Gut

Leading article – Molecular biology series

Molecular biology and gastric carcinoma

The molecular study of gastric carcinoma has much to offer the medical scientist, pathologist, clinician, and patient. Morphological classifications are not completely satisfactory in predicting biological and clinical behaviour. Molecular diagnostic markers are needed to discriminate between inflammation and neoplasia¹ in biopsy and cytology specimens, to show invasion in those biopsies currently difficult to interpret,² to define disease subgroups with differing natural history or response to various treatments, and to identify high risk groups for surveillance. It is also hoped that molecular analysis of the disease will identify new therapeutic targets for more precise and effective treatments with fewer side effects.

The genes underlying inherited susceptibility to gastric carcinoma have not yet been identified, and the molecular mechanisms of cancer promotion by environmental influences, such as diet and *Helicobacter pylori* infection, await elucidation. Some progress has been made, however, in identifying abnormalities of oncogenes, tumour suppressor genes, and growth factors including their receptors and related gene products. This will provide a structural framework for analysing molecular mechanisms of disease development. Comparisons can now be made between gastric carcinoma and other cancers (particularly colorectal cancer), between diffuse and intestinal morphological types of gastric carcinoma, and between early and advanced cases. Many of the problems which have limited previous progress are now being resolved.

Which chromosomes are involved?

Early cytogenetic studies were done on malignant effusions from very advanced cases,³ so it was unsurprising that a large variety of chromosomal aberrations were found, differing from case to case. More recent studies of solid tumour material have also found marked heterogeneity both between cases and within each case, but two studies^{4,5} have reported frequent breakpoints at 3p21, which is the site of deletions in lung cancer and may involve a phosphatase tumour suppressor. Rodriguez *et al* found translocations and deletions of 11p13–15 (often abnormal in other solid tumours) in four of five cases, two involving reciprocal translocations of 3p21. They observed the same rearrangement in adenocarcinomas of the lower oesophagus, supporting the view that these cancers are similar in pathogenesis to gastric carcinomas, as they usually arise in the metaplastic glandular mucosa of Barrett's oesophagus. A cytogenetic analysis of nine cases

of Barrett's metaplasia, however, did not find chromosomal abnormalities at either the 11p13–15 or 3p21 sites.⁶

Chromosomal losses important in the pathogenesis of cancers can be studied by using DNA polymorphism marker techniques to identify separately maternal and paternal alleles (comparable with HLA typing). Patients are informative if their constitutional DNA (from peripheral blood, or hair roots) shows two patterns – one for each allele. Allele loss is indicated if one of these disappears in tumour tissue, and may signify loss of a tumour suppressor gene. Studies on gastric carcinomas were relatively few and only analysed small numbers of tumours, most finding a low level of deletion. The high proportion of stromal cells in many gastric cancers, particularly the diffuse type, may have caused a dilution of tumour DNA and consequent failure to detect allele loss. A recent study by Sano *et al* selected cases comprising over 50% of tumour cells microscopically assessed on immediately adjacent tissue, and found a much higher rate of allele loss.⁷ Deletion of 5q was found only in well differentiated cases and not in undifferentiated tumours. The 5q sites correspond with the sites of the familial adenomatous polyposis (APC) gene, also deleted in some spontaneous colon carcinomas, and the adjacent mutated in colon carcinoma (MCC) gene. In contrast, both types of gastric tumour showed a 60% rate of allele loss on 17p at the site of the p53 gene. Extrapolating from this, we would then expect to find mutations of the p53 gene in both cancer types, but 5q gene mutations only in differentiated carcinomas. Paradoxically, Horii *et al* have found APC gene mutations in only diffuse or poorly differentiated types, but not in differentiated intestinal types of gastric carcinoma, in a study of 44 tumours.⁸ It will be interesting to see if this curious paradox is clarified by other studies in progress.

Allele loss has also been reported on the 18q chromosomal site of the deleted in colon cancer (DCC) gene in a series of intestinal type gastric carcinomas (diffuse cancers were not examined).⁹ 18q deletions were found in over 60% of informative cases including many early intramucosal cases, compared to 17p losses (p53 gene) found at a lower rate and in more advanced cases. Thus the overall pattern of allele loss in gastric cancers shows many similarities with colon cancer, with losses at 5q, 18q, and 17p, with 17p loss associated with progression.

Ras oncogenes and proteins

The *ras* protein p21 is encoded by three *ras* genes, *Ha-ras*,

K-ras, and *N-ras*, and is thought normally to have a role in signal transduction, proliferation, and differentiation. Many tumours have single base mutations or increased expression of one or more of these genes and amplification of *K-ras* has also been reported. Studies of Chinese populations have shown *H-ras* mutations in about a third of gastric carcinomas, associated with presence of distant metastases and short survival,¹⁰ but other studies of different populations have failed to find a significant rate of *ras* mutation.¹¹⁻¹³ Differences could be caused by the methodology, or different carcinogenic mechanisms possibly related to diet or pathogen prevalence, since different carcinogens have been shown to target different sites on the genome. Studies of *ras* expression show raised p21 protein in tumours, using immunohistochemistry,¹⁴ Western blotting,¹⁵ radioimmunoassay,¹⁶ and *in situ* hybridisation.¹⁷ There have been a variety of *ras* antibodies available, however, and many studies do not identify which antibodies were used, making comparison of conflicting results difficult. Most report increased immunostaining in intestinal type gastric carcinomas compared with the diffuse type^{18,19} and in advanced compared with early cancers.^{15,17} Correlation of *ras* immunostaining with depth of invasion, metastases, and worse prognosis has been reported in a large survey of 171 cases,¹⁵ but not in smaller studies.¹⁸

Ras overexpression, however, does not appear to be specific for carcinoma. *Ras* immunocytochemistry has been evaluated for diagnostic use on cytology brush smears.²⁰ While all cancers were positive, so was one peptic ulcer, and this technique obviously cannot be used to discriminate neoplasia from reactive atypia. High levels have been reported in dysplasia, intestinal metaplasia, regenerating epithelium adjacent to peptic ulceration, and even in normal mucosa adjacent to neoplastic lesions.^{14,17-20} Cerniak *et al* observed immunopositivity in mucosa adjacent to diffuse but not intestinal types of carcinoma, and also in dysplasia and intestinal metaplasia adjacent to intestinal type carcinomas.¹⁹ They argued that this supported the view that intestinal carcinomas arise from intestinal metaplasia or dysplasia, while diffuse cancers arise *de novo* from morphologically normal epithelium, with increased *ras* expression occurring at an early stage of carcinogenesis in both. It is not possible, however, to show a temporal sequence from samples of the same resection specimen. Overexpression of *ras* in the adjacent mucosa may be in response to a trophic stimulus from the tumour, and thus may follow rather than precede tumour development. An interesting study by Yamamoto *et al* on 174 gastric carcinomas showed increased coexpression of *ras* and TGF- α (a growth factor), correlating with stage, grade, depth of invasion, presence of lymphatic metastases, and worse prognosis.²¹ This raises the possibility that an important action of *ras* on gastric cells may be to upregulate production of TGF- α , and differences in *ras* expression between tumours may reflect different proliferation rates rather than different patterns of differentiation.

P53 tumour suppressor gene

P53 mutations are the most common defect found in human cancer, and several studies have found a high proportion of abnormalities in gastric carcinoma by direct DNA analysis²² and by immunohistochemistry²³ to detect the mutant protein which is usually not degraded as rapidly as the normal p53 protein and therefore accumulates in the cell. Abnormalities have been found in both intestinal and diffuse cases, more commonly in metastases than primary cancers.²²⁻²⁵ Advanced cancers have a higher rate of p53 abnormality than early cancers, and p53 immunopositivity is associated with poorer survival.²³ One study found that all cases with mutant p53

were aneuploid, and no diploid tumour had a p53 mutation,²² which supports the view that the role of normal p53 is to prevent cells with damaged DNA from replicating.²⁶ P53 abnormalities, however, are not always late events in gastric carcinogenesis, as immunopositivity has been shown in early cancers, and in dysplasia.²⁷ Some mutations, such as deletions causing a frame shift, result in a truncated protein which is not detected by immunohistochemistry, and certain mutations are only weakly stabilising. Conversely immunopositivity may occur in the absence of mutation, possibly caused by increased transcription in rapidly proliferating cells, or inactivation of a factor required for degradation.²⁸ It is currently unclear which analysis immunopositivity or DNA mutation, gives the more useful information about biological and clinical behaviour. Further evaluation of p53 as a prognostic and diagnostic marker for use on biopsies and cytology specimens is merited.

Growth factors, receptors and related genes

Abnormalities of several growth factor/receptor systems have been found in gastric carcinoma. Those from the fibroblast growth factor system seem to be more associated with diffuse carcinomas, whereas intestinal carcinomas tend to have a higher rate of abnormality of the genes related to epidermal growth factor and their receptors. The *K-sam* oncogene was originally isolated from a cell line from a diffuse carcinoma, and comprises a rearranged fibroblast growth factor receptor gene²⁹ (It is also related to another oncogene, *ret*, found in thyroid papillary carcinomas). Amplification has been reported in 10/48 undifferentiated (diffuse) carcinomas compared with 0/35 well differentiated (intestinal) carcinomas, thus adding to the evidence that the two main morphological types of gastric carcinoma have a different molecular pathogenesis. With the ligands of the fibroblast growth factor group, the evidence is less clear. The *hst-1* oncogene encodes fibroblast growth factor 4, and is often coamplified with the *int-2* oncogene encoding fibroblast growth factor 3, but there are conflicting reports as to how often they are coamplified in gastric carcinoma.^{30,31} Basic fibroblast growth factor 2 overexpression has been observed in gastric cancer, more frequently in scirrhous tumours.³² Thus there is some evidence that the epithelial stromal interactions observed in the scirrhous diffuse cancers are at least partially mediated through the fibroblast growth factor system.

Intestinal tumours tend to show a higher frequency of overexpression of receptors of the epidermal growth factor system than diffuse or undifferentiated tumours. This has mainly been shown with the receptors EGFR, ERBB2, and ERBB3, and is sometimes but not always associated with amplification of the receptor gene.³³⁻³⁵ The situation with the numerous ligands is less clear, and raised levels of TGF α and epidermal growth factor have been shown in both intestinal and diffuse carcinomas,^{36,37} although some studies show a higher frequency of increased expression in tumours of intestinal type.³⁸ While the more advanced tumours in most studies have higher levels of expression of both receptors and ligands than early cancers,³⁹ there are conflicting reports as to their prognostic value. Overexpression has also been found in non-neoplastic tissue.

These studies of growth factors and their receptors are of great interest regardless of any prognostic predictive power. It is believed that herein may lie a mechanism of cancer promotion by *Helicobacter pylori*. There are complex interactions with the trefoil peptides – a group of peptides including pS2 and hSP associated with cells involved in gastrointestinal inflammatory repair,⁴⁰ which may be of significance in neoplasia as well as inflammation.⁴¹ Both the fibroblast growth factor and epidermal growth factor systems offer therapeutic targets both for inhibition of tumour

growth, and for development of receptor-specific targeting mechanisms.⁴²

Differentiated gastric carcinomas – a paradox?

Most human cancers with well differentiated and poorly differentiated subtypes show a pattern with loss of differentiation paralleled by increased aggressiveness, and a higher rate of genetic abnormalities such as aneuploidy and allele loss. In early gastric cancer (T1), however, the raised tubulopapillary Pen A subtype is morphologically well differentiated but has the worst prognosis of any early gastric cancer, and the highest rate of aneuploidy, while early gastric carcinomas of diffuse type are virtually always cured by surgery. Advanced intestinal carcinomas have been shown to have a higher rate of allele loss than diffuse and poorly differentiated carcinomas at several chromosomal sites, and are more frequently aneuploid. Yet diffuse cancers have a worse prognosis when they become advanced. Some of these paradoxical observations may be the result of artefactual underrepresentation of tumour cells in poorly differentiated and diffuse cases in some of the studies, and the inconsistency of histological subtyping between the various researchers. Nevertheless gastric cancer appears to be an intriguing model for studying the interactions between differentiation, proliferation, and carcinogenesis in epithelial cells.

Clearly knowledge of the molecular pathogenesis of gastric carcinoma is at an early stage. Differences have been found between intestinal type and diffuse carcinomas, and between early and advanced cancers. Some studies have shown correlations with prognosis. Abnormalities have been found in dysplasia and intestinal metaplasia in intestinal type cancers, and in adjacent mucosa in diffuse cancers. At present we cannot recommend direct application of molecular techniques for routine diagnostic use, although some areas such as p53 immunohistochemistry merit further clinical evaluation. We confidently predict, however, an exciting future for the exploration of this complex and fascinating disease.

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- Williams GT. Early gastric cancer. In: Filipe MI, Jass JR, eds. *Gastric carcinoma*. Edinburgh: Churchill Livingstone, 1986: 172–96.
- Isacson PG. Biopsy appearances easily mistaken for malignancy in gastro-intestinal endoscopy. *Histopathology* 1982; **6**: 377–89.
- Sandberg AA. Solid tumours and metastatic cancer – tumours of the alimentary tract. In: Sandberg AA, ed. *The chromosomes in human cancer and leukaemia*. New York: Elsevier, 1980: 468–84.
- Ochi H, Douglass HO, Sandberg AA. Cytogenetic studies in primary gastric cancer. *Cancer Genet Cytogenet* 1986; **22**: 295–307.
- Rodriguez E, Rao PH, Ladanyi M, et al. 11p13–15 is a specific region of chromosome rearrangement in gastric and esophageal adenocarcinomas. *Cancer Res* 1990; **50**: 6410–6.
- Garewal HS, Sampliner R, Liu Y, Trent JM. Chromosomal rearrangements in Barrett's oesophagus: a premalignant lesion of esophageal adenocarcinoma. *Cancer Genet Cytogenet* 1989; **42**: 281–96.
- Sano T, Tsujino T, Yoshida K, et al. Frequent loss of heterozygosity on chromosomes 1q, 5q, and 17p in human gastric carcinomas. *Cancer Res* 1991; **51**: 2926–31.
- Horii A, Nakatsuru S, Miyoshi Y, et al. The APC gene responsible for Familial Adenomatous Polyposis is mutated in human gastric cancer. *Cancer Res* 1992; **52**: 3231–3.
- Uchino S, Tsuda H, Noguchi M, et al. Frequent loss of heterozygosity at the DCC locus in gastric cancer. *Cancer Res* 1992; **52**: 3099–102.
- Deng G, Liu X, Wang J. Correlation of mutations of oncogene c-Ha-ras at codon 12 with metastases and survival of gastric cancer patients. *Oncogene Res* 1991; **6**: 33–8.
- Fujida K, Ohuchi N, Yao T, et al. Frequent overexpression, but not activation by point mutation of ras genes in primary human gastric cancers. *Gastroenterology* 1987; **93**: 1339–45.
- Jiang W, Kahn SM, Guillem JG, Lu SH, Weinstein IB. Rapid detection of ras oncogenes in human tumours: applications to colon, oesophageal and gastric cancer. *Oncogene* 1989; **4**: 923–8.
- Victor T, Du Toit R, Jordaan AM, Bester AJ, van Helden PD. No evidence for point mutations in codons 12, 13, and 61 of the ras gene in a high incidence area for esophageal and gastric cancers. *Cancer Res* 1990; **50**: 4911–4.
- Yoshida K, Hamatani K, Koide H, et al. Preparation of anti-ras Mr 21,000 protein monoclonal antibodies and immunohistochemical analyses on expression of ras genes in human stomach and thyroid cancers. *Cancer Res* 1988; **48**: 5503–9.
- Tahara E, Yasui W, Taniyama K, Ochiai A, Yamamoto T, Nakajo S, et al. Ha-ras oncogene product in human gastric carcinoma: correlation with invasiveness, metastasis, or prognosis. *Jpn J Cancer Res* 1986; **77**: 517–22.
- de Blasi F, del Sal G, Hand PH. Evidence of enhancement of the ras oncogene protein product (p21) in a spectrum of human tumours. *Br J Cancer* 1989; **43**: 431–5.
- Ohuchi N, Hand PH, Merio G, et al. Enhanced expression of c-Ha-ras p21 in human stomach adenocarcinomas defined by immunoassays using monoclonal antibodies and in situ hybridisation. *Cancer Res* 1987; **47**: 1413–20.
- Yoshida K, Hamatani K, Koide H, et al. Analysis of ras gene expression in stomach cancer by anti-ras p21 monoclonal antibodies. *Cancer Detect Prev* 1988; **12**: 369–76.
- Czerniak B, Herz F, Gorczyca W, Koss L. Expression of ras oncogene p21 protein in early gastric carcinoma and adjacent gastric epithelia. *Cancer* 1989; **64**: 1467–73.
- Czerniak B, Herz F, Koss LG, Schlom J. Ras oncogene p21 as a tumour marker in the cytodagnosis of gastric and colonic carcinomas. *Cancer* 1987; **60**: 2432–6.
- Yamamoto T, Hattori T, Tahara E. Interaction between transforming growth factor alpha and c-Ha-ras p21 in progression of human gastric carcinoma. *Pathol Res Pract* 1988; **183**: 663–9.
- Tamura G, Kihana T, Nomura K, et al. Detection of frequent p53 mutations in primary gastric cancer by cell sorting and polymerase chain reaction single-strand conformation analysis. *Cancer Res* 1991; **51**: 3056–8.
- Martin HM, Filipe MI, Morris RW, et al. P53 expression and prognosis in gastric carcinoma. *Int J Cancer* 1992; **50**: 859–62.
- Kim JH, Takahashi T, Chiba I, et al. Occurrence of p53 abnormalities in gastric carcinoma tumours and cell lines. *J Natl Cancer Inst* 1991; **83**: 938–43.
- Yamada Y, Yoshida T, Hayashi J, et al. P53 gene mutations in gastric cancer metastases and in gastric cancer cell lines derived from metastases. *Cancer Res* 1991; **51**: 5800–5.
- Kastan MB, Onyekwere O, Sidransky D, Vogelstein B, Craig RW. Participation of p53 protein in the cellular response to DNA damage. *Cancer Res* 1991; **51**: 6304–11.
- Brito MJ, Filipe MI, Lane D, Morris R. P53 overexpression in early gastric carcinoma and precancerous lesions. *J Pathol* 1992; **167**: 95A.
- Wynford-Thomas D. P53 in tumour pathology: can we trust immunocytochemistry? *J Pathol* 1992; **166**: 331–2.
- Nakatani H, Sakamoto H, Yoshida T, et al. Isolation of an amplified DNA sequence in stomach cancer. *Jpn J Cancer Res* 1990; **81**: 707–10.
- Yoshida MC, Wada M, Satoh H, et al. Human HST1 (HSTF1) gene maps to chromosome band 11q13 and co-amplifies with the INT2 gene in human cancer. *Proc Natl Acad Sci USA* 1988; **85**: 4861–4.
- Tsuda T, Tahara E, Kajiyama G, et al. High incidence of co-amplification of hst-1 and int-2 genes in human esophageal carcinomas. *Cancer Res* 1989; **49**: 5505–8.
- Tamimoto H, Yoshida K, Yokozaki H, et al. Expression of basic fibroblast growth factor in human gastric carcinomas. *Virchows Arch [B] Cell Pathol* 1991; **61**: 263–7.
- Lemoine NR, Jain S, Silvestre F, et al. Amplification and overexpression of the EGF receptor and c-erbB2 protooncogenes in human stomach cancer. *Br J Cancer* 1991; **64**: 79–83.
- Falck VG, Gullick WJ. c-erbB2 oncogene product staining in gastric adenocarcinoma. An immunohistochemical study. *J Pathol* 1989; **159**: 107–11.
- Lemoine NR, Prigent SA, Hughes CM, et al. Expression of c-erbB-3 protein in normal human adult and fetal tissues. *J Pathol* 1992; **167**: 133A.
- Muller W, Borchard F. Expression of transforming growth factor alpha in gastric carcinoma and normal gastric mucosa cells. *Cancer* 1992; **69**: 2871–5.
- Hirayama D, Takahiro F, Satonaka K, et al. Immunohistochemical study of epidermal growth factor and transforming growth factor β in the penetrating type of early gastric cancer. *Hum Pathol* 1992; **23**: 681–5.
- Nasim MM, Thomas DM, Alison MR, Filipe MI. Transforming growth factor α expression in normal gastric mucosa, intestinal metaplasia, dysplasia, and gastric carcinoma – an immunohistochemical study. *Histopathology* 1992; **20**: 339–43.
- Yasui W, Hata J, Yokozaki H, et al. Interaction between epidermal growth factor and its receptor in progression of human gastric carcinoma. *Int J Cancer* 1988; **41**: 211–7.
- Wright NA, Poulson R, Stamp GWH, et al. Epidermal growth factor (EGF/URO) induces expression of regulatory peptides in damaged human gastrointestinal tissues. *J Pathol* 1990; **162**: 279–84.
- Theisinger B, Welter C, Seitz G, et al. Expression of the breast cancer associated gene pS2 and the pancreatic spasmodic polypeptide gene (hSP) in diffuse type of stomach carcinoma. *Eur J Cancer* 1991; **27**: 770–3.
- Wright PA, Lemoine NR. The molecular basis of gastric cancer. In: Lemoine NR, ed. *Genes, cancer and the surgeon*. Oxford: Blackwell Scientific (in press).