of gastric cancer. More excitingly, Magnusson et al. found that distinct HLA class IIDQ and DR alleles are associated with the development of gastric cancer and infection with *H. pylori*. The DQA1*0102 is associated with protection from *H. pylori* infection, whereas the DRB*1601 is associated with cancer development, particularly, *H. pylori*-negative diffuse type gastric cancer. These host factors as well as *H. pylori* strain may determine why some individual infected with *H.pylori* develop gastric cancer while others do not.

The most important factor implicated in gastric carcinogenesis is genetic instability including microsatellite instability (MSI) and chromosomal instability. MSI due to epigenetic inactivation of the hMLH1 is found in 15 to 39% of sporadic intestinal type gastric cancer, of which 70% are associated with loss of hMLH1 by hypermethylation of the hMLH1 promoter. Intestinal type gastric cancers with MSI often occur in patients over 73 years of age and often occur in the antrum pylori. They are also associated with abundant lymphocyte infiltration, a putative favorable prognosis, and multiple tumors. In addition, MSI at the locus D1S191 is found in 26% of intestinal metaplasia and 46% of intestinal type gastric cancer. An identical pattern of MSI at the locus D1S191 is detected in both intestinal type cancer and the adjacent intestinal metaplasia, suggesting the sequential development of intestinal adenocarcinoma from intestinal metaplasia.

On the other hand, diffuse type gastric cancers with MSI occur mostly in patients under 35 years of age, and are frequently accompanied by scirrhous type carcinoma. This type of cancer, however, harbors no germline mutation of hMLH1 and hMSH2 and no alteration at BAT-RII, but is frequently associated with LOH on chromosome 17q21 including the *BRCA1* gene. Loss of the *BRCA1* by promoter methylation may have implications for the genesis of diffuse type gastric cancer.

Chromosomal instability leading to DNA aneuploidy is also an underlying factor in cancer. Telomere length is necessary for maintaining chromosomal stability. Recent evidence indicates that in the absence of telomerase, telomere shortening can bring about telomere dysfunction that causes both DNA breaks and chromosome gain or loss. Conversely, telomerase can inhibit chromosomal instability. Most intestinal type gastric cancers have remarkably shortened telomere length, associated with high levels of telomerase activity and significant expression of human telomerase reverse transcriptase (hTERT). More importantly, over 50% of intestinal metaplasia, as well as adenoma, express low levels of telomerase activity. We have found that *H. pylori* infection is a strong trigger for hyperplasia of hTERT positive cells in intestinal metaplasia, followed by increased telomerase activity and telomere reduction. Therefore, telomere reduction and telomerase activation play the most critical roles in an initial step of gastric carcinogenesis.

Mutations in the *APC* gene participate in chromosomal instability. Recently Kaplan et al. reported that mutation in *APC* may be responsible for chromosomal instability in colon cancer. It remains to be examined whether gastric cancer cells carrying a truncated *APC* gene are defective in chromosome segregation. APC protein directly binds to a kinetochore protein and is an avid *in vitro* substrate of the mitotic check-point protein Bub1. There

is no mutation in the *hBub1* gene in gastric cancer.

3.3 Abnormal Growth Factor/Cytokine Network in Gastric Cancer

Gastric cancer cells express a broad spectrum or growth factors, cytokines or both, including TGF-α, TGF-β, EGF, amphiregulin (AR), cripto, heparin binding (HB)-EGF, PDGF, IGF II, basic fibroblast growth factor (bFGF), IL-1α, IL-6, IL-8, and OPN. These growth factors and cytokines function as autocrine, paracrine, and juxtacrine modulators for the growth of cancer cells, and they organize the complex interaction between cancer cells and stromal cells which play a key role in morphogenesis, invasion, neovascularization, and metastasis (Fig. 4). Interestingly, the expression pattern of these growth factors and cytokines by cancer cells differs in the two histological types of gastric carcinomas. The EGF family including EGF, TGFα, cripto and AR are commonly overexpressed in intestinal type carcinoma, whereas TGFβ, IGF II, and bFGF are predominantly overexpressed in diffuse type carcinoma. Coexpression of EGF/TGF-α, EGFR and cripto correlates well with the biological malignancy, as these factors induce metalloproteinases. Overexpression

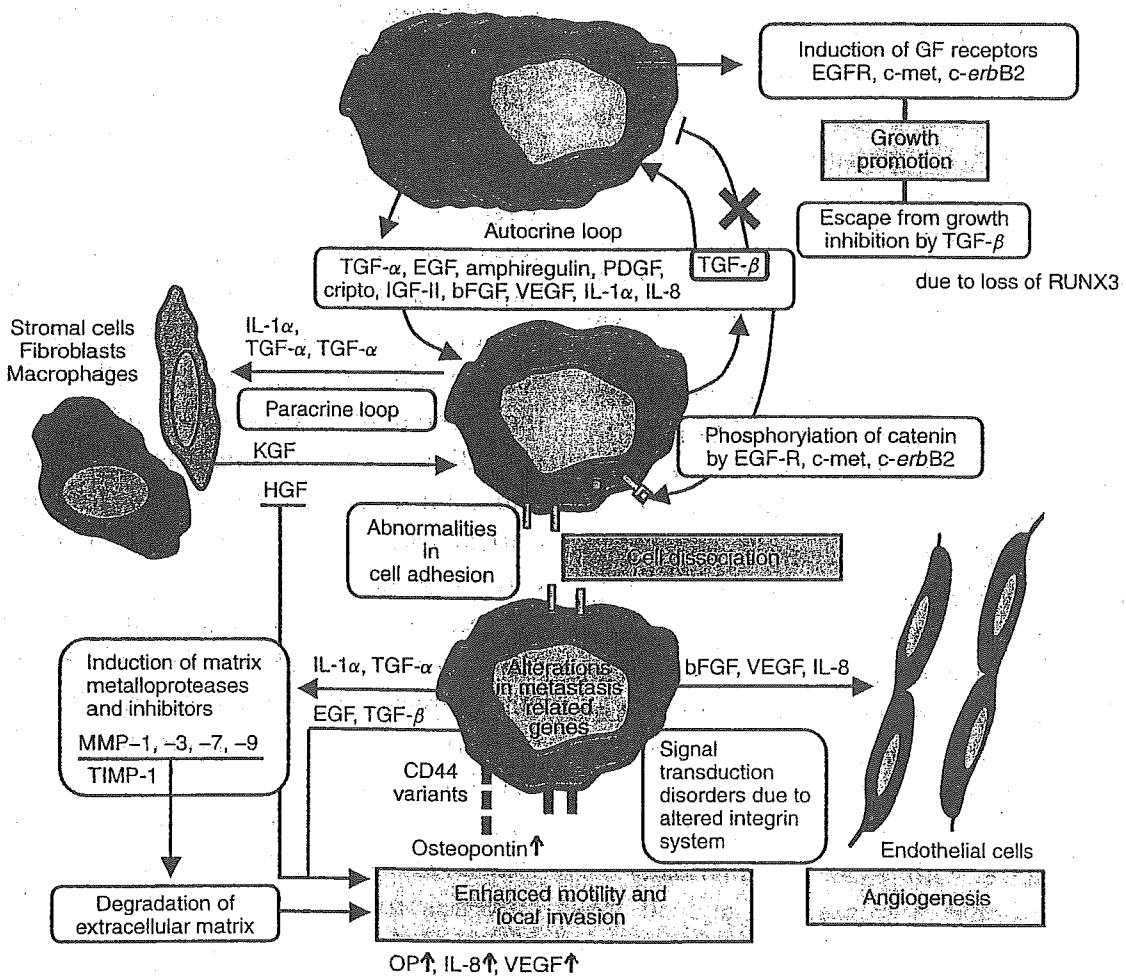

Fig. 4 Cancer-stromal interaction in gastric cancer through growth factors and cytokines.

of cripto is frequently associated with intestinal metaplasia and gastric adenoma. Akagi et al. have recently shown that gastric cancer cells express neutrophilin-1(NRP-1), which acts as a coreceptor for VEGF-165 and increases its affinity for VEGF receptor 2 endothelial cells. EGF induces both NRP-1 and VEGF expression, suggesting that regulation of NRP-1 expression in gastric cancer is intimately associated with EGF/EGFR system.

IL-1α is a cytokine mainly produced by activated macrophages through NF-κB activation and mediates many of the local and systemic responses to infection and inflammation. Gastric cancer cells also produce it. We have found that IL-1α acts as an autocrine growth factor for oral and gastric carcinoma cells and plays a pivotal role as a trigger for induction of EGF and EGFR expression. The expression of IL-1α by tumor cells is induced by either IL-1α, EGF, or TGF-α, while IL-α upregulates the expression of TGF-α and EGFR by tumor cells themselves, indicating that an intimate interplay between IL-1α and EGF/receptor system stimulates the growth of gastric cancer cells. In addition to IL-1α, IL-6 is also an autocrine growth stimulator for gastric cancer cells. The expression of IL-1α by tumor cells is induced by IL-6, while IL-1α increases the expression of IL-6 by tumor cells themselves. Currently, Fukayama's group reported that IL-1β may act as an autocrine growth factor in a human Epstein-Barr virus-associated gastric carcinoma.

IL-8, a member of the CXC chemokine family, induces haptotactic migration and proliferation of melanoma cells and angiogenesis. More importantly, gastric cancer cell lines express mRNA and protein for IL-8 and IL-8 receptors (IL-8RA and IL-8RB). More than 80% of primary tumors coexpress IL-8 and IL-8 receptor; this coexpression correlates directly with tumor vascularity and tumor progression. IL-8 enhances the expression of EGFR, type IV collagenase (MMP-9), VEGF, and IL-8 itself by tumor cells, while IL-8 decreases expression of E-cadherin. Moreover, IL-8 increases MMP-9 activity and the ability of gastric cancer cells to invade through Matrigel. IL-8 may play an important role in the growth and progression of gastric carcinoma by autocrine and paracrine mechanisms.

In addition to IL-8, VEGF and bFGF participate mainly in neovascularization in gastric cancer. We have shown that eight gastric cancer cell lines secrete VEGF into conditioned media. EGF or IL-1α upregulates VEGF expression by tumor cells, whereas interferon-γ downregulates it. VEGF promotes angiogenesis and the progression of gastric carcinoma, especially intestinal type. VEGF-C produced by tumor cells participates in the development of lymph node metastasis. On the other hand, bFGF produced by tumor cells is frequently associated with angiogenesis and extensive fibrosis in diffuse type carcinoma, particularly those of the scirrhous type. Interestingly, Nakazawa et al. reported that keratinocyte growth factor (KGF) produced by gastric fibroblasts specifically binds to K-sam on tumor cells and then stimulates proliferation of cancer cells, resulting in the development of the scirrhous type of gastric cancer. KGF from gastric fibroblasts may underline the remarkable proliferation of scirrhous gastric cancer cells in a paracrine manner.

Stromal cells, especially fibroblasts stimulated by growth factors or cytokines such as IL-1α, TGF-α, and TGFβ, secrete HGF/SF, which can function in a paracrine manner as a morphogen or mitogen of tumor cells. For example, in the case

of a cancer cell clone maintaining expression of cell adhesion molecules, HGF/SF promotes tubular formation of tumor cells, resulting in intestinal type gastric cancer. Conversely, in the case of a clone with reduced expression of cell adhesion molecules, HGF/SF can act as a mitogen and induce scattering of tumor cells, resulting in diffuse type gastric cancer.

OPN, also termed Eta-1 (early T-lymphocyte activation-1), which is a reported protein ligand of CD44, is overexpressed in 73% of gastric cancer. The co-expression of OPN and CD44 v9 in tumor cells correlates with the nodal metastasis in diffuse type gastric cancer. The *CD44* gene contains at least 20 exons, 12 of which can be alternatively spliced to make up a wide variety of molecular variants. All gastric cancer cell lines and primary tumors show overexpression of abnormal CD44 transcripts containing the intron 9 sequence. The intestinal metaplasia also expresses these variants but normal gastric mucosa does not express them. Currently, Medico et al. reported that OPN is an autocrine mediator of HGF induced invasive growth.

4 Genetic and Epigenetic Alterations and Abnormal Growth Factor/Cytokine Network in Colorectal Cancer

Cancer of the colon and rectum is the fourth most common cancer in the world. In 1996, an estimated 875 000 new cases were diagnosed worldwide, accounting for 8.5% of all new cancers. Approximately 98% of malignant colorectal tumors are adenocarcinoma. Rectal tumors account for about 27%, while almost 50% occur proximal to the splenic flexure.

The accumulation of multiple genetic and epigenetic alterations in tumor-suppressor genes, oncogenes and DNA mismatch repair genes takes place in the multistep process of colorectal carcinogenesis. Inactivation of APC, p53, and DCC and K-ras mutations are involved in a major genetic pathway for colorectal tumorigenesis showing the course of malignant progression from normal mucosal cells through adenomas (adenoma–carcinoma sequence) (Fig. 5). This section will make an overview of multiple

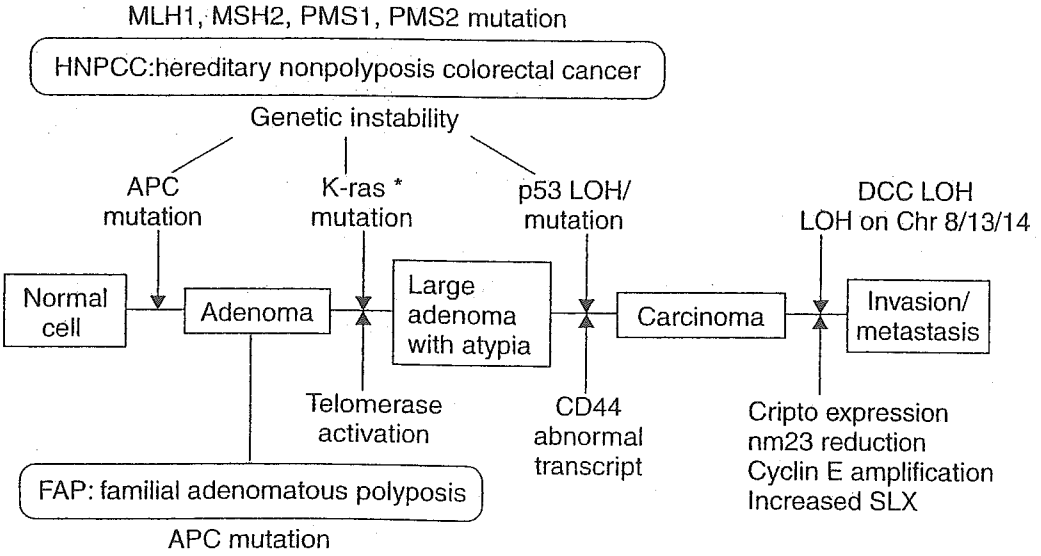

Fig. 5 A major genetic pathway for colorectal carcinogenesis. (*K-ras mutation is infrequent in flat adenoma)

genetic and epigenetic alterations responsible for colorectal carcinogenesis and abnormal growth factor/cytokine network, which is implicated in the progression and metastasis of colorectal cancer.

4.1 Genetic and Epigenetic Alterations in Colorectal Cancer

The *APC* gene, first isolated as a causative gene for familial adenomatous polyposis (FAP), encodes a large protein of 2843 amino acids, which forms a complex with α-and β-catenins and may mediate cell adhesion, cytoskeletal anchoring, and signal transduction. The *APC* gene is abnormal in the germline of FAP patients. LOH and mutations of the *APC* gene occur in 60% of sporadic colorectal adenomas and adenocarcinomas. These tumors harbor loss of the *APC* gene in one allele and mutation of the gene in the remaining allele, supporting Knudson's two-hit theory. The characteristic of the mutation is a base substitution that leads to a stop codon (nonsense mutation), which occurs in about 70% of FAP kindred. The APC alterations are found even in small adenomas with mild atypia. The tumor-suppressor function of APC has also been demonstrated in mouse models. These results and animal models show that inactivation of APC is an initiating genetic event for colorectal tumorigenesis.

The major tumor-suppression activity of APC is regulation of β-catenin. APC's association with β-catenin and promotion of the degradation of β-catenin are most relevant to its tumor-suppressor function. APC binds not only β-catenin but also glycogen synthase kinase-3β (GSK3β) and Axin. APC and Axin serve as a scaffold to facilitate the phosphorylation of β-catenin by GSK3β. The phosphorylated β-catenin is ubiquitinated by β-Trcp and the ubiquitinated β-catenin is then degraded by proteasome. Mutant APC proteins cannot regulate the degradation of β-catenin, resulting in excessive β-catenin that interacts with T-cell factor (TCF)-4 and translocates into the nucleus. The β-catenin/TCF-4 complex then activates the expression of many genes including *c-myc*, matrix metalloproteinase-7 (*MMP-7*), peroxisome proliferator-activated receptor δ (*PPARδ*) and cyclin D1, leading to promotion of colon tumorigenesis. In fact, overexpression of *c-myc*, *PPARδ* and cyclin D1 is observed in colorectal cancer. A recent study of PPARδ deficient (Ppard−/−) mice has shown that colon tumor formation is significantly greater in mice nullizygous for PPAR, suggesting that PPARδ attenuates colon carcinogenesis. It is contrary to previous reports suggesting that activation of PPARδ is causally associated with colon polyp formation. Further work is necessary to clarify the role of PPARδ in colon carcinogenesis.

On the other hand, somatic mutations of β-catenin are detected in both human and rodent colorectal tumors that do not have the APC mutation. All the β-catenin mutations found in colorectal cancers occur at the critical region for phosphorylation of β-catenin by GSK3β. The mutant β-catenin is resistant to APC-mediated degradation.

Mutation in the *K-ras* oncogene is involved in the progression from small adenoma with mild atypia to large adenoma with severe atypia. About 40 to 50% of large adenomas with severe atypia and adenocarcinomas contain K-ras point mutations at codon 12 or 13, compared to only 10% of small adenomas with mild atypia. Conversely, the frequency of K-ras mutation is lower (<10%) in superficial-type or flat adenomas even in the presence

of significant atypia. Much evidence indicates that mutation of K-ras alone can bring about a hyperplastic lesion that has a limited potential to progress to larger tumors. Mutant K-ras can, however, promote tumor progression in lesions initiated by APC mutation.

LOH of the *p53* gene locus is detected in about 80% of colorectal adenocarcinoma, and most harbor inactivation of the *p53* gene in both alleles. Because only 5 to 20% of the adenomas have p53 inactivation, it must play a crucial key in the transition from adenoma to adenocarcinoma. There are hot spots for point mutations in the highly conserved regions such as codon 175, 248, and 273, where G:C to A:T transitions occur. Abnormal accumulation of p53 protein detected by immunohistochemistry is frequently associated with deeply invasive carcinomas and carcinomas with metastasis. Almost all mutant p53 proteins derived from cancers have altered sequence-specific DNA binding and transcription activities.

Overexpression of *c-erbB2* has been reported in 80 to 100% of colorectal cancers. The amplification of *c-erbB2*, which is common in intestinal type gastric cancer, occurs in about 10% of colorectal carcinomas. Moreover, the cyclin E gene, a positive regulator of cell cycle progression, is amplified in about 10% of colorectal carcinomas. The overexpression of cyclin E is detected in 5% of adenomas and in 20% of adenocarcinomas. Among adenomas, a significant correlation is observed between cyclin E expression and the grade of atypia. Overexpression of cyclin E is prominent in carcinoma invading the submucosa or deeper compared to those limited to the mucosal layer. Cyclin E expression is thus a candidates molecular biomarker for predicting malignant progression of colorectal as well as gastric cancers. Reduction in p27 expression participates in progression and poor prognosis of colorectal cancer as well.

LOH of 18q including *DCC*, *Smad4*, and *Smad2* genes is frequently associated with advanced colorectal cancer. Among them, *DCC* and *Smad4* play important roles in colon cancer progression. LOH of the *DCC* gene is rare in adenoma but frequent (about 70%) in adenocarcinoma. LOH of *DCC* increases as the tumor invades deeply, and almost all the metastatic liver tumors show this LOH. Moreover, reduced expression of *DCC* in colorectal cancer is correlated with a poor prognosis. *DCC* encodes a receptor for netrin-1, but its function in normal colon epithelial cells remains unclear. *Smad4* encodes a protein that plays a critical role in the TGF-β signal transduction pathway. Although *Smad4* was isolated as a tumor-suppressor gene for pancreatic cancer, somatic mutations of *Smad4* frequently take place in advanced colon cancer, suggesting that Smad4 inactivation confers progression of colorectal cancer. In addition, *Smad4* germline mutations are responsible for juvenile polyposis, an autosomal disease that has high susceptibility for hamartomatous polyposis and gastrointestinal cancer.

In addition to these molecular events, and as mentioned for gastric cancer, telomere reduction may result in chromosomal instability and telomerase reactivation. The colorectal adenomas and adenocarcinomas share shorter telomeres than those in normal tissues. We have found that more than 90% of colorectal adenocarcinomas express extremely high levels of telomerase activity regardless of tumor staging and histological differentiation. All the adenomas also exhibit considerable levels of telomerase activity. Telomerase activity and stabilization of telomeres occur concomitantly with the acquisition of

immortality, contributing to an early stage of colorectal carcinogenesis.

Beside the major genetic pathway (adenoma–carcinoma sequence), an alternative genetic pathway exists for colorectal carcinogenesis. The so-called *de novo* carcinogenesis exhibits no adenoma–carcinoma sequence but develops directly from the colorectal epithelial cells that share p53 inactivation, followed by APC inactivation. K-ras mutation is not detected in *de novo* carcinogenesis. This type of colorectal carcinogenesis is frequently found in Japanese patients.

Tab. 4 Differences between MSI positive and MSI negative colorectal cancers.

	MSI +	*MSI –*
Location	Proximal	Distal
Ploidy	Near diploid	Aneuploid
Chromosomal instability	Rare	Common
Mutation Frequency		
P53	Low	High
APC	Low	High
TGFβ RII	High	Low
BAX	High	Low
Methylation	High	Low
Survival	Better	Worse
Hereditary syndrome	HNPCC	FAP

4.2 Factors Associated with Increased Incidence of Colorectal Cancer

Genetic and epigenetic alterations in DNA mismatch repair genes including hMHL1 and hMSH2 raise MSI that has implications for predisposition to colorectal cancer. The MSI occurs in 10 to 15% of sporadic colorectal cancer. These sporadic, mostly right-side colon cancers with MSI are associated with hypermethylation of the CpG islands in the hMLH1 promoter, resulting in loss of hMLH1, loss of function of other genes such as *p16*, defective mismatch repair, and widespread MSI. Hereditary nonpolyposis colorectal cancer (HNPCC), caused by inherited germline mutations in hMLH1 and hMLH2, accounts for 3 to 10% of colorectal cancer. Genes coding for TGFβ type II receptor, insulin-like growth factor (IGF)2 receptor, proapoptotic protein BAX, cell cycle regulator E2F-4, and mismatch repair proteins MSH3 and MSH6 are mutated in HNPCC or MSI sporadic colon cancer.

Some differences between MSI positive and MSI negative colorectal cancers are presented in Table 4. Tumor location, ploidy, mutation frequency, methylation, and survival are different between MSI positive and MSI negative tumors, although there are overlaps between the two.

In addition, sporadic colon cancer with MSI is also associated with altered expression of IGF2, namely, loss of imprinting (LOI) of IGF2. Importantly, the normal colonic mucosa exhibits aberrant hypermethylation and LOI of IGF2 as a sign of a field defect. However, Feinberg et al. have recently reported that hypomethylation of H19 and IGF2 is a mechanism for LOI and is found in both colorectal cancers and normal mucosa from the same patients. Moreover, they reported that LOI of IGF2 provides a potential heritable biomarker for colon cancer predisposition.

NF-κB activation is also associated with chronic inflammatory bowel diseases (IBD) and colorectal cancer. As mentioned in Sect. 3.2, NF-κB activation leads to production of enzymes such as iNOS and COX2 and enhanced expression of growth factors and cytokines. IBD including ulcerative colitis and Crohn's disease

induce persistent NF-κB activation in tissue macrophages and epithelial cells of the colonic mucosa. Both inflammatory bowel diseases are well known to increase the risk of colorectal cancer. The link between COX2 and colorectal cancer is supported strongly by epidemiological and experimental evidence. COX2 is overexpressed in adenomas and carcinomas of the colon. COX2-null mice are resistant to colorectal carcinogenesis. Long-term consumption of aspirin or other COX inhibitors has been reported to reduce the relative risk of colorectal cancer. These results indicate that COX2 contributes to colorectal tumorigenesis.

4.3 Abnormal Growth Factor/Cytokine Network in Colorectal Cancer

Colorectal carcinomas express multiple growth factors, such as EGF and TGFα and their receptors thus creating autocrine loops. TGFα and EGF are overexpressed in colorectal adenomas and the majority of colorectal carcinomas. Coexpression of TGFα, EGF or both, and EGFR is well correlated with high grade of malignancy and metastasis.

As described in gastric cancer, NRP-1 induced by EGF is expressed in all of colorectal cancer tissues and cell lines but not in the adjacent nonmalignant colonic mucosa. A recent study of NRP-1 in colon cancer suggests that NRP-1 may contribute to colon cancer angiogenesis and that EGF and mitogen-activated protein kinase signaling may play an important role in NRP-1 regulation in colon cancer cells.

The cripto gene was originally identified in undifferentiated human embryonal carcinoma cells and encodes a 37 amino acid region that shares structural homology with other members of the EGF family. However, cripto does not bind to the EGFR and its receptor has not been identified. Strong expression of mRNA and protein for cripto is found in 60 to 80% of colorectal cancers but not in normal colorectal mucosa. It is detected in 40% of tubular adenomas and 86% of tubulovillous adenomas, respectively. These findings suggest that cripto expression may be involved in the early stages of malignant transformation. Amphiregulin (AR) is another member of the EGF family that utilizes EGFR. About half of colorectal carcinomas as well as 60% of adenomas express AR. It has been confirmed that cripto and AR act as autocrine growth stimulators for colorectal cancer cell lines. Because AR is also expressed in normal colorectal epithelium, AR may participate in the regulation of growth of normal as well as colorectal cancer cells.

TGFβ-1 is expressed in over half of colorectal cancers. Interestingly, high levels of TGFβ-1 expression in tumor cells and elevated plasma levels of mRNA are associated with advanced Dukes' stage, suggesting that there is a correlation between TGFβ overexpression and tumor progression. Moreover, circulating TGFβ-1 may serve as a predictor of liver metastasis after resection of colorectal cancer. In addition, TGFβ produced by cancer cells stimulates angiogenesis by inducing thymidine phosphorylase, regulation of extracellular matrix adhesion molecules such as carcinoembryonic antigen (CEA), and by the enhanced secretion of gelatinase B, a matrix degrading enzyme.

VEGF and bFGF are also expressed strongly in colorectal cancer in contrast to normal colorectal epithelium and adenoma. The expression of bFGF is higher in Dukes stage D than in Dukes stage B colorectal cancer. Moreover, bFGF and

VEGR are elevated in the serum of patients with aggressive advanced colorectal cancer. These circulating growth factors as well as TGFβ may be useful biomarkers for understanding angiogenesis and malignancy. On the other hand, the expression of VEGF-C and VEGF-D correlates with lymph-node metastasis in colorectal carcinoma, and these expressions are heterogeneous and elevated at the invasive edge of tumors.

Activation of the pp60src protein kinase activity occurs during colorectal tumorigenesis. The kinase activity of pp60src is highly regulated and is induced by many growth factors. Recently, pp60src has been reported to be essential for the induction of VEGF by hypoxia. The specific activity of pp60src is higher in colorectal polyp than in normal mucosa, and is further increased in colorectal carcinoma and in metastatic colon tumor in liver. Further study is needed to clarify the mechanism for increased pp60src activity during colorectal carcinogenesis.

The abnormal transcripts of the *CD44* gene are also expressed in all of colorectal cancers. As mentioned in gastric cancer, the *CD44* gene consists of at least 20 exons, of which 10 are alternatively spliced to make up variants. Among several *CD44* variants, aberrant transcripts with retention of intron 9 are best for distinguishing carcinoma tissues from normal tissues in the colorectum. However, the variants do not correlate with nodal or distant metastasis.

A candidate suppressor gene of tumor metastasis, *nm23*, encodes nucleotide diphosphate kinase and c-myc transcription factor (PuF). Although most of colorectal cancers express nm23 at higher levels than the corresponding normal mucosa, an inverse correlation is observed between nm23 expression and tumor staging. Moreover, reduced expression of nm23 is associated with distant metastasis.

Another candidate for a molecular marker that indicates metastatic potential is cell surface carbohydrate, sialyl-dimeric Le antigens. Both sialyl Lex (SLX) and sialyl Lea (SLA or Ca 19-9) as ligands bind to E-selectin, also known as ELAM-1, one of the adhesion molecules on activated endothelial cells. SLX and SLA may participate in distant metastasis through interaction between cancer cells and endothelial cells. The expression of SLX in colorectal carcinomas shows significant correlation with liver metastasis and poor prognosis.

5 Conclusion

A large number of molecular events are involved in the development and progression of gastrointestinal carcinomas. Among them, common and distinct events of genetic and epigenetic alterations are observed in esophageal, gastric, and colorectal cancers. MSI confers the initial step of gastric and colorectal carcinomas, while it is less involved in esophageal SCC. Chromosomal instability (telomere reduction) and telomerase activation participate commonly in the very early stage of gastrointestinal carcinogenesis. p53 inactivation and RUNX3 loss by promoter hypermethylation are also common events, although RUNX3 loss is less in colorectal cancer. APC LOH and DCC LOH are commonly detected in the majority of the three gastrointestinal cancers, although APC mutations occur mainly in colorectal cancer. K-ras mutation is often found in colorectal cancer, whereas it is extremely rare in esophageal SCC and gastric cancer. Amplification of the cyclin D1 gene

is preferentially found in esophageal SCC, while the gene amplification of cycline E is frequently associated with both gastric and colorectal adenocarcinomas. Reduced expression of the CDK inhibitors such as p16 and p27 is often found in gastrointestinal cancers. In gastric cancer, the pattern of genetic and epigenetic alterations also differs depending on the two histological types, intestinal or well-differentiated type and diffuse or poorly differentiated type. The amplification of *c-met* and *K-sam* genes and the mutation/loss of the E-cadherin gene as well as *RARβ2* loss occur preferentially in diffuse type, whereas the amplification of *c-erbB2* gene, pS2 reduction, p16 loss and hMLH1 loss as well as APC mutation is predominantly found in intestinal type.

In addition to these events, gastrointestinal cancer cells express a broad spectrum of the growth factor/cytokine receptor systems that organize complex interactions between cancer cells and stromal cells, which confer cell growth, apoptosis, morphogenesis, angiogenesis, progression and metastasis. However, these abnormal growth factor/cytokine networks are also evidently different among esophageal, gastric, and colorectal cancers, respectively. Importantly, NF-κB activation induced by inflammation may act as a key player for induction of growth factor/cytokine networks in gastrointestinal cancers.

Overall, the observations on the molecular events involving growth factors and oncogenes in gastrointestinal cancers will no doubt provide a deeper understanding of prevention, molecular diagnosis, and therapeutics of these cancers. In fact, by applying these molecular events of gastrointestinal cancers to routine clinical practice, we have implemented molecular pathological diagnosis of gastrointestinal cancer in collaboration with Hiroshima City Medical Clinical Laboratory since 1993. We have analyzed more than 10 000 cases of gastrointestinal biopsy and surgery and then obtained additional information on differential diagnosis, biological malignancy, and tumor multiplicity. We believe this approach will better serve science, but more importantly, patient care.

See also Growth Factors; Oncology, Molecular.

Bibliography

Books and Reviews

Correa, P., Miller, M., Mannick, E.E. (1997) Oxidative Damage During the Gastric Precancerous Process, in: Tahara, E. (Ed.) *Molecular Pathology of Gastroenterological Cancer*, Springer-Verlag, Tokyo, Japan, pp. 23–29.

Fenoglio-preiser, C., Noffsinger, A., Stemmermann, G. (2004) Pathology and Natural History of Gastric Cancer, in: Abbruzzese, J.L., Evans, D.B., Willett, C.G., Fenoglio-Preiser, C. (Eds.) *Gastrointestinal Oncology*, Oxford University Press, New York, pp. 281–293.

Fidler, I.J. (1997) Critical Determinants of Human Colon Cancer Metastasis, in: Tahara, E. (Ed.) *Molecular Pathology of Gastroenterological Cancer*, Springer-Verlag, Tokyo, Japan, pp. 15–21.

Grizzle, W.E., Shibata, D., Manne, U., Myers, R.B., Frost, A.R. (1999) Molecular and Histopathologic Changes in the Development of Colorectal Neoplasia, in: Srivastava, S. Henson, D.E. Gazdar, A. (Eds.) *Molecular Pathology of Early Cancer*, IOS Press, Amsterdam, Netherlands, pp. 135–170.

Mao, L., El-Naggar, A.K. (1999) Molecular Changes in the Multistage Pathogenesis of Head and Neck Cancer, Srivastava, S., et al. (Eds.) *Molecular Pathology of Early Cancer*, IOS Press, Amsterdam, Netherlands, pp. 189–205.

Nakamura, Y., Tokino, T., Isomura, M., Inazawa, J., Aoki, T., Mori, T., Shimada, M.,

Miura, K., Suzuki, K. (1997) Multistep Carcinogenesis of Esophageal Carcinoma, in: Tahara, E. (Ed.) *Molecular Pathology of Gastroenterological Cancer*, Springer-Verlag, Tokyo, Japan, pp. 15–21.

Su, L.K. (2004) Molecular Biology of Colorectal Cancer, in: Abbruzzese, J.L., et al. (Eds.) *Gastrointestinal Oncology*, Oxford University Press, New York, pp. 635–644.

Tahara, E. (1997) Cell Growth Regulation and Cancer: Stromal Interaction, in: Sugimura, T. Sasaki, M. (Eds.) *Gastric Cancer*, Oxford University Press, Tokyo, Japan, pp. 100–108.

Tahara, E. (2004) Genetic Pathways of Two Types of Gastric Cancer, in: Buffler, P., et al. (Eds.) *Mechanisms of Carcinogenesis: Contributions of Molecular epidemiology*, IARC Scientific Publications No. 157, International agency for Research on Cancer, Lyon, France, pp. 327–349.

Tahara, E., Yasui, W., Yokozaki, H. (1996) Abnormal Growth Factor Networks in Neoplasia, in: Pusztai, L., et al. (Eds.) *Cell Proliferation in Cancer: Regulatory Mechanisms of Neoplastic Cell Growth*, Oxford Medical Publications, New York, pp. 131–153.

Tahara, E., Yokozaki, H., Yasui, W. (1993) Growth Factors in Gastric Cancer, in: Nishi, M. Ichikawa, H. Nakajima, T. Maruyama, K. Tahara, E. (Eds.) *Gastric Cancer*, Springer-Verlag, Tokyo, Japan, pp. 209–217.

Tahara, E., Yokozaki, H., Yasui, W. (1999) Stomach–Genetic and Epigenetic Alterations of Preneoplastic and Neoplastic Lesions, in: Srivastava, S. Henson, D.E. Gazdar, A. (Eds.) *Molecular Pathology of Early Cancer*, IOS Press, Amsterdam, Netherlands, pp. 341–361.

Tahara, H., Tahara, E. Jr., Tahara, E., Ide, T. (1997) Telomeres and Telomerase in Gastrointestinal Cancer, in: Tahara, E. (Ed.) *Molecular Pathology of Gastroenterological Cancer*, Springer-Verlag, Tokyo, Japan, pp. 245–259.

Tselepis, C., Jankowski, J. (2004) Molecular Biology of Esophageal Cancer, in: Abbruzzese, J.L., et al. (Eds.) *Gastrointestinal Oncology*, Oxford University Press, New York, pp. 155–166.

Yasui, W., Yokozaki, H., Tahara, E. (1997) Molecular Diagnosis of Gastrointestinal Cancer, in: Tahara, E. (Ed.) *Molecular Pathology of Gastroenterological Cancer*, Springer-Verlag, Tokyo, Japan, pp. 187–207.

Yokozaki, H., Yasui, W., Tahara, E. (2001) Genetic and Epigenetic Changes in Stomach Cancer, in: Jeon, K.W. (Ed.) *International Review of Cytology Vol.20: A Survey of Cell Biology*, Academic Press, New York, pp. 49–95.

Yoshida, K., Yasui, W., Kagawa, Y., Tahara, E. (1997) Multiple Genetic Alterations and Abnormal Growth Factor Network in Human Esophageal Carcinomas, in: Tahara, E. (Ed.) *Molecular Pathology of Gastroenterological Cancer*, Springer-Verlag, Tokyo, Japan, pp. 31–41.

Primary Literature

Akagi, M., Kawaguchi, M., Liu, W., McCarty, M.F., Takeda, A., Fan, F., Stoeltzing, O., Parikh, A.A., Jung, Y.D., Bucana, C.D., Mansfield, P.F., Hicklin, D.J., Ellis, L.M. (2003) Induction of neuropilin-1 and vascular endothelial growth factor by epidermal growth factor in human gastric cancer cells, *Br. J. Cancer* **88**, 796–802.

Akhtar, M., Cheng, Y., Magno, R.M., Ashktorab, H., Smoot, D.T., Meltszer, S.J., Wilson, K.T. (2001) Promoter methylation regulates *Helicobacter pylori*-stimulated cycloxygenase-2 expression in gastric epithelial cells, *Cancer Res.* **61**, 2399–2403.

Akiyama, Y., Maesawa, C., Ogasawara, S., Terashima, M., Masuda, T. (2003) Cell-type-specific repression of the maspin gene is disrupted frequently by demethylation at the promoter region in gastric intestinal metaplasia and cancer cells, *Am. J. Pathol.* **163**, 1911–1919.

Aoki, T., Takeda, S., Yanagisawa, A. (1994) APC and p53 mutations in *de novo* colorectal adenocarcinomas, *Hum. Mutat.* **3**, 342–346.

Arber, N., Hibshoosh, H., Moss, S.F., Sutter, T., Zhang, Y., Begg, M., Wang, S., Weinstein, I.B., Holt, P.R. (1996) Increased expression of cyclin D1 is an early event in multistage colorectal carcinogenesis, *Gastroenterology* **110**, 669–674.

Arber, N., Gammon, M.D., Hibshoosh, H., Britton, J.A., Zhang, Y., Schonberg, J.B., Roterdam, H., Fabian, I., Holt, P.R., Weinstein, I.B. (1999) Overexpression of cyclin D1 occurs in both squamous carcinomas and adenocarcinomas of the esophagus and in adenocarcinomas of the stomach, *Hum. Pathol.* **30**, 1087–1092.

Bodmer, W.F., Bailey, C.J., Bodmer, J. et al. (1987) Localization of the gene for familial

adenomatous polyposis on chromosome 5, *Nature* 328, 614–616.

Bronner, C.E., Baker, S.M., Morrison, P.T., Warren, G., Smith, L.G., Lescoe, M.K., Kane, M., Earabino, C., Lipford, J., Lindblom, A. et al. (1994) Mutation in the DNA mismatch repair gene homologue *hMLH1* is associated with hereditary nonpolyposis colon cancer, *Nature* 368, 258–261.

Carvalho, F., Seruca, R., David, L., Amorin, A., Seixas, M., Bennett, E., Clausen, H., Sobrinho-Simoes, M. (1997) MUC1 gene polymorphism and gastric cancer–an epidemiological study, *Glycoconjugate J.* 14, 107–111.

Chen, L., Matsubara, N., Yoshino, T., Nagasaka, T., Hoshizima, N., Shirakawa, Y., Naomoto, Y., Isozaki, H., Riabowol, K., Tanaka, N. (2001) Genetic alterations of candidate tumor suppressor ING1 in human esophageal squamous cell cancer, *Cancer Res.* **61**, 4345–4349.

Cho, B., Lee, H., Jeong, S.W., Bang, Y.J., Lee, H.J., Hwang, K.S., Kim, H.Y., Lee, Y.S., Kang, G.H., Jeoung, D.I. (2003) Promoter hypomethylation of a novel cancer/testis antigen gene CAGE is correlated with its aberrant expression and is seen in premalignant stage of gastric carcinoma, *BBRC* **307**, 52–63.

Chong, J.M., Sakuma, K., Sudo, M., Osawa, T., Ohara, E., Uozaki, H., Shibahara, J., Kuroiwa, K., Tominaga, S., Hipp, Y., Aburatani, H., Funata, N., Jukayama, M. (2002) Interleukin-1β expression in human gastric carcinoma with Epstein-Barr Virus Infection, *J. Virol.* **76**, 6825–6831.

Crawford, H.C., Fingleton, B.M., Rudolph-Wwen, L.A., Goss, K.J., Rubinfeld, B., Polakis, P., Matrisian, L.M. (1999) The metalloproteinase matrilysin is a target of β-catenin transactivation in intestinal tumors, *Oncogene* **18**, 2883–2891.

Cruz-Correa, M., Cui, H., Giardiello, F.M., Powe, N.R., Hylind, L., Robinson, A., Hutcheon, D.F., Kafonek, D.R., Brandenburg, S., Wu, Y., He, X., Feinberg, A.P. (2004) Loss of imprinting of insulin growth factor II gene: a potential heritable biomarker for colon neoplasia predisposition, *Gastroenterology* **126**, 964–970.

Cui, H., Onyango, P., Brandenburg, S., Wu, Y., Hsieh, C.L., Feinberg, A.P. (2002) Loss of imprinting in colorectal cancer linked to hypomethylation of *H19* and *IGF2*, *Cancer Res.* **62**, 6442–6446.

Cui, H., Cruz-Correa, M., Giardiello, F.M., Hutcheon, D.F., Kafonek, D.R., Brandenburg, S., Wu, Y., He, X., Powe, N.R., Feinberg, A.P. (2003) Loss of *IGF2* imprinting: a potential marker of colorectal cancer risk, *Science* **299**, 1753–1755.

Cunningham, J.M., Christensen, E.R., Tester, D.J., Kim, C.Y., Roche, P.C., Burgart, L.J., Thibodeau, S.N. (1998) Hypermethylation of the hMLH1 promoter in colon cancer with microsatellite instability, *Cancer Res.* 58, 3455–3460.

Eden, A., Gaudet, F., Waghmare, A., Jaenisch, R. (2003) Chromosomal instability and tumors promoted by DNA hypomethylation, *Science* **300**, 455.

El-Omar, E.M., Rabkin, C.S., Gammon, Md., Vaughan, T.L., Risch, H.A., Schonberg, J.B., Stanford, J.L., Mayne, S.T., Goedert, J., Blot, W.J., Fraumeni, J.F. Jr., Chow, W.H. (2000) Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms, *Gastroenterology* **124**, 1193–1201.

El-Omar, E.M., Carrington, M., Chow, W.H., McColl, K.E., Bream, J.H., Young, H.A., Herrera, J., Lissowska, J., Yuan, C.C., Rothman, N., Lanyon, G., Martin, M., Fraumeni, J.F. Jr., Rabkin, C.S. (2000) Interleukin-1 polymorphisms associated with increased risk of gastric cancer, *Nature* **404**, 398–402.

Eppert, K., Scherer, S.W., Ozcelik, H., Pirone, R., Hoodless, P., Kim, H., Tsui, L.C., Bapat, B., Gallinger, S., Andrulis, I.L., Thomsen, G.H., Wrana, J.L., Attisano, L. (1996) *MADR2* maps to 18q21 and encodes a TGFβ-regulated MAD-related protein that is functionally mutated in colorectal carcinoma, *Cell* **86**, 543–552.

Fearon, E.R., Vogelstein, B. (1990) A genetic model for colorectal tumorigenesis, *Cell* **61**, 759–767.

Feilchenfeldt, J., Brundler, M.A., Soravia, C., Totsch, M., Meier, C.A. (2004) Peroxisome proliferator-activated receptors (PPARs) and associated transcription factors in colon cancer: reduced expression of PPARgammacoactivator 1 (PGC-1), *Cancer Lett.* **203**, 25–33.

Feinberg, A., Tycho, B. (2004) The history of cancer epigenetics, *Nat. Rev. Cancer* 4, 143–153.

Goto, T., Haruma, K., Kitadai, Y., Ito, M., Yoshimura, M., Sumii, K., Hayakawa, N.,

Kajiyama, G. (1999) Enhanced expression of inducible nitric oxide synthase and nitrotyrosine in gastric mucosa of gastric cancer patients, *Clin. Cancer Res.* 5, 1411–1415.

Guilford, P.J., Hopkins, J.B., Grady, W.M., Markowitz, S.D., Willis, J., Lynch, H., Rajput, A., Wiesner, G.L., Lindor, N.M., Burgart, L.J., Toro, T.T., Lee, D., Limacher, J.M., Shaw, D.W., Findlay, M.P., Reeve, A.E. (1999) E-cadherin germline mutations define an inherited cancer syndrome dominated by diffuse gastric cancer, *Hum. Mutat.* 14, 249–255.

Harman, F.S., Nicol, C.J., Marin, H.E., Ward, J.M., Gonzalez, F.J., Peters, J.M. (2004) Peroxisome proliferator-activated receptor-delta attenuates colon carcinogenesis, *Nat. Med.* 10, 481–483.

Hayashi, K., Goodison, S., Urquidi, V., Tarin, D., Lotan, R., Tahara, E. (2003) Differential effects of retinoic acid on the growth of isogenic metastatic and non-metastatic breast cancer cell lines and their association with distinct expression of retinoic acid receptor β isoforms 2 and 4, *Int. J. Oncol.* 22, 62–629.

Hayashi, K., Yokozaki, H., Naka, K., Yasui, W., Lotan, R., Tahara, E. (2001) Overexpression of retinoic acid receptor β induces growth arrest and apoptosis in oral cancer cell lines, *Jpn. J. Cancer Res.* 92, 42–50.

Hayashi, K., Yokozaki, H., Goodison, S., Oue, N., Suzuki, T., Lotan, R., Yasui, W., Tahara, E. (2001) Inactivation of retinoic acid receptor β by promoter CpG hypermethylation in gastric cancer, *Differentiation* 68, 13–21.

He, T.C., Chan, T.A., Vogelstein, B., Kinzler, K.W. (1999) PPARδ is an APC-regulated target nonsteroidal anti-inflammatory drugs, *Cell* 99, 335–345.

Higashi, H., Tsutsumi, R., Fujita, A., Yamazaki, S., Asaka, M., Azuma, T., Hatakeyama, M. (2002) Biological activity of the *Helicobacter pylori* virulence factor CagA is determined by variation in the tyrosine phosphorylations site, *PNAS* 99, 14428–14433.

Hollstein, M., Sidransky, D., Vogelstein, B., Harris, C.C. (1991) *p53* mutations in human cancers, *Science* 253, 49–53.

Hollstein, M.C., Metcalf, R.A., Welsh, J.A., Montesano, R., Harris, C.C. (1990) Frequent mutation of the *p53* gene in human esophageal cancer, *Proc. Natl. Acad. Sci. USA* 87, 9958–9961.

Huang, J.Q., Sridhar, S., Chen, Y., Hunt, R.H. (1998) Meta-analysis of the relationship between *Helicobacter pylori* seropositivity and gastric cancer, *Gastroenterology* 114, 1169–1179.

Imoto, I., Yuki, Y., Sonoda, I., Ito, T., Shimada, Y., Imamura, M., Inazawa, J. (2003) Identification of *ZASC1* encoding a Krüppel-like zinc finger protein as a novel target for 3q26 amplification in esophageal squamous cell carcinomas, *Cancer Res.* 63, 5691–5696.

Imoto, I., Yang, Z.Q., Pimkhaokham, A., Tsuda, H., Shimada, Y., Imamura, M., Ohki, M., Inazawa, J. (2001) Identification of *cIAP1* as a candidate target gene within an amplicon at 11q22 in esophageal squamous cell carcinomas, *Cancer Res.* 61, 6629–6634.

Ionov, Y.M., Peinado, A., Malkhosyan, S., Shibata, D., Perucho, M. (1993) Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis, *Nature* 363, 558–561.

Irby, R.B., Mao, W., Coppola, D., Kang, J., Loubeau, J.M., Trudeau, W., Karl, R., Fujita, D.J., Jove, R., Yeatman, T.J. (1999) Activating SRC mutation in a subset of advanced human colon cancers, *Nat. Genet.* 21, 187–190.

Ito, Y. (2004) Oncogenic potential of the RUNX gene family: 'overview', *Oncogene* 23, 4198–4208.

Jiang, W., Zhang, Y.J., Kahn, S.M., Hollstein, M.C., Santella, R.M., Lu, S.H., Harris, C.C., Montesano, R., Weinstein, I.B. (1993) Altered expression of the cyclin D1 and retinoblastoma genes in human esophageal cancer, *Proc. Natl. Acad. Sci. USA* 90, 9026–9030.

Kaplan, K.B., Burds, A.A., Swedlow, J.R., Bekir, S.S., Sorger, P.K., Nathke, I.S. (2001) A role for the adenomatous polyposis coli protein chromosome segregation, *Nat. Cell. Biol.*. 3, 429–432.

Karin, M., Cao, Y., Greten, F.R., Li, Z.W. (2002) NF-κB in cancer: from innocent bystander to major culprit, *Nat. Rev. Cancer* 2, 301–310.

Kato, J.Y., Matsushime, H., Heibert, S.W., Ewen, M.E., Sherr, C.J. (1993) Direct binding of cyclin D to the retinoblastoma gene product (pRb) and pRb phosphorylation by the cyclin D-dependent kinase, CDK4, *Gene Dev.* 7, 331–342.

Kim, T.Y., Lee, H.J., Hwang, K.S., Lee, M., Kim, J.W., Bang, Y.J., Kang, G.H. (2004) Methylation of RUNX3 in various types of

human cancers and premalignant stages of gastric carcinoma, *Lab. Invest.* 84, 479–484.

Kinzler, K.W., Vogelstein, B. (1996) Lessons from hereditary colorectal cancer, *Cell* 87, 159–170.

Kitahara, K., Yasui, W., Yokozaki, H., Semba, S., Hamamoto, T., Hisatsugu, T., Tahara, E. (1996) Expression of cyclin D1, CDK4 and $p27^{KIP1}$ is associated with the $p16^{MTS1}$ gene status in human esophageal carcinoma cell lines, *J. Exp. Ther. Oncol.* 1, 7–12.

Kuniyasu, H., Yoshida, K., Yokozaki, H., Yasui, W., Ito, H., Toge, T., Ciardiello, F., Persico, M.G., Saeki, T., Salomon, D.S., Tahara, E. (1991) Expression of *cripto*, a novel gene of the epidermal growth factor family, in human gastrointestinal carcinomas, *Jpn. J. Cancer Res.* 82, 969–973.

Levine, A.J. (1997) p53, the cellular gatekeeper for growth and division, *Cell* 88, 323–331.

Li, M., Song, S., Lippman, S.M., Zhang, X.K., Liu, X., Lotan, R., Xu, X.C. (2002) Induction of retinoic acid receptor-beta suppresses cyclooxygenase-2 expression in esophageal cancer cells, *Oncogene* 21, 411–418.

Li, Q.L., Ito, K., Sakakura, C., Fukamachi, H., Inoue, K., Chi, X.Z., Lee, K.Y., Nomura, S., Lee, C.W., Han, S.B., Kim, H.M., Kim, W.J., Yamamoto, H., Yamashita, N., Yano, T., Ikeda, T., Itohara, S., Inazawa, J., Abe, T., Hagiwara, A., Yamagishi, H., Ooe, A., Kaneda, A., Sugimura, T., Ushijima, T., Bae, S.C., Ito, Y. (2002) Causal relationship between the loss of RUNX3 expression and gastric cancer, *Cell* **109**, 113–124.

Liu, C., Kato, Y., Zhang, Z., Do, V.M., Yankner, B.A., He, X. (1999) β-Trcp couples β-catenin phosphorylation-degradation and regulates *Xenopus* axis formation, *Proc. Natl. Acad. Sci. USA* **96**, 6273–6278.

Lotan, R., Xu, X.C., Lippman, S.M., Ro, J.Y., Lee, J.S., Lee, J.J., Hong, W.K. (1995) Suppression of retinoic acid receptor-beta in premalignant oral lesions and its up-regulation by isotretinoin, *N. Engl. J. Med.* 332, 1405–1410.

Lynch, H.T., Grady, W., Lynch, J.F., Tsuchiya, K.D., Wiesner, G., Markowitz, S.D. (2000) E-cadherin mutations-based genetic counseling and hereditary diffuse gastric carcinoma, *Cancer Genet. Cytogenet.* 122, 1–6.

Lynch, H.T., Smyrk, T., McGinn, T., Lanspa, S., Cavalieri, J., Lynch, J., Slominski-Castor, S., Cayouette, M.C., Priluck, I., Luce, M.C. (1995) Attenuated familial adenomatous polyposis (AFAP). A phenotypically and genotypically distinctive variant of FAP, *Cancer* 76, 2427–2433.

Magnusson, P.K.E., Enroth, H., Eriksson, I., Held, M., Nyren, O., Engstrand, L., Hansson, L.E., Gyllenstem, U.B. (2001) Gastric cancer and human leukocyte antigen, *Cancer Res.* 61, 2684–2689.

Markowitz, S., Wang, J., Myeroff, L., Parsons, R., Sun, L., Lutterbaugh, J., Fan, R.S., Zborowska, E., Kinzler, K.W., Vogelstein, B., Brattain, M., Willson, J.K.V. (1995) Inactivation of the type II TGF-β receptor in colon cancer cells with microsatellite instability, *Science* 268, 1336–1338.

Medico, E., Gentile, A., Celso, C.L., Williams, T.A., Gambarotta, G., Trusolino, L., Comoglio, P.M. (2001) Osteopontin is an autocrine mediator of hepatocyte growth, *Cancer Res.* 61, 5861–5868.

Montesano, R., Hollstein, M., Hainaut, P. (1996) Genetic alterations in esophageal cancer and their relevance to etiology and pathogenesis: a review, *Int. J. Cancer* 69, 225–235.

Mukhopadhyay, D., Tsiokas, L., Zhou, X.M., Foster, D., Brugge, J.S., Sukhatme, V.P. (1995) Hypoxic induction of human vascular endothelial growth factor expression through c-Src activation, *Nature* 375, 577–581.

Myeroff, L.L., Parsons, R., Kim, S.J., Hedrick, L., Cho, K.R., Orth, K., Mathis, M., Kinzler, K.W., Lutterbaugh, J., Park, K., Bang, Y.J., Lee, H.Y., Park, J.G., Lynch, H.T., Roberts, A.B., Vogelstein, B., Markowitz, S.D. (1995) A transforming growth factor β receptor type II gene mutation common in colon and gastric but rare in endometrial cancers with micro-satellite instability, *Cancer Res.* 55, 5545–5547.

Nakagawa, H., Chadwick, R.B., Peltomaki, P., Plass, C., Nakamura, Y., de la Chapelle, A. (2001) Loss of imprinting of the insulin-like growth factor II gene occurs by biallelic methylation in a core region of H19-associated CTCF-binding sites in colorectal cancer, *PNAS* 98, 591–596.

Nakamura, T., Watanabe, M., Yamanaka, M., Hirokawa, Y., Suzuki, H., Ito, H., Yatani, R., Shiraishi, T. (2001) The role of epigenetic modifications in retinoic acid receptor β2 gene expression in human prostate cancers, *Lab. Invest.* 81, 1049–1057.

Nakazawa, K., Yashiro, M., Hirakawa, K. (2003) Keratinocyte growth factor produced by gastric

fibroblasts specifically stimulates proliferation of cancer cells from scirrhous gastric carcinoma, *Cancer Res.* **63**, 8848–8852.

Olschwang, S., Hamelin, R., Laurent-Puig, P., Thuille, B., Rycke, Y.D., Li, Y.J., Muzeau, F., Girodet, J., Salmon, R.J., Thomas, G. (1997) Alternative genetic pathways in colorectal carcinogenesis, *Proc. Natl. Acad. Sci. USA* **94**, 12122–12127.

Parikh, A.A., Fan, F., Liu, W.B., Ahmad, S.A., Stoeltzing, O., Reinmuth, N., Bielenberg, D., Bucana, C.D., Klagsbrun, M., Ellis, L.M. (2004) Neuropilin-1 in human colon cancer: expression, regulation, and role in induction of angiogenesis, *Am. J. Pathol.* **164**, 2139–2151.

Parkin, D.M., Pisani, P., Ferlay, J. (1999) Estimates of the worldwide incidence of 25 major cancers in 1990, *Int. J. Cancer* **80**, 827–841.

Pellman, D. (2001) Cancer. A CINtillating new job for the APC tumor suppressor, *Science* **291**, 2555–2556.

Picard, E., Seguin, C., Monhoven, N., Rochette-Egly, C., Siat, J., Borrelly, J., Martinet, Y., Martinet, N., Vignaud, J.M. (1999) Expression of retinoid receptor genes and proteins in non-small-cell lung cancer, *J. Natl. Cancer Inst.* **91**, 1059–1066.

Prinz, C., Schoniger, M., Rad, R., Becker, I., Keiditsch, E., Wagenpfeil, S., Classen, M., Rosch, T., Schepp, W., Gerhard, M. (2001) Key importance of the Helicobacter pylori adherence factor blood group antigen binding adhesion during chronic gastric inflammation, *Cancer Res.* **61**, 1903–1909.

Qiu, H., Lotan, R., Lippman, S.M., Xu, X.C. (2000) Lack of correlation between expression of retinoic acid receptor-beta and loss of heterozygosity on chromosome band 3p24 in esophageal cancer, *Genes Chromosomes Cancer* **28**, 196–202.

Qiu, H., Zhang, W., El-Naggar, A.K., Lippman, S.M., Lin, P., Lotan, R., Xu, X.C. (1999) Loss of retinoic acid receptor-beta expression is an eary event during esophageal carcinogenesis, *Am. J. Pathol.* **155**, 1519–1523.

Sano, H., Kawahito, Y., Wilder, R.L., Hashiramoto, A., Mukai, S., Asai, K., Kimura, S., Kato, H., Kondo, M., Hla, T. (1995) Expression of cyclooxygenase-1 and -2 in human colorectal cancer, *Cancer Res.* **55**, 3785–3789.

Sato, N., Maitra, A., Fukushima, N., van Heek, N.T., Matsubayashi, H., Iacobuzio-Donahue, C.A., Rosty, C., Goggins, M. (2003) Frequent hypomethylation of multiple genes overexpressed in pancreatic ductal adenocarcinoma, *Cancer Res.* **63**, 4158–4166.

Schlessinger, J. (2000) New roles for Src kinases in control of cell survival and angiogenesis, *Cell* **100**, 293–296.

Serrano, M., Hannon, G.J., Beach, D. (1993) A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4, *Nature* **366**, 704–707.

Shibata, D., Peinado, M.A., Ionov, Y., Malkhosyan, S., Perucho, M. (1994) Genomic instability in repeated sequences is an early somatic event in colorectal tumorigenesis that persists after transformation, *Nat. Genet.* **6**, 273–281.

Shibata, D., Reale, M.A., Lavin, P., Silverman, M., Fearon, E.R., Steele, G. Jr., Jessup, J.M., Loda, M., Summerhayes, I.C. (1996) The DCC protein and prognosis in colorectal cancer, *N. Engl. J. Med.* **335**, 1727–1732.

Shibata, H., Toyama, K., Shioya, H., Ito, M., Hirota, M., Hasegawa, S., Matsumoto, H., Takano, H., Akiyama, T., Toyoshima, K., Kanamaru, R., Kanegae, Y., Saito, I., Nakamura, Y., Shiba, K., Noda, T. (1997) Rapid colorectal adenoma formation initiated by conditional targeting of the *Apc* gene, *Science* **278**, 120–123.

Shimizu, N., Inada, K., Nakanishi, H., Tsukamoto, T., Ikehara, Y., Kaminishi, M., Kuramoto, S., Sugiyama, A., Katsuyama, T., Tatematsu, M. (1999) *Helicobacter pylori* infection enhances glandular stomach carcinogenesis in Mongolian gerbils treated with a chemical carcinogen, *Carcinogenesis* **20**, 669–676.

Shimizu, N., Ikehara, Y., Inada, K., Nakanishi, H., Tsukamoto, T., Nozaki, K., Kaminishi, M., Kuramoto, S., Sugiyama, A., Katsuyama, T., Tatematsu, M. (2000) Eradication diminishes enhancing effects of *Helicobacter pylori* infection on glandular stomach carcinogenesis in Mongolian gerbils, *Cancer Res.* **60**, 1512–1514.

Shinmura, K., Kohno, T., Takahashi, M., Sasaki, A., Ochiai, A., Guilford, P., Hunter, A., Reeve, A.E., Sugimura, H., Yamaguchi, N., Yokota, J. (1999) Familial gastric cancer: clinicopathological characteristics, RER phenotype and germline p53 and E-cadherin mutations, *Carcinogenesis* **20**, 1127–1131.

Shtutman, M., Zhurinsky, J., Sincha, I., Albanese, C., D'Amico, M., Pestell, R., Ben-Ze'ev, A. (1999) The cyclin D1 gene is a target

of the β-catenin/LEF-1 pathway, *Proc. Natl. Acad. Sci. USA* **96**, 5522–5527.

Sonoda, I., Imoto, I., Inoue, J., Shibata, T., Shimada, Y., Chin, K., Imamura, M., Amagasa, T., Gray, J.W., Hirohashi, S., Inazawa, J. (2004) Frequent silencing of *low density lipoprotein receptor-related protein 1B (LRP1B)* expression by genetic and epigenetic mechanisms in esophageal squamous, *Cancer Res.* **64**, 3741–3747.

Souza, R.F. (2002) Molecular and biologic basis of upper gastrointestinal malignancy–esophageal carcinoma, *Surg. Oncol. Clin. N. Am.* **11**, 257–272.

Su, L.K., Vogelstein, B., Kinzler, K.W. (1993) Association of the APC tumor suppressor protein with catenins, *Science* **262**, 1734–1737.

Tahara, E. (1990) Growth factors and oncogenes in human gastrointestinal carcinomas, *J. Cancer Res. Clin. Oncol.* **116**, 121–131.

Tahara, H., Kuniyasu, H., Yokozaki, H., Yasui, W., Shay, J.W., Ide, T., Tahara, E. (1995) Telomerase activity in preneoplastic and neoplastic gastric and colorectal lesions, *Clin. Cancer Res.* **1**, 1245–1251.

Takaguchi, M., Achanzar, W.E., Qu, W., Li, G., Waalkes, M.P. (2003) Effects of cadmium on DNA-(Cytosine-5) methyltransferase activity and DNA methylation status during cadmium-induced cellular transformation, *Exp. Cell Res.* **286**, 355–365.

Takubo, K., Nakamura, K., Izumiyama, N., Mafune, K., Tanaka, Y., Miyashita, M., Sasajima, K., Kato, M., Oshimura, M. (1997) Telomerase activity in esophageal carcinoma, *J. Surg. Oncol.* **66**, 88–92.

Tanaka, S., Mori, M., Akiyoshi, T., Tanaka, Y., Mafune, K., Wands, J.R., Sugimachi, K. (1997) Coexpression of Grb7 with epidermal growth factor receptor or Her2/erbB2 in human advanced esophageal carcinoma, *Cancer Res.* **57**, 28–31.

Termuhlem, P.M., Curley, S.A., Talamonti, M.S., Saboorian, M.H., Gallick, G.E. (1993) Site-specific differences in pp60c-src activity in human colorectal metastases, *J. Surg. Res.* **54**, 293–298.

Thibodeau, S.N., Bren, G., Schaid, D. (1993) Microsatellite instability in cancer of the proximal colon, *Science* **260**, 816–819.

Toyota, M., Ohe-Toyota, M., Ahuja, N., Issa, J.P. (2000) Distinct genetic profiles in colorectal tumors with or without the CpG island methylator phenotype, *Proc. Natl. Acad. Sci. USA* **97**, 710–715.

Tsuda, T., Tahara, E., Kajiyama, G., Sakamoto, H., Terada, M., Sugimura, T. (1989) High incidence of coamplification of *hst*-1 and *int*-2 genes in human esophageal carcinomas, *Cancer Res.* **49**, 5505–5508.

Tsushima, H., Ito, N., Tamura, S., Matsuda, Y., Inada, M., Yabuuchi, I., Imai, Y., Nagashima, R., Misawa, H., Takeda, H., Matsuzawa, Y., Kawata, S. (2001) Circulating transforming growth factor β1 as a predictor of liver metastasis after resection in colorectal cancer, *Clin. Cancer Res.* **7**, 1258–1262.

Ueda, T., Sasaki, H., Kuwahara, Y., Nezu, M., Shibuya, T., Sakamoto, H., Ishii, H., Yanagihara, K., Mafune, K., Makuuchi, M., Terada, M. (1999) Deletion of the carboxylterminal exons of K-sam/FGFR2 by short homology-mediated recombination, generating preferential expression of specific messenger RNAs, *Cancer Res.* **59**, 6080–6086.

Vogelstein, B., Fearon, E.R., Hamilton, S.R., Kern, S.E., Preisinger, A.C., Leppert, M., Nakamura, Y., White, R., Smits, A.M., Bos, J.L. (1988) Genetic alterations during colorectal-tumor development, *N. Engl. J. Med.* **319**, 525–532.

Wan, H., Oridate, N., Lotan, D., Hong, W.K., Lotan, R. (1999) Overexpression of retinoic acid receptor β in head and neck squamous cell carcinoma cells increases their sensitivity to retinoid-induced suppression of squamous differentiation by retinoids, *Cancer Res.* **59**, 3518–3526.

Weinberg, R.A. (1998) Bumps on the road to immortality, *Nature.* **396**, 23–24.

Xu, M., Jin, Y.L., Fu, J., Huang, H., Chen, S.Z., Qu, P., Tian, H.M., Liu, Z.Y., Zhang, W. (2002) The abnormal expression of retinoic acid receptor-beta, p53 and Ki67 protein in normal, premalignant and malignant esophageal tissues, *World J. Gastroenterol.* **8**, 200–202.

Xu, X.C., Sneige, N., Liu, X., Nandagiri, R., Lee, J.J., Lukmanji, F., Hortobagyi, G., Lippman, S.M., Dhingra, K., Lotan, R. (1997) Progressive decrease in nuclear retinoic acid receptor bet messenger RNA level during breast carcinogenesis, *Cancer Res.* **57**, 4992–4996.

Yamasaki, R., Yokota, K., Okada, H., Hayashi, S., Mizuno, M., Yoshino, T., Hirai, Y., Saitou, D.,

Akagi, T., Oguma, K. (2004) Immune response in *Helicobacter pylori*-induced low-grade gastric-mucosa-associated lymphoid tissue (MALT) lymphoma, *J. Med. Microbiol.* 53, 21–29.

Yasui, W., Yokozaki, H., Shimamoto, F., Tahara, H., Tahara, E. (1999) Molecular-pathological diagnosis of gastrointestinal tissues and its contribution to cancer histopathology, *Pathol. Int.* 49, 763–774.

Yasui, W., Kuniyasu, H., Yokozaki, H., Semba, S., Shimamoto, F., Tahara, E. (1996) Expression of cyclin E in colorectal adenomas and adenocarcinomas: correlation with expression of Ki-67 and p53 protein, *Virchows Arch.* 429, 13–19.

Yonemura, Y., Endo, Y., Fujita, H., Fushida, S., Ninomiya, I., Bandou, E., Taniguchi, K., Miwa, K., Ohoyama, S., Sugiyama, K., Sasaki, T. (1999) Role of vascular endothelial growth factor C expression in the development of lymph node metastasis in gastric cancer, *Clin. Cancer Res.* 5, 1823–1829.

Yu, H.P., Wu, S.Q., Lu, W.H., Li, Y.Y., Li, F., Wang, X.L., Su, Y.H. (2004) Telomerase activity and expression of telomerase genes in squamous dysplasia and squamous cell carcinoma of the esophagus, *J. Surg. Oncol.* 86, 99–104.

Harvesting Chemical Energy: *see* Metabolic Basis of Cellular Energy

Mechanisms of Carcinogenesis: Contributions of Molecular Epidemiology
Patricia Buffler, Jerry Rice, Robert Baan, Michael Bird and Paolo Boffetta, eds
IARC Scientific Publications No. 157
International Agency for Research on Cancer, Lyon, 2004

Genetic Pathways of Two Types of Gastric Cancer

Eiichi Tahara

Summary

Multiple genetic and epigenetic alterations in oncogenes, tumour-suppressor genes, cell-cycle regulators, cell adhesion molecules, DNA repair genes and genetic instability as well as telomerase activation are implicated in the multistep process of human stomach carcinogenesis. However, particular combinations of these alterations differ in the two histological types of gastric cancer, indicating that well-differentiated or intestinal-type and poorly differentiated or diffuse-type carcinomas have distinct carcinogenetic pathways. In the multistep process of well-differentiated-type carcinogenesis, the genetic pathway can be divided into three subpathways: an intestinal metaplasia→adenoma→carcinoma sequence, an intestinal metaplasia→carcinoma sequence and *de novo*. In the multistep process of well-differentiated-type or intestinal-type gastric carcinogenesis, infection with *Helicobacter pylori* may be a strong trigger for hyperplasia of hTERT-positive 'stem cells' in intestinal metaplasia. Genetic instability and hyperplasia of hTERT-positive stem cells precede replication error at the D1S191 locus, DNA hypermethylation at the D17S5 locus, *pS2* loss, *RARβ* loss, *CD44* abnormal transcripts and *p53* mutation, all of which accumulate in at least 30% of incomplete intestinal metaplasias. All of these epigenetic and genetic alterations are common events in intestinal-type gastric cancer. An adenoma→carcinoma sequence is found in about 20% of gastric adenomas with *APC* mutations. In addition to these events, *p53* mutation and loss of heterozygosity (LOH), reduced *p27* expression, *cyclin E* expression and the presence of c-*met* 6.0-kb transcripts allow malignant transformation from the above precancerous lesions to intestinal-type gastric cancer. *DCC* loss, *APC* mutations, 1q LOH, *p27* loss, reduced tumour growth factor (TGF)-β type I receptor expression, reduced *nm23* expression and c-*erb*B gene amplification are frequently associated with an advanced stage of intestinal-type gastric cancer. The de-novo pathway for carcinogenesis of well-differentiated gastric cancer involves LOH and abnormal expression of the *p73* gene that is responsible for the development of foveolar-type gastric cancers with pS2 expression.

On the other hand, LOH at chromosome 17p, mutation or LOH of *p53* and mutation or loss of E-cadherin are preferentially involved in the development of poorly differentiated gastric cancers. In addition to these changes, gene amplification of K-*sam*, and c-*met* and *p27* loss as well as reduced nm23 obviously confer progression, metastasis and diffusely productive fibrosis. Mixed gastric carcinomas composed of well-differentiated and poorly differentiated components exhibit some but not all of the molecular events described so far for each of the two types of gastric cancer.

Besides these genetic and epigenetic events, well-differentiated and poorly differentiated gastric cancers also organize different patterns of interplay between cancer cells and stromal cells through the growth factor/cytokine receptor system, which plays an important role in cell growth, apoptosis, morphogenesis, angiogenesis, progression and metastasis.

Meta-analysis of epidemiological studies and animal models show that both intestinal and diffuse types of gastric cancer are equally associated with *H. pylori* infection. However, *H. pylori* infection may play a role only in the initial steps of gastric carcinogenesis. Differences in *H. pylori* strain, patient age, exogenous or endogenous carcinogens and genetic factors such

as DNA polymorphism and genetic instability may be implicated in two distinct major genetic pathways for gastric carcinogenesis.

Introduction

Striking advances in molecular dissection of precancerous and cancerous lesions of the stomach indicate that genetic and epigenetic alterations in oncogenes, tumour-suppressor genes, DNA-repair genes, cell-cycle regulators, telomeres and telomerase, as well as genetic instability at microsatellite foci are involved in the multistep process of human stomach carcinogenesis (Sano *et al.*, 1991; Tahara, 1993; Tahara *et al.*, 1996a).

There are several histological classifications of gastric cancer. Lauren (1965) divided gastric cancer into two types, intestinal and diffuse, and the Japan Research Society for Gastric Cancer (JRSGC, 1999) classified it into five common types. The JRSGC classification is similar to that of the World Health Organization (Hamilton & Aaltonen, 2000). In this chapter, we use a two-type classification: the intestinal or well-differentiated type (which includes the papillary and tubular adenocarcinomas of the JRSGC classification), and the diffuse or poorly differentiated type (which includes the diffuse and signet-ring cell carcinomas of the JRSGC classification).

The genetic and epigenetic changes found in gastric carcinoma differ, depending upon the histological type of gastric cancer, indicating that different carcinogenetic pathways exist for intestinal and diffuse types of carcinomas (Table 1; Figures 1 and 2). In addition, cancer–stromal interaction through the growth factor/cytokine receptor system, which plays a pivotal role in morphogenesis, cancer progression and metastasis, is also much different between the two types of gastric carcinoma (Tahara *et al.*, 1993, 1994).

This chapter provides a detailed overview of the molecular machinery that underlies stomach carcinogenesis.

Oncogenes

Several proto-oncogenes, including c-*met*, K-*sam* and c-*erb*B2, are frequently activated in gastric carcinomas. The amplification of the c-*met* gene encoding a receptor for hepatocyte growth factor/scatter factor is found in 19% of intestinal and 39% of diffuse gastric cancers, frequently accompanied by diffusely productive fibrosis of the scirrhous type (Kuniyasu *et al.*, 1992). Most gastric carcinomas express two different c-*met* transcripts, one of 7.0 kb and the other of 6.0 kb. Expression of the 6.0-kb c-*met* transcript, which is expressed preferentially in cancer cells, correlates well with tumour staging, lymph node metastasis and depth of tumour invasion (Kuniyasu *et al.*, 1993). Soman *et al.* (1991) reported that the *tpr-met* rearrangement is expressed in gastric carcinomas and gastric precancerous lesions. However, we have not detected the *tpr-met* rearrangement in any gastric cancer or intestinal metaplasia.

The K-*sam* (KATO-III cell-derived stomach cancer amplified) gene has at least four transcriptional variants. Type II encodes a receptor for keratinocyte growth factor (Katoh *et al.*, 1992). Type II transcript is expressed only in carcinoma cells (not in cell lines from sarcomas). K-*sam* is preferentially amplified in 33% of advanced diffuse or scirrhous-type gastric carcinomas, but not in intestinal-type gastric carcinomas (Hattori *et al.*, 1990). Moreover, K-*sam* is never seen in esophageal or colorectal carcinomas. Gastric cancers that overexpress K-sam protein are associated with a less favourable prognosis.

In contrast to K-*sam*, c-*erb*B2 is preferentially amplified in 20% of intestinal gastric cancers but not in diffuse-type gastric cancer (Yokota *et al.*, 1988; Kameda *et al.*, 1990). Overexpression of c-*erb*B2 associated with gene amplification is closely correlated with a poor prognosis and liver metastasis (Oda *et al.*, 1990; Yonemura *et al.*, 1991). The amplification of c-*erb*B1 and c-*erb*B3 is found in 3% (Kameda *et al.*, 1990) and 0% (Katoh & Terada, 1993), respectively, of gastric cancers.

K-*ras* mutation is found in gastric intestinal metaplasias, adenomas and intestinal-type adenocarcinomas (Sano *et al.*, 1991; Lee *et al.*, 1995; Isogaki *et al.*, 1999), although its incidence is low (10–18%). However, K-*ras* mutation is not seen in diffuse-type gastric cancer. The *hst*-1 gene, isolated from a surgical specimen of human gastric cancer by the NIH/3T3 transformation assay, is rarely amplified in gastric cancer (2% of cases) (Yoshida *et al.*, 1988).

Table 1. Genetic and epigenetic alterations found in two types of gastric cancer

Genetic and epigenetic alterations	Incidence of cases with indicated alterations (%)	
	Well-differentiated[a]	Poorly differentiated[a]
Tumour suppressors		
p53 LOH, mutation	60	75
p73 LOH	53[b]	24
APC LOH, mutation	40–60	0
DCC LOH	50	0
LOH of chromosome 1q	44	0
LOH of chromosome 7q	53	33
LOH of chromosome 17q	0	40[c]
Loss of *pS2* expression	49	31
Loss of *RARβ*	64	0
Cell-cycle regulators		
Cyclin E amplification	33	7
Cyclin E overexpression	26	27
CDC25B overexpression	33	73
Loss of p16 expression	12	31
Loss of p27 expression	46	69
Oncogenes		
K-*ras* mutation	10	0
c-*met* amplification	19	39
K-*sam* amplification	0	33
c-*erbB2* amplification	20	0
Adhesion molecules		
E-cadherin mutation/loss	0	50
CD44 aberrant transcript	100	100
Microsatellite instability	20–40	20–70[c]
Histone deacetylation	61	82
Telomere/telomerase		
Telomere reduction	62	53
Telomerase activity	100	90
TERT expression	100	86

[a] According to the criteria of the JRSGC classification of gastric cancer
[b] Preferentially found in foveolar-type adenocarcinoma
[c] Preferentially found in patients younger than 35 years of age
LOH, loss of heterozygosity