of (commercially available) c-erbB-2 antibodies to detect c-erbB-2 protein over-expression in archival paraffin-embedded tissue samples. In particular, polyclonal antibody A485 was found to provide the highest level of concordance with Northern and Western blot analyses as measures of c-erbB-2 gene expression. Moreover, the concomitant use of two or more antibodies yielded in part complementary findings on c-erbB-2 over-expression. Yet nearly all of the former studies invariably used a single antibody other than A485. Finally, another drawback of these previous studies is that almost no early carcinomas were included, whilst the early cancers included were mostly from Asian origin. Since early carcinomas are thought to evolve into advanced carcinomas, it is questionable whether data from advanced carcinomas can be readily extrapolated to early carcinomas. As mentioned previously, it may well be that Asian and Caucasian (early) gastric carcinomas are quite dissimilar in molecular profiles, due to differences in racial background and/or geographical exposure to various carcinogens. Japanese investigators found c-erbB-2 gene amplification in 40% of (intestinal-type) carcinomas, more than twice the number in a comparable European study. Similarly, c-erbB-2 over-expression could not be demonstrated in a series of 45 Caucasian early gastric carcinomas, using polyclonal antibody A485 and monoclonal antibody 3B5.

**MDM2**

Recent studies indicate that Murine Double Minute-2 (MDM2) gene amplification with concomitant MDM2 protein over-expression may negatively regulate the transcriptional activating function of wild-type p53, may overcome wild-type p53-mediated suppression of transformed cell growth, and may increase the tumorigenic potential of NIH3T3 cells. Interestingly, MDM2 gene amplification/over-expression and p53 gene mutation apparently constitute two distinct, mutually exclusive, mechanisms of wild-type p53 inactivation, since MDM2 amplification and/or over-expression have only been found in sarcomas and various other tumours without p53 gene mutation. Whereas data on MDM2 over-expression in gastric carcinogenesis are scant in the literature, it has been reported that MDM2 over-expression, as assessed by immunohistochemistry, apparently does not occur in early gastric carcinomas and precursor lesions. Hence, it may well be that functional inactivation of p53 by MDM2 over-expression does not play a role in gastric carcinogenesis and is restricted to a subset of malignant tumours for as yet unclear reasons.

**c-met**

The met proto-oncogene was originally identified as an oncogene rearranged following treatment of an osteosarcoma cell line (HOS) with N'-methyl-N'-nitro-N-nitrosoguanidine (MNNG). The gene, located on chromosome 7, encodes a transmembrane tyrosine kinase identified as the receptor of Hepatocyte Growth Factor/Scatter Factor (HGF/SF). HGF/SF is a unique growth factor with mitogenic and motogenic properties, as well as the ability to induce epithelial cell invasion in collagen matrices in vitro. Moreover, it appears that met/HGF also plays a role in tumour metastasis in vivo. Activation of c-met can be due to gene amplification with subsequent over-expression, to defective post-translational processing of the precursor protein or due to oncogenic rearrangement between two distinct genetic loci, namely tpr and met, leading to a novel 5.0 kb hybrid RNA transcript encoding a 65 kDa fusion protein.

Several observations suggest that c-met may play a role in gastric carcinogenesis. First, amplification with concomitant over-expression of c-met has been observed in the gastric carcinoma cell line GTL-16. Second, amplification/over-expression of c-met has been reported to occur in up to 50% of gastric carcinomas. Third, the tpr -met oncogenic rearrangement has been found in gastric carcinoma and its precursor lesions. Despite small numbers included, tpr -met RNA was present at all stages of the carcinogenic process.

**c-myc**

Data on the c-myc gene, which encodes a nuclear transcriptional factor, are scant. Yet, although rare, both c-myc amplification and over-expression have been reported in up to 11% of gastric carcinomas (n=19) and in up to 19% of xenografts in nude mice (n=16). Moreover, although reported to occur in advanced carcinomas only, no clear-cut relationship was found with prognosis. It may well be that c-myc over-expression indicates increased proliferation rather than malignant phenotype since c-myc over-expression has been found in non-neoplastic gastric lesions as well.

**SAM**

By use of an in-gel DNA renaturation method, an amplified DNA sequence was demonstrated in KATO-III, a cell line from a signet-ring-cell carcinoma of the stomach. Interestingly, SAM amplification could not be demonstrated in eight other gastric carcinoma cell lines, nor in 11 cell lines derived from cancers at other sites. Subsequent analysis of 24 gastrectomy specimens showed a 30- to 50-fold SAM amplification in three (13%) gastric carcinomas, all
Alterations in tumour suppressor genes

\textbf{p53}

Although first thought to be a dominant-acting onco-
genome, it has now been firmly established that p53 is a tumour suppressor gene that plays an important role in the regulation of gene expression, in cell-cycle progression and in cellular (apoptotic) response mechanisms to DNA damage. In the normal physiological situation, wild-type p53 protein acts as a transcription factor via binding to specific binding motifs in target genes such as p21\textsuperscript{waf1/cip1}. In the absence of normally functioning wild-type p53 protein, cells may progress through the cell cycle despite genomic injury. As a result, daughter cells may arise with ever increasing levels of genetic aberrations. Inactivation of the p53 gene is mainly caused by mutations in the highly conserved region spanning exons 5–8. The majority of these inactivating mutations are nonsense mutations, leading to mutant p53 proteins, usually detectable by immunohistochemistry. Approximately 10–20% are nonsense mutations leading to stop codon formation with subsequent premature termination of translation. To date, various studies have reported p53 gene mutations to occur in up to 60% of cases with base transitions G:C to A:T being the most frequent genetic change in p53 in gastric carcinomas. Mutations at codons 173 and 251 appear to be preferentially found in gastric carcinomas, and have not been observed in cancers at other sites. With regard to the chronology of accumulation of p53 protein accumulation during gastric carcinogenesis, immunohistochemical studies uniformly report accumulation from dysplasia onwards. In contrast, the only study to date which has used p53 gene mutational analysis (single-strand conformation polymorphism with direct DNA sequencing) as an adjunct to immunohistochemistry has suggested that p53 gene mutation is an early pathogenic event in gastric carcinogenesis. However, missense mutations were found only from dysplasia onwards. Moreover, the mutations found in three out of eight cases of IM did not change the amino-acid sequence of the p53 protein, thereby leaving the p53 protein in its wild-type form. Finally, it was not stated whether or not the intron 5 mutation in IM affected the splice-site acceptor region. Apparently, p53 is not involved in the (early) progression from metaplastic to dysplastic epithelium, but is involved in the malignant transformation from dysplasia onwards. From a clinical point of view, immunohistochemical studies have reported p53 protein accumulation in gastric carcinoma to be correlated with a higher propensity for lymph-node metastasis, and to be correlated with an unfavourable clinical outcome independent of tumour stage as measured by early relapse and death. It remains unclear, however, how these findings might be incorporated into routine daily clinical practice.

\textbf{APC}

The Adenomatous Polyposis Coli gene, located at chromosome 5q, is a site of frequent allelic loss in up to 40% of gastric carcinomas, particularly of the well-differentiated type. Although the APC gene is difficult to examine for mutations, APC gene mutations have been found in up to 44% of carcinomas, depending on the histological type. Since APC gene mutations have also been found in 20% of gastric adenomas, it has been postulated that, analogous to colonic carcinogenesis, these mutations are a relatively early event in gastric carcinogenesis.

\textbf{DCC}

Studies on the Deleted-in-Colon-Cancer (DCC) gene in gastric carcinoma are sparse in the literature. Loss of heterozygosity of the DCC locus on chromosome 18q has been reported in up to 61% of gastric carcinomas, although contradictory results have been reported as well.

\textbf{Alterations in mismatch repair genes}

Alterations (germline mutations) in early DNA mismatch repair (MMR) genes were first described in Hereditary Non-Polyposis Colon Cancer. The phenotypic manifestation of these alterations was
shown to be microsatellite instability, characterized by alterations in the length of simple nucleotide repeats (e.g. CACACA... etc. or C\(n\), most commonly of di- and trinucleotide origin, with subsequent shifts in electrophoretic mobility. Although highly characteristic for HNPCC, recent studies have shown that microsatellite instability is not limited to colonic carcinoma, but can also be found in other tumours such as breast, bladder and lung carcinomas. With regard to gastric carcinoma, several studies have reported microsatellite instability in up to 39% of cases, although early gastric carcinomas and precursor lesions were hardly examined. However, whether these tumours develop through accumulation of somatic mutations or whether a germline mutation in a mismatch repair gene is already present, remains to be elucidated.

Concluding remarks

Despite advances in diagnostic and therapeutic modalities, we expect the overall 5-year survival rate of patients with gastric carcinoma to remain poor, since most patients will continue to present at an advanced stage of disease. Moreover, the observed shifts in tumour histology and tumour site within the stomach may further worsen final outcome. Unfortunately, although the unravelling of the molecular alterations in gastric carcinogenesis continues at a steady pace, implementation of its results into daily clinical practice has been thwarted by insufficient and sometimes clearly contradictory results. Finally, in view of the probably pivotal role of \(H. pylori\) in gastric carcinogenesis, and in view of the fact that gastric carcinoma ultimately results from the accumulation of genetic damage to dominant-acting oncogenes and recessive-acting tumour suppressor genes, future research should be directed towards the unravelling of how and at what stage \(H. pylori\) might be involved in inducing these genetic changes.

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