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*Guest editorial* *

# Molecular mechanism of stomach carcinogenesis

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**Abstract.** Gene changes in multiple oncogenes, multiple growth factors and multiple tumor-suppressor genes are observed in stomach cancer. Among them, those most commonly implicated in both well-differentiated adenocarcinoma and poorly differentiated adenocarcinoma are inactivation (mutations and allele loss) of the p53 gene, and activation (abnormal expression and amplification) of the c-*met* gene. Moreover, they occur at an early stage of stomach carcinogenesis. In addition, loss of heterozygosity (LOH) on chromosome 5q (APC locus) is frequently associated with well-differentiated adenocarcinoma. LOH on chromosome 18q (DCC locus) and LOH of the *bcl*-2 gene also are common events of well-differentiated adenocarcinoma. LOH on chromosomes 1q and 7q may be involved in the progression of well-differentiated adenocarcinoma. Conversely, the development of poorly differentiated adenocarcinoma, in addition to changes in p53 and c-*met* genes, requires reduction or dysfunction of cadherin. Overexpression of *bcl*-2 protein is observed in poorly differentiated adenocarcinoma or signet-ring cell carcinoma. Moreover, the K-*sam* gene is amplified preferentially in poorly differentiated adenocarcinoma or scirrhous carcinoma. K-*sam* amplification in scirrhous carcinoma often occurs independently of c-*met* gene amplification. LOH on chromosome 1p also is relatively common in poorly differentiated adenocarcinoma. Exceptionally, signet-ring cell carcinoma shares APC mutations. There are some differences in expression of the growth-factor/receptor system between well-differentiated adenocarcinoma and poorly differentiated adenocarcinoma. Moreover, interaction between cell-adhesion molecules in tumor cells expressing c-*met* and hepatocyte growth factor (HGF) from stromal cells is linked with morphogenesis of two histological types of stomach cancer. Intestinal metaplasia and adenoma of the stomach also contain p53 mutations and K-*ras* mutations or *tpr-met* rearrangement. Taken together, different genetic pathways of stomach carcinogenesis may exist for poorly differentiated and well-differentiated stomach cancers. Some of the latter may develop by a cumulative series of gene alterations similar to those of colorectal cancer.

**Key words:** Oncogenes – Tumor-suppressor genes – Growth-factor/receptor system – Stomach carcinogenesis – Colon carcinogenesis

## Introduction

Recent rapid progression in the field of cancer research reveals that conversion of normal cells to so-called clinical cancer is needed for a multistage process that is intimately associated with an accumulation of multiple gene changes including both oncogenes and tumor-suppressor genes (Marx 1989; Fearon and Vogelstein 1990). Stomach cancer is no exception and shows multiple gene alterations including the p53, APC, DCC and c-*met* genes. Surprisingly, the incidence of activation in the c-*met* gene is very high compared to that of K-*ras* mutations or other oncogene activations. Moreover, intestinal metaplasia and adenoma of the stomach show genetic changes in stomach cancer.

Stomach cancer has marked heterogeneity of morphology and function, as does lung cancer. However, it is histologically classified into two types: the well-differentiated or intestinal type and the poorly differentiated type, as well as the diffuse type. Interestingly, the scenario of multiple gene changes in stomach cancer differs depending on the two histological types, indicating that the two types of stomach cancer may have different genetic pathways.

The aim of this editorial, based on our recent data, is to describe the molecular mechanism of the two types of stomach carcinogenesis.

## Multiple oncogenes

Stomach cancer involves alterations in multiple oncogenes encoding tyrosine kinase receptors, such as c-*met*, K-*sam*

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*Abbreviation:* LOH, loss of heterozygosity

and c-*erb*B-2. Among these proto-oncogenes, the one most frequently implicated in stomach cancer is the c-*met* gene. Deletion, amplification and overexpression or abnormal expression of the c-*met* gene are frequently associated with stomach cancer and closely correlated with tumor progression and metastasis (Tahara 1992; Kuniyasu et al. 1992).

The c-*met* gene was initially identified in NIH 3H3 cells transfected with DNA from the human osteosarcoma cell line (HOS) transformed with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidines (Cooper et al. 1984; Dean et al. 1985). Subsequent analyses on the proto form of this gene revealed that the c-*met* gene encodes hepatocyte growth factor (HGF) receptor composed of a 50-kDa $\alpha$ subunit disulfide-linked to a 145-kDa $\beta$ subunit to form a 190-kDa heterodimer (Park et al. 1987; Naldini et al. 1991; Bottaro et al. 1991). Loss of heterozygosity (LOH) of the c-*met* gene, which is located on chromosome 7q31, is observed in 50% of the well-differentiated adenocarcinomas of the stomach. This is involved in deletion of other regions in chromosome 7q36-ter, indicating that the region containing the c-*met* gene is widely deleted. However, this chromosomal defect occurs independently of c-*met* gene amplification. Chromosome 7q LOH will be described later, in the chapter on tumor-suppressor genes. Conversely, the c-*met* gene is amplified in approximately 40% of scirrhous gastric carcinomas. Interestingly, all of five scirrhous carcinoma cell lines, including HSC-39, KATO-III, NTAS, NKPS and HSC-43 cells, share gene amplification (Kuniyasu et al. 1992). Moreover, a good correlation is observed between c-*met* amplification and the clinical stage or patient prognosis. More importantly, a 6000-base (6.0-kb) transcript of the c-*met* gene is expressed only in stomach cancer of both cell lines and tumor tissues (Kuniyasu et al. 1992). In addition, the expression of this abnormal transcript of 6.0 kb is closely linked with the tumor staging, lymph node metastasis and depth of tumor invasion. It is extremely interesting that 60% of stomach cancers expressing the 6.0-kb transcript of the c-*met* gene share p53 abnormalities.

Various transcripts of the c-*met* gene have been reported with sizes of 9.0 kb, 7.0 kb, and 6.0 kb (Park et al. 1987; Di Renzo et al. 1991). However, it has not been fully elucidated whether structural gene changes or abnormalities in RNA splicing are involved in the multiplicity of the transcripts. Two types of c-*met* gene rearrangement have been so far reported. One is found on a der (7) chromosome, which accounts for an unbalanced translocation between chromosomes 7 and 1 (Park et al. 1988; Testa et al. 1990). This rearrangement is found in MNNG-HOS cells and may cause the 6.0-kb transcript. The other type of rearrangement is in the *tpr-met* gene, which expresses a 5.0-kb transcript (Park et al. 1986; Tempest et al. 1986; Gonzatti-Haces et al. 1988).

Interestingly, *tpr-met* protein, $p65^{tpr\text{-}met}$ induces meiotic maturation in *Xenopus* oocytes and activates maturation-producing factor through a *mos*-dependent pathway (Daar et al. 1991). Recently, Soman et al. (1991) reported that the *tpr-met* rearrangement was expressed in stomach cancers as well as in chronic gastritis.

In addition to c-*met*, the K-*sam* gene, which encodes a tyrosine kinase receptor belonging to the fibroblast growth factor (FGF) receptor (Nakatani et al. 1991; Hattori et al. 1990), is amplified preferentially in poorly differentiated adenocarcinoma or scirrhous carcinoma. It is significant that, in cases of scirrhous carcinoma, K-*sam* amplification often occurs independently of c-*met* amplification. Moreover, there is a tendency for the K-*sam* gene to be amplified in tumors of women under 40 years old and for the c-*met* gene to be amplified in tumors of men over 50 years old. However, the stomach cancer cell lines, KATO-III, in which the K-*sam* gene was first identified as amplified DNA, and HSC-39, both scirrhous cancer cell lines, contain amplifications of both K-*sam* and c-*met* genes. As to gene expression, various transcripts of the K-*sam* gene have been reported with sizes of 4.5 kb, 4.0 or 3.5 kb, 3.2 kb and 1.6 or 1.8 kb (Katoh et al. 1992). Their neucleotide sequences indicate that the K-*sam* gene encodes secreted as well as transmembrane receptor kinase. A 4.5-kb transcript encoding a full-length transmembrane receptor or human *bek* product is observed in normal gastric mucosa and brain tissues. A 3.5-kb transcript, which is truncated in the 3' noncoding region, is detected in KATO-III cells and stomach cancer tissues.

On the other hand, amplificatioin and overexpression of c-*erb*B-2 is often associated with well-differentiated adenocarcinoma (Kameda et al. 1990). Moreover, c-*erb*B-2 gene amplification is frequently detected in distant metastatic tumors rather than in primary tumors (Tsujino et al. 1990), suggesting that c-*erb*B-2 gene amplification is not an initiating event in stomach carcinogenesis but occurs late in tumor progression and metastasis. However, on the basis of our recent studies, it is also likely that the primary tumor contains a small clone of gene-amplified tumor cells, which has a selective advantage in progression or metastasis. c-*met* and K-*sam* genes are also amplified in metastatic tumors of the liver, lung, ovary and lymph nodes.

Genes that regulate programmed cell death or apoptosis evidently contribute to the development of human cancer. The *bcl*-2 oncogene encodes an inner mitochondrial membrane protein, which blocks programmed cell death (Hockenbery et al. 1990). Except in the lymphoid and hematopoietic tissues, *bcl*-2 protein expression is observed preferentially in neurons and epithelial cells that reveal apoptotic cell turnover (Hockenbery et al. 1991). Interestingly, LOH of the *bcl*-2 gene is frequently associated with well-differentiated adenocarcinoma. However, it is not involved in poorly differentiated adenocarcinoma or signet-ring cell carcinoma. *bcl*-2 mRNA is expressed at various levels in gastric carcinoma tissues and cell lines. In well-differentiated adenocarcinoma, no tumor reveals higher levels of *bcl*-2 mRNA than do normal tissues, while 30% of poorly differentiated adenocarcinomas express higher levels of *bcl*-2 mRNA as compared to the normal tissues. Furthermore, *bcl*-2 protein is expressed at high levels by HSC-39 cells containing signet-ring cancer cells. These observations suggest that well-differentiated adenocarcinoma may often induce apoptosis through allele loss of the *bcl* gene, whereas poorly differentiated adenocarcinoma or scirrhous carcinoma may have a long-survival clone of tumor cells because of overexpression of *bcl*-2 protein.

### Multiple growth factors and IL-1$\alpha$

Stomach cancer expresses a broad spectrum of the growth factors, gut hormones and cytokines that play a crucial role in the growth of tumor cells (Tahara 1990). Transforming

growth factor $\alpha$ (TGF$\alpha$), the most primitive growth factor, acts as a positive autocrine growth factor for gastric carcinoma, regardless of histological type. TGF$\alpha$ and epidermal growth factor (EGF) successively evoke cascade phenomena that favor tumor progression, invasion and extracellular matrix formation (Yoshida et al. 1990). TGF$\beta$1, a negative growth regulator, is also commonly expressed by tumor cells (Yoshida et al. 1989).

Our recent study on a gastric carcinoma cell line TMK-1, which expresses TGF$\beta$1, has demonstrated that TGF$\beta$1 inhibits DNA synthesis by TMK-1 in a dose-dependent manner up to 200 pM and that the type I receptor for TGF$\beta$1 is mainly linked to the growth-inhibitory signal by a decrease in retinoblastoma protein phosphorylation by $p34^{cdc2}$ without suppression of c-*myc* expression (Ito et al. 1992 a).

TGF$\beta$ receptors are subclassified into three types: I, II, III. As indicated by recent studies on expression cloning of the TGF$\beta$ type II and type III receptors (Lin et al. 1992; Wang et al. 1991), type I and type II receptors function as signal-transducing molecules. But type III receptor, also called $\beta$-glycan (Lopez-Casillas et al. 1991), may regulate the ligand-binding ability or surface expression of the other types of receptor. Interestingly, over 80% of gastric carcinoma tissues show a reduction in TGF$\beta$ type I receptor and a low level of TGF$\beta$-inhibitory-element-binding protein, regardless of histological type (Ito et al. 1992 b). Moreover, the reduction in type I receptor in the tumor tissue is correlated with depth of tumor invasion, as almost all the tumors that invade the deep layer were found to have lower levels of type I receptor than normal mucosa (Ito et al. 1992 b). These results indicate that most advanced gastric cancers escape from growth inhibition by TGF$\beta$1 through the reduction in type I receptor.

*cripto*, a novel gene of the EGF family, was cloned from an undifferentiated human teratocarcinoma cell line NTERA2 clone D1-(NT2D1) (Ciccodicola et al. 1989). The *cripto* gene shares structural homology with human EGF, human TGF$\alpha$ and amphiregulin. Moreover, *cripto* has transforming activity in transfected mouse NIH3H3 fibroblasts (Ciccodicola et al. 1989) and in mouse NOG-8 mammary epithelial cells (Ciardiello et al. 1991). The interaction of *cripto* with EGF receptor, c-*erb*B-2 or c-*erb*B-3 has not been investigated. *cripto* is expressed in the intestines and kidney, but not in the salivary gland, esophagus or breast. An exciting observation is that most well-differentiated stomach cancers as well as colorectal cancers express the *cripto* gene at higher levels than those in normal gastric mucosa (Kuniyasu et al. 1991). Moreover, *cripto* protein is overexpressed in 44% of early gastric cancers even those under 1 cm in diameter. *cripto* overexpression is also correlated with tumor stage and patient prognosis. More importantly, a good correlation exists between *cripto* expression and the grade of gastric intestinal metaplasia in which absorptive cells display strong immunoreactivity to *cripto* protein (Tahara 1992). It is likely that *cripto* overexpression in gastric intestinal metaplasia may participate in the pathogenesis of well-differentiated adenocarcinoma of the stomach.

On the other hand, overexpression of TGF$\beta$1, platelet-derived growth factor (PDGF), insulin-like growth factor II (IGF-II) and basic fibroblast growth factor (FGF) is frequently associated with poorly differentiated adenocarcinoma (Tsuda et al. 1989; Yoshida et al. 1989; Tahara 1990; Tanimoto et al. 1991). In particular, the development of scirrhous gastric carcinoma, which corresponds to diffusely infiltrating carcinoma or Borrmann's type IV carcinoma of the stomach, requires synchronous overexpression of TGF$\beta$1, PDGF, IGF-II and basic FGF in the same tumor. This reflects the close interaction of tumor cells and stroma cells, including fibroblasts, endothelial cells, macrophages and lymphocytes.

Surprisingly, the IL-1$\alpha$ gene is expressed at various levels by most of gastric carcinoma cell lines, among which MKN-7 secretes a large amount of IL-1$\alpha$ into the conditioned medium (Tahara 1992). Tumor-derived IL-1$\alpha$ may bring about an increase in proteolytic enzyme activity, cell-adhesion molecules and interleukin production and decrease production of the extracellular matrix by stromal cells. Therefore, multiple growth factors and cytokines produced by tumor cells not only may serve autocrine or paracrine growth and motility of tumor cells themselves but also may bind to each of the receptors on stromal cells, leading to fibrosis, angiogenesis, activation of cytokine network and suppression of T cell function. On the other hand, fibroblasts, endothelial cells, macrophages and lymphocytes stimulated by tumor cells may cause the secretion of multiple growth factors and cytokines, resulting in proliferation, enhanced motility and cell death or necrosis of tumor cells.

With regard to the complex interaction between tumor cells and stromal cells, human hepatocyte growth factor (HGF), which is a ligand for the c-*met* protein (Bottaro et al. 1991), plays an important role in progression and morphogenesis of stomach cancer. HGF, a heparin-binding polypeptide, is a heterodimeric molecule composed of a 69-kDa $\alpha$-subunit and a 34-kDa $\beta$-subunit and is derived from a single-chain precursor of 728 amino acids (Nakamura et al. 1989; Miyazawa et al. 1991). Interestingly, HGF reveals high sequence homology with plasminogen and has four kringle domains in its $\alpha$-chain, which is linked with the ligand-binding activity of the protein (Chan et al. 1991). HGF stimulates the tyrosine kinase activity of c-*met* receptor (Naldini et al. 1991). Although HGF was initially identified as a mitogen for hepatocytes, HGF is expressed by stromal fibroblasts and stimulates proliferation of a wide variety of epithelial cells that express c-*met* protein (Prat et al. 1991). Moreover, HGF is essentially identical with scatter factor, which enhances cell motility and stimulates the dispersion of epithelial and vascular endothelial cells (Bhargava et al. 1992). In addition, HGF has the property of epithelial morphogenesis (Montesano et al. 1991). Therefore, HGF is a multifunctional polypeptide that may act as a paracrine mediator of epithelial/mesenchymal interaction in enhancement of cell growth (mitogen), enhancement of cell motility (motogen) and promotion of epithelial tubules (morphogen).

More recently, we have found that the human stomach fibroblast cell line ST-Fib as well as the human lung fibroblast cell l ine MRC-5 expresses high levels of HGF mRNA and secretes HGF into the conditioned medium. The gastric cancer cell line HSC-39 also expresses the HGF gene but the HGF content in the conditioned medium is not detectable by enzyme-linked immunosorbent assay. Interestingly, treatment of ST-Fib cells with TGF$\alpha$, TGF$\beta$ and IL-1$\alpha$ induces enhancement of HGF secretion, suggesting that TGF$\alpha$,

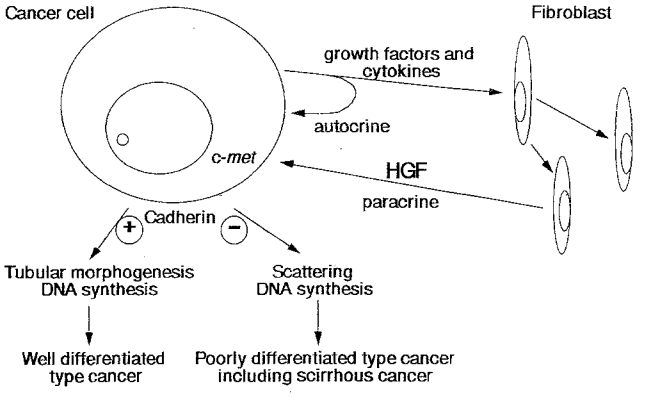


**Fig. 1.** Interaction between cancer cells and stromal cells implicated in morphogenesis of two types of stomach cancer. HGF, hepatocyte growth factor

TGF$\beta$ and IL-1$\alpha$ produced by tumor cells are triggers for enhanced secretion of HGF from stromal fibroblast (Fig. 1). In fact, gastric cancer cell lines TMK-1 (a poorly differentiated adenocarcinoma) and MKN-28 (a well-differentiated adenocarcinoma) secrete IL-1$\alpha$ (2.1 pg/ml) and TGF$\beta$1 (0.2 ng/ml) respectively into the conditioned medium.

Moreover, the number of TMK-1 and MKN-28 cells grown on collagen gel is increased with co-culture of ST-Fib cells or treatment of human HGF. Simultaneously, TMK-1 cells show marked scattering, whereas this is not the case for MKN-28 cells. Both cell lines express the same levels of c-*met* protein and have a high-affinity receptor with a $K_d$ of 100–450 pM and 1600–2900 sites/cell. Western blot analysis for phosphotyrosine also indicates that increased phosphorylation of 170-kDa protein occurs in both the cell lines with co-culture of ST-Fib cells or HGF treatment. Why is scattering induced by HGH only in TMK-1 cells? The predominant difference between the two cell lines is E-cadherin expression. TMK-1 cells reveal weak E-cadherin expression, while MKN-28 cells maintain strong expression of E-cadherin at the cell-cell adhesion sites as in normal gastric epithelial cells. The overall results provide a plausible hypothesis for the morphogenesis of stomach cancer through interaction of HGF in stromal cells and cell adhesion molecules in c-*met*-overexpressing tumor cells (Fig. 1). Stromal fibroblasts, stimulated by tumor-derived growth factors or cytokines, may secrete HGF in a paracrine manner which can bind to c-*met* protein on tumor cells. If this happens, in the case of a clone maintaining expression of E-cadherin, HGF could enhance the growth of tumor cells and promote tubular formation, leading to the well-differentiated type of stomach cancer. Conversely, in the case of a clone having diminished expression or loss of E-cadherin, HGF may cause scattering and proliferation of tumor cells, resulting in the poorly differentiated type of stomach cancer. In fact, in gastric carcinoma tissues, most of the well-differentiated adenocarcinomas express E-cadherin uniformly at the cell-cell border of tumor cells, whereas poorly differentiated adenocarcinomas or scirrhous carcinomas reveal weak expression or no expression of E-cadherin (Shimoyama and Hirohashi 1991; Tahara 1992). However, poorly differentiated adenocarcinoma sometimes shows no correlation between E-cadherin expression and intracellular adhesiveness, suggesting that E-cadherin, even if strongly expressed by tumor cells, does not function as a cell adhesion molecule in tumor cells. As to the mechanism of E-cadherin involvement in tumor invasion or metastasis, the following should be taken into account: (a) decrease in synthesis of E-cadherin, (b) decrease in expression of E-cadherin mRNA and (c) disturbance in function of E-cadherin due to tyrosine phosphorylaltion of the cadherin-catenin complex or loss of catenin (Nagafuchi et al. 1991; Matsuyoshi 1992).

Interestingly, Faletto et al. (1992) have proposed that the cluster or patch distribution of c-*met* receptor protein on the cell surface may be related to the motogenic response to HGF. It is of considerable interest whether the clusters or patches of c-*met* receptor kinase may be linked with tyrosine phosphorylation of the cadherin-catenin complex.

## Multiple tumor-suppressor genes

Stomach cancer shows frequent loss or inactivation of multiple tumor-suppressor genes including p53, APC and DCC. LOH on chromosome 17p (p53 locus) and mutation of the p53 gene are observed in over 60% of gastric carcinomas, regardless of histological type (Sano et al. 1991; Tamura et al. 1991; Yokozaki et al. 1992). Moreover, this occurs from the early stage of progression. A recent review has concluded that the majority of p53 mutations in human cancers take place in evolutionarily highly conserved domains in exons 5–8 of the p53 gene, and mutational spectra vary among cancer types (Harris 1991). More recently, we have demonstrated that the p53 mutation spectrum in primary gastric carcinoma is characterized by (a) frequent mutation at an A · T pair (50%), (b) extremely high transversion incidence (46%), (c) no transition at CpG, and (d) no G · C to T · A transversion (Yokozaki et al. 1992). Previously published p53 mutations in gastric carcinoma (Kim et al. 1991; Tamura et al. 1991; Yamada et al. 1991) also tend to show the above-mentioned characteristics. Moreover, there are some differences in the p53 mutation spectrum between well-differentiated and poorly differentiated adenocarcinomas (Yokozaki et al. 1992). In well-differentiated adenocarcinoma, which is frequently accompanied by atrophic gastritis and intestinal metaplasia, frequent mutations are at an A · T pair, which may partly account for DNA depurination from irritants to mucosa, including ethanol or scalding temperature and chemical carcinogens, such as urethan, contained in alcoholic beverages as discussed in esophageal cancer by Hollstein et al. (1990, 1991). Bile reflux also may have implications for the p53 mutation spectrum. On the other hand, in poorly differentiated adenocarcinoma, which frequently occurs in younger age groups with relatively well preserved gastric mucosa, G · C to A · T transitions predominate. Carcinogenic *N*-nitrosoamines cause predominantly G · C to A · T base substitutions and are candidates for stomach carcinogens (Sugimura et al. 1970). Pickled vegetables and dried salted fish contain *N*-nitrosoamines, which can also be produced from amines with nitrites in the acidic environment of the stomach. In addition, an analysis of p53 mutation spectra in early gastric cancer showing multiple p53 point mutations suggests that external or internal carcinogens may cause different mutations at different clones, out of which the clone having a crit-

ical point mutation can expand selectively to reach a finally advanced stage of malignancy (Yokozaki et al. 1992). As far as p53 mutations in gastric cancer cell lines are concerned, MKN-1, -7, -28, HSC-39 and TMK-1 cells contain missense mutations but no mutations at "hot spots", codons 175, 248, 273 or 282, each of which is a CpG dinucleotide (Mattar et al. 1992). YCC-3 and NCI-N87 cells, however, have a CpG transition at codons 175 and 248 respectively (Kim et al. 1991). KATO-III cells have lost both alleles of the p53 gene. Both LOH and mutation of the p53 gene are frequently associated with p53 protein accumulation. p53 overexpression is detected in about 50% of gastric carcinomas regardless of histological types (Yokozaki et al. 1992; Martin et al. 1992). Moreover, patients with p53 expression reveal a worse prognosis than those without p53 expression (Martin et al. 1992).

LOH on chromosome 5q, where the APC gene is located, is detected in 60% of early well-differentiated adenocarcinomas, but not in poorly differentiated adenocarcinoma (Tahara 1992). Recently, the APC gene, which is responsible for familial adenomatous polyposis (FAP), has been isolated (Kinzler et al. 1991; Groden et al. 1991; Joslyn et al. 1991). The APC gene has been regarded as one of the tumor-suppressor genes, as it is somatically mutated in sporadic colorectal cancers (Nishisho et al. 1991). FAP patients often develop adenoma and adenocarcinoma of the stomach and duodenum. Interestingly, we have learned that Nakamura has detected frequent mutations of the APC gene in well-differentiated adenocarcinoma of the stomach. Moreover, signet-ring cell carcinoma on occasion has APC mutations (Horii et al. 1992). These observations suggest that inactivation of the APC gene after allele loss and mutation is one of the critical alterations in the pathway of carcinogenesis of well-differentiated stomach cancer.

In addition to the APC gene, LOH on chromosome 18q (DCC locus) is frequently found (50%) in well-differentiated adenocarcinoma (Tahara 1992). Such a LOH occurs in more than 70% of colorectal carcinoma (Fearon et al. 1990). Interestingly, in stomach cancer, LOH on chromosome 18q (DCC locus) may precede LOH on 17p (p53 locus) (Uchino et al. 1992). Considering these findings and alterations in the p53 and APC genes, it is likely that well-differentiated stomach cancer develops by a genetic pathway similar to those of colorectal carcinomas.

What is more, LOH on chromosome 1q is also frequently detected in well-differentiated adenocarcinoma, whereas LOH on chromosome 1p is relatively common in poorly differentiated adenocarcinoma (Sano et al. 1991). These findings suggest that tumor-suppressor genes exist on normal chromosome 1. In fact, we have confirmed suppression of tumorigenicity in MKN-28 cells by introduction of normal chromosome 1. In addition, as described in the chapter of oncogenes, chromosome 7q LOH containing the c-*met* gene is often associated with advanced cases of well-differentiated adenocarcinoma.

Genetic abnormalities in human stomach cancer are summarized in Table 1. Inactivation (allele loss and mutation) of the p53 tumor-suppressor gene and activation (amplification, abnormal expression) of the c-*met* gene are common events in two types of stomach cancer. K-*ras* mutation, although its incidence is low compared with that of colorectal or pancreatic cancer (Laurent-Puig et al. 1991; Lemoine et al. 1992),

**Table 1.** Genetic alterations in two types of stomach cancer

| Genetic alterations | Frequency of occurrence (%) | |
|---|---|---|
| | Poorly differentiated type cancer | Well-differentiated type cancer |
| Mutation | | |
| K-*ras* | – | 9 |
| p53 | 66 | 50 |
| APC | – (+)[a] | (+) |
| Deletion | | |
| p53 | 76 | 60 |
| Chromosome 5q (APC locus) | – | 60 |
| Rb | 7 | 5 |
| *bcl*-2 | – | 43 |
| Chromosome 18q (DCC locus) | – | 50 |
| Chromosome 1q | – | 44 |
| Chromosome 1p | 38 | 25 |
| Chromosome 7q (c-*met* locus) | – | 50 |
| Amplification | | |
| c-*met* | 39[b] | 19 |
| K-*sam* | 33[b] | – |
| c-*erb*B2 | – | 18 |
| Abnormal expression | | |
| c-*met* 6.0 kb | 73[b] | 50 |

[a] Signet-ring cell carcinoma
[b] Scirrhous carcinoma

c-*erb*B-2 amplification and *bcl*-2 deletion take place only in well-differentiated types of cancer, whereas K-*sam* amplification is found only in poorly differentiated types or scirrhous cancer. The chromosome 1p LOH is frequently associated with poorly differentiated cancer, whereas inactivation (allele loss and mutation) of the APC gene and LOH of DCC are often observed in the well-differentiated type. In addition, allele losses on chromosomes 1q and 7q may be involved in the progression of well-differentiated adenocarcinoma. The exception is that signet-ring cell carcinoma on occasion has APC mutations.

Therefore, common and different alterations in oncogenes and tumor-suppressor genes are observed between two types of stomach cancer, suggesting that the accumulation of these genetic changes is responsible for two types of stomach carcinogenesis. In fact, most gastric carcinomas have at least five gene changes including both oncogenes and tumor-suppressor genes (Tahara 1992).

## Genetic alterations in precancerous lesions

Recently Correa (1991) has proposed a hypothesis of multistage carcinogenesis of the stomach. The progression from normal epithelial cells to tumor cells may require at least six stages including superficial gastritis, chronic atrophic gastritis, intestinal metaplasia of the small-intestinal type followed by the colonic type, dysplasia and carcinoma. These sequential changes in gastric mucosa may arise over a period of

many years through a variety of exogenous and endogenous factors that evidently cause genetic alterations similar to those observed in gastric carcinomas. Interestingly, the *tpr-met* rearrangement, which is the fusion of a *tpr* (translocated promoter region) locus on chromosome 1 to the 5' region of the *met* gene on chromosome 7, is frequently expressed in chronic atrophic gastritis and intestinal metaplasia (Soman et al. 1991). However, no evident correlation is found between the level of *tpr-met* expression and progression of the disease. Recently, *Helicobacter pylori* has been recognized as a cause of chronic gastritis (Asaka et al. 1992). It remains to be elucidated whether *H. pylori* infection has implications for *tpr-met* expression.

The mutations in codon 12 of the K- and H-*ras* genes are also detected in antral gastritis and intestinal metaplasia of the small-intestinal type (Soman et al. 1991). We also have found a mutation in codon 12 of the K-*ras* gene in gastric intestinal metaplasia, associated with adenocarcinoma in adenoma having the same mutation of the K-*ras* gene and LOH of p53 and APC genes (Tahara 1992). Immunohistochemical analyses of the expression of *ras* p21 indicate that overexpression of *ras* p21 is frequently observed in well-differentiated adenocarcinoma, adenoma and intestinal metaplasia of the stomach (Yoshida et al. 1989). However, the *ras* mutation in stomach cancer is found in under 10% of well-differentiated adenocarcinomas, while the K-*ras* mutation is not detected in poorly differentiated adenocarcinoma.

An exciting observation is that p53 mutations occur in intestinal metaplasia and adenoma of the stomach (Tahara 1992). In the case of intestinal metaplasia, their incidence is about 10%, being almost equal to that of K-*ras* mutations. Moreover, p53 mutations are detected in 30% of gastric adenomas and are often silent (Tohdo et al. 1992). Histologically, gastric adenomas having frameshift or missense mutations reveal severe dysplasia. But we have observed no allele loss of the p53 gene in gastric adenoma and intestinal metaplasia.

Hastie et al. (1990) reported that telomere reduction was often involved in colorectal adenomas and carcinomas, and also closely correlated with ageing in blood DNA. Human telomeric DNA comprises ~2–20 (kb) of the tandemly repeated sequence $(TTAGGG)_n$ and protects DNA from degradation and end-to-end joining (Blackburn 1991; Gray et al. 1991). Telomere DNA is synthesized by the ribonucleoprotein reverse transcriptase, telomerase, which gradually decreases with increased age or cell division number (Hastie et al. 1990; Blackburn 1991). Gastric intestinal metaplasia as well as gastric carcinoma shows telomere reduction as compared with the length of telomere-repeat arrays in normal gastric mucosa (Tahara 1992). Hence, reduction or loss of telomeres in intestinal metaplasia could lead to chromosome instability or rearrangements subsequently observed in gastric tumors.

What is more exciting is that deletion of the 2.2-kb gene occurs in almost all intestinal metaplasias and well-differentiated adenocarcinomas of the stomach (Tahara 1992). This gene bears 60% homology with debrisoquine 4-hydroxylase. We are now performing cDNA deoxynucleotide sequencing for the identification of this gene.

Genetic alterations in precancerous lesions, together with the results of changes in multiple oncogenes, multiple growth factors and multiple tumor-suppressor genes, indicate that well-differentiated and poorly differentiated stomach cancers, including the scirrhous type, may have different genetic pathways as illustrated in Fig. 2.

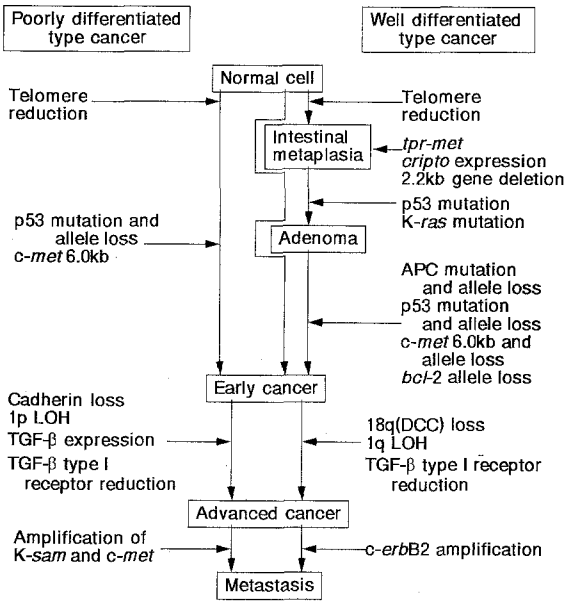


**Fig. 2.** Genetic pathway of two types of stomach cancer. LOH, loss of heterozygosity

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