esophageal SCC, accompanied by overexpression of EGFR. The frequency of K-ras mutation is very low in esophageal SCC, whereas it takes place in 50% of sporadic colorectal carcinoma. c-*erb*B2 is amplified in esophageal adenocarcinoma but not in esophageal SCC. Recently, Inazawa's group reported that *ZASC1* encoding a Krüppel-like zinc finger protein is involved in the pathogenesis of esophageal SCC as one of the targets for 3q26 amplification. *CIAP1*, a number of the *IAP* (antiapoptotic) gene family, may also be a target for 11q22 amplification.

Telomerase, a ribonucleoprotein enzyme, is necessary for cancer cells to maintain their telomere and to become immortal. Results of a 1998 study on cell immortalization show, however, that activation of telomerase alone is not enough to immortalize certain epithelial cells, and that inactivation of the p16/Rb pathway is needed. More than 80% of gastrointestinal carcinomas exhibit high level of telomerase activity and overexpression of human telomerase reverse transcriptase (hTERT). The expression of hTERT is closely associated with activation of telomerase *in vitro* and *in vivo*. It is of interest to note that telomerase activity as well as hTERT expression is detected in about 45% of dysplasia and in 90% of SCC of the esophagus. Telomerase activation may also play a critical role in early stage of esophageal SCC.

Recently, Chen et al. reported that LOH at 13q 33–34 including *ING1*, a candidate tumor-suppressor gene, was observed in about 60% of esophageal SCC, associated with mutation as well as loss of ING1 protein. ING1, a novel growth inhibitor, cooperates directly with p53 in growth regulation by modulating the ability of p53 to act as a transcriptional activator. Genetic or epigenetic alterations in ING1 may be also involved with esophageal SCC. Sonoda et al. reported that loss of *LRP1B* (low density lipoprotein receptor–related protein 1*B*) often occurs in esophageal SCC.

These results overall indicate that accumulation of the above-mentioned genetic and epigenetic alterations is involved in the multistep carcinogenesis and progression of esophageal SCC. Inactivation of tumor-suppressor genes on 3p (ex. RARβ2) and p53, and telomerase activity may be important for converting normal stratified squamous epithelium to dysplasia. Because p16 inactivation and 9q LOH are found occasionally in mild dysplasia, but frequently in severe dysplasia and in carcinoma *in situ*, these alterations may have implications for transformation to malignancy. Amplification of cyclin D1and *EGFR* genes, inactivation of tumor-suppressor genes on 5q, 13q, and 18q, and abnormal expression of growth factor/cytokine receptor system may confer progression and metastasis of esophageal SCC. The genetic progression model of esophageal SCC (Fig. 1) is quite similar to that of head and neck SCC

2.2 Abnormal Growth Factor/Cytokine Network in Esophageal SCC

Esophageal SCC cells express a variety of growth factor/ receptor and cytokines including the EGF family, PDGF, transforming growth factor β (TGFβ), interleukin (IL)-1α and IL-6. Among them, the EGF/TGFα receptor system plays a major role in the cell growth and progression of esophageal SCC through signaling of receptor-linked tyrosine kinases.

In many cancer cells, both EGF and TGFα act as autocrine growth factors through EGFR which is encoded by the proto-oncogene *c-erbB1*.

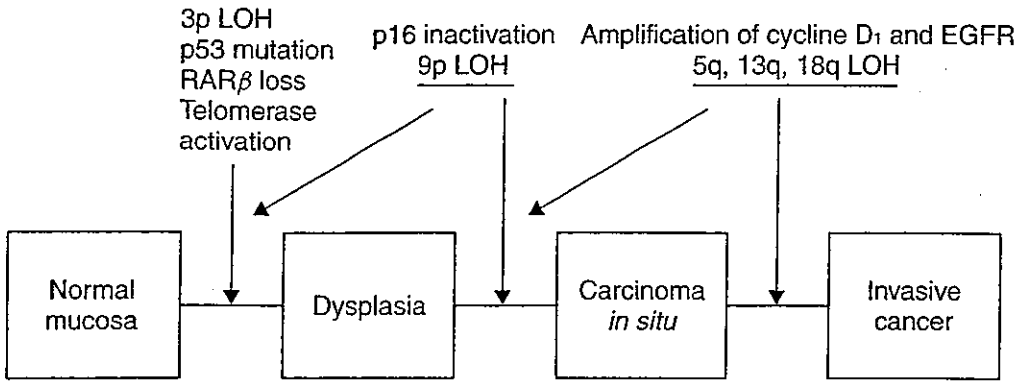

Fig. 1 Genetic progression model of esophageal SCC.

Membrane-bound TGFα binds to EGFR on the surface of contiguous cells and induces receptor autophosphorylation, leading to signal transduction, which is regarded as juxtacrine mitogenetic stimulation. In addition, EGF or TGFα induce the expression of TGFα and EGFR, thus indicating the presence of an autocrine loop of EGF/TGFα/receptor system. We have found that EGF is expressed in 30% of esophageal SCC while TGFα and EGFR are expressed in 77 and 89% of esophageal SCC, respectively. Coexpression of TGFα and EGFR is closely correlated with dysplastic progression and high grade of malignancy. The number of EGFR receptors is about 10 times higher than that in gastric carcinomas, which might be one of the reasons for the rapid growth of esophageal SCC compared to that of other carcinomas.

Esophageal SCC expresses PDGF A- and B-chains and PDGF B-receptor, suggesting the existence of a multiautocrine loop in the growth and progression of tumor cells. What is interesting is that EGF or TGFα increase the expression of PDGF A- and B-chain in esophageal SCC cell lines.

Grb7, a ligand for both EGFR and c-*erb*B2, is also implicated in esophageal SCC. Tanaka et al. found overexpression of Grb7in 44 % of the tumors and coexpression of Grb7 with EGFR or c-*erb*B-2 in 32% of the advanced cases, suggesting that Grb7 functions as an extracellular stimulus for progression of esophageal SCC.

EGF or TGFα produced by the tumor cells can make not only an autocrine loop of EGF/TGFα receptor system for tumor growth stimulation but also an induction of matrix metalloproteinases such as intestinal collagenase and stromelysin to evoke a cascade of cellular events that are involved in extracellular matrix degradation and tumor invasion. Moreover, one of the important substrates of EGFR kinase is β-catenin, a regulatory protein for cadherin. Upon phosphorylation of β-catenin, the cells are dissociated by loss of cadherin function. A phosphorylated form of β-catenin is detected in tumor cells of esophageal SCC and adenocarcinomas. β-catenin is also involved as a downstream transcriptional activator of the Wnt signaling pathway. Recently, β-catenin has been shown to induce cyclin D1 expression, suggesting that free β-catenin may be implicated in an increase in cell cycling.

In addition to phosphorylation of β-catenin, downregulation of E-cadherin and α-catenin occurs in both esophageal SCC and adenocarcinomas, while upregulation of P-cadherin takes place in

esophageal SCC. The reduced expression of α-catenin correlates more with invasion and nodal metastasis than E-cadherin reduction in esophageal SCC.

Above all, esophageal SCC exhibit multiple autocrine growth factor-receptor loops that may participate not only in cell growth but also in tumor invasion and metastasis, associated with reduction of cell adhesion molecules. Understanding the biology of esophageal cancer is indispensable to precise diagnosis and proper cancer treatment.

3 Genetic and Epigenetic Alterations and Abnormal Growth Factor/Cytokine Network in Gastric Cancer

Gastric cancer is the most common cancer worldwide and is second only to lung cancer as a cause of cancer mortality. Most recent world estimates indicate that 798 000 new cases are diagnosed and 628 000 deaths occur annually from gastric cancer. The highest incidence is still observed in Japan because of the remarkable increase in the aged population over 60 years old. Most gastric cancers arise distally from the antrum and pylorus, but about 20% involve the cardia and fundus and approximately 10% involve the stomach diffusely.

There are several histological classifications of gastric cancer. Lauren divided gastric cancer into two types, intestinal and diffuse, and the Japan Research Society for Gastric Cancer classified it into five common types. The JRSGC classification is similar to that of the World Health Organization. This article will use a two-type classification: the intestinal or well-differentiated type and the diffuse or poorly differentiated type.

The genetic and epigenetic changes found in gastric carcinoma differ depending on the histological type of gastric cancer, indicating that different carcinogenetic pathways exist for the intestinal and diffuse types of carcinomas. 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 different between the two types of gastric carcinomas.

Meta-analysis of epidemiological studies and animal models show that both intestinal and diffuse types of gastric cancer are equally associated with *Helicobacter pylori* (*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.

The next paragraph will describe the genetic pathways of the two types of gastric cancer and the abnormal growth factor/cytokine network that organizes remarkably complex interplay between cancer cells and stroma cells in gastric cancer.

3.1 Genetic and Epigenetic Alterations in Gastric Cancer

Genetic and epigenetic alterations in oncogenes, tumor-suppressor genes, cell-cycle regulators, cell adhesion molecules, DNA repair genes, and genetic instability as well as telomerase activation, are responsible for tumor genesis and progression of gastric cancer (Tables 2 and 3; Figs. 2 and 3). Among them, inactivation of various genes including *p16*, *hMLH1*, cadherin1(*CDH1*),

Tab. 2 Genetic and epigenetic alterations found in intestinal and diffuse types of gastric cancer (1).

Genetic and epigenetic alterations	*Incidence of cases with indicated alterations [%]*	
	Intestinal type	*Diffuse type*
Tumor suppressors		
p53 LOH, mutation	60	75
p73 LOH	53[a]	24
APC LOH, mutation	40–60	0
DCC LOH	50	0
LOH of Chr. 1q	44	0
LOH of Chr. 7q	53	33
LOH of Chr. 17q	0	40[b]
Loss of pS2 expression	49	31
Loss of RARβ2	50	73[c]
Loss of RUNX3	37	40
Cell cycle regulators		
Cyclin E amplification	33	7
Cyclin E overexpression	26	27
CDC25B overexpression	33	73
Loss of p16 expression	50[d]	10
Loss of p27 expression	46	69

[a] Preferentially found in foveolar-type adenocarcinoma.
[b] Preferentially found in patients younger than 35 years of age.
[c] $p = 0.0335$; Fisher's exact test.
[d] $p = 0.0023$; Fisher's exact test.

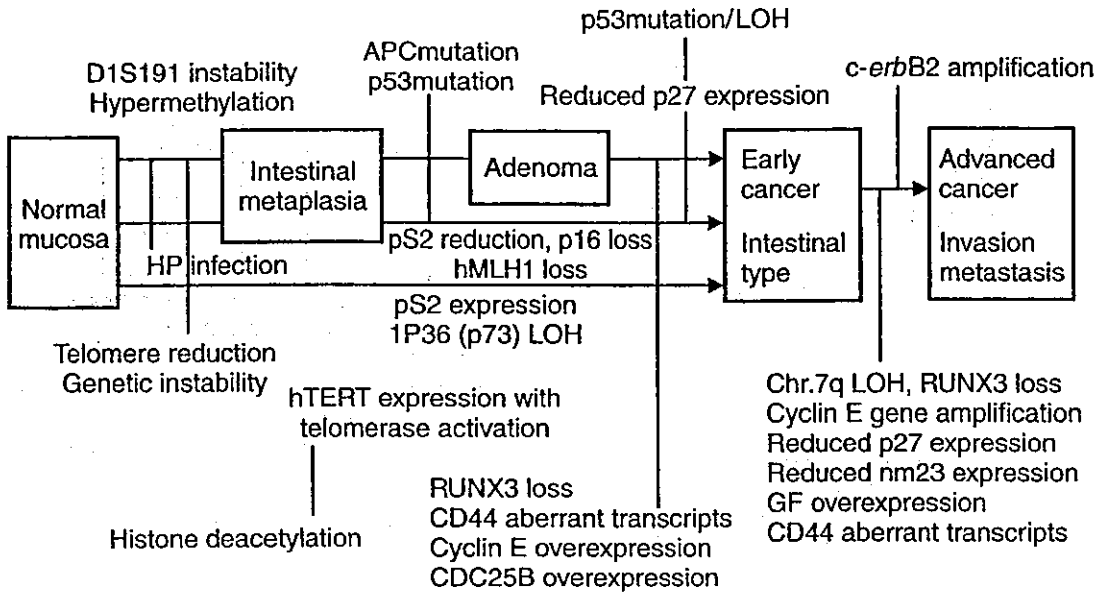

Fig. 2 Multiple genetic and epigenetic alterations during human gastric carcinogenesis (intestinal type).

Tab. 3 Genetic and epigenetic alterations found in intestinal and diffuse types of gastric cancer (2).

Genetic and epigenetic alterations	*Incidence of cases with indicated alterations [%]*	
	Intestinal type	*Diffuse type*
Oncogenes		
K-ras mutation	10	0
c-met amplification	19	39
K-sam amplification	0	33
c-*erb*B2 amplification	20	0
Adhesion molecules		
E-cadherin, mutation	0	50
Loss of CDH1	55	79[b]
CD44 aberrant transcript	100	100
Microsatellite instability (hMLH1 methylation)	20–40 (5–20)	20–70[a] (0)
Histone deacetylation	61	82
Telomere/Telomerase		
Telomere reduction	62	53
Telomerase activity	100	90
TERT expression	100	86

[a] Preferentially found in patients younger than 35 years of age.
[b] $p = 0.0175$; Fisher's exact test.

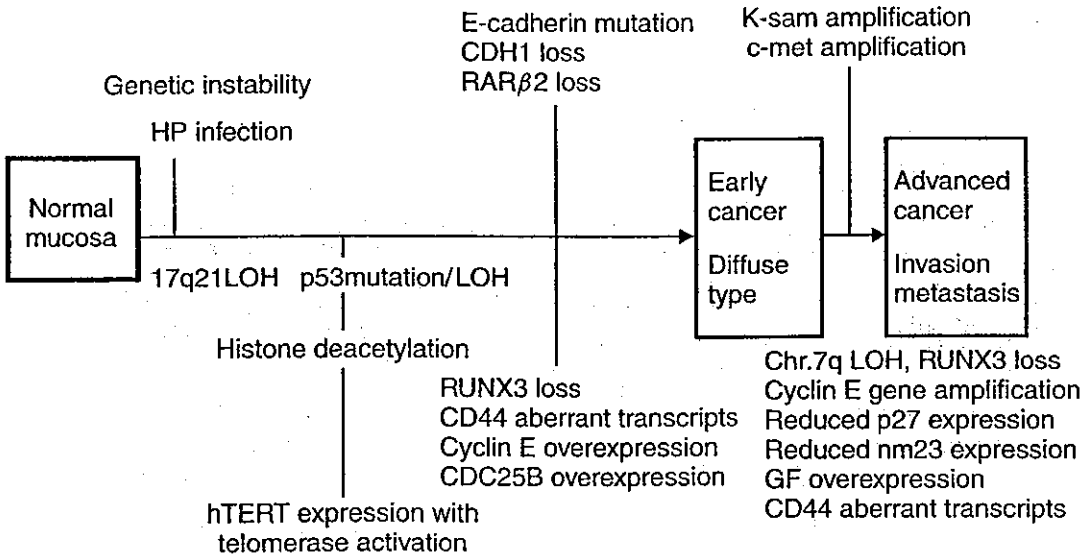

Fig. 3 Multiple genetic and epigenetic alterations during human gastric carcinogenesis (diffuse type).

RARβ2, *pS2* and *RUNX3* by DNA methylation is involved in two distinct major genetic pathways of gastric cancer. Hypermethylation of the p16 and of hMLH1 promoters is preferentially associated with intestinal type, whereas concordant hypermethylation of the CDH1 and RARβ2 promoters predominantly occurs in diffuse type gastric carcinoma. Loss of RUNX3 and pS2 expression by promoter methylation is a common event in both types of gastric carcinoma. The scenario of these epigenetic alterations indicates that there are at least two types of CpG island methylator phenotypes in intestinal and diffuse types of gastric cancer. Recently, Cho et al. reported that promoter hypomethylation of a novel cancer/testis antigen gene CAGE was found in 35% of chronic gastritis and in 78% of gastric cancer.

In addition to promoter hypermethylation or hypomethylation, acetylated histone H4 is obviously reduced in the majority of gastric carcinoma. Histone H4 is progressively deacetylated from the early stage (precancerous lesions) to the late stage (invasion and metastasis) in gastric carcinogenesis. Since there is no difference in the level of acetylated histone H4 between the intestinal and diffuse types of gastric cancer, histone H4 deacetylation is a common event in both types of gastric cancer.

In the multistep process of intestinal type carcinogenesis, the genetic pathways can be divided into three subways: an intestinal metaplasia → adenoma → carcinoma sequence, an intestinal metaplasia → carcinoma sequence and *de novo*. Infection with *H. pylori* may be a strong trigger for hyperplasia of hTERT-positive "stem cell" in intestinal metaplasia. Genetic instability and hyperplasia of hTERT positive stem cells precede replication error at the D1S191, DNA hypermethylation at the D17S5 locus, pS2 loss, RARβ2 loss, RUNX3 loss, CD44 abnormal transcripts and p53 mutation, all of which accumulate in at least 30% of incomplete intestinal metaplasia. All of these epigenetic and genetic changes are common events in intestinal type gastric cancer. An adenoma → carcinoma sequence is found in about 20% of gastric adenoma with APC mutations. In addition to these events, p53 mutation and LOH, RUNX3 loss, reduced p27 expression, cyclin E overexpression and abnormal transcript of c-met allow malignant transformation from the above precancerous lesions to intestinal type gastric carcinoma (Fig. 2). DCC loss, APC mutations, 1qLOH, p27 loss, reduced TGFβ receptor, reduced *nm23* and *c-erbB2* gene amplification are frequently associated with an advanced stage of intestinal-type gastric carcinoma. The *de novo* gastric carcinoma involves LOH and abnormal expression of *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, LUNX3 loss, and mutation or loss of E-cadherin are preferentially involved in the development of diffuse type gastric carcinoma. These events may occur simultaneously or in the relatively short term in superficial gastritis induced by *H. pylori* infection. In addition to these alterations, gene amplification of *K-sam* and *c-met*, RUNX3 loss, 7q LOH, cyclin E gene amplification, p27 loss as well as reduced nm23 confer progression, and metastasis, frequently associated with productive fibrosis. Mixed gastric carcinomas composed of intestinal and diffuse components exhibit some but not all of the molecular events described

so far for each of the two types of gastric cancer (Fig. 3).

Several proto-oncogenes, including *c-met*, *K-sam*, and *c-erbB2*, are frequently activated in gastric cancer. The amplification of the *c-met* gene encoding a receptor for hepatocyte growth factor/scatter factor (HGF/SF) is found in 19% of intestinal type and 39% of diffuse type gastric cancers. Most of gastric carcinomas express two different c-met transcripts, one of 7.0 kb and another of 6.0 kb. Expression of the 6.0 kb c-met transcript correlates well with tumor staging, lymph node metastasis, and depth of tumor invasion. The *K-sam* gene has at least four transcriptional variants. Type II transcript, which is expressed only in carcinoma cells, encodes a receptor for keratinocyte growth factor (KGF). *K-sam* is preferentially amplified in 33% of advanced diffuse or scirrhous type gastric cancer. But *K-sam* gene amplification is never seen in esophageal or colorectal carcinomas. In contrast to *K-sam*, *c-erbB2* is preferentially amplified in 20% of intestinal type gastric cancer but not in diffuse type gastric cancer. Overexpression of *c-erbB2* associated with gene amplification confers a poor prognosis and liver metastasis. The amplification of *c-erbB1* (EGFR) and *c-erbB3* is seen in 3 and 0%, respectively, of gastric cancer.

RUNX3, a Runt domain transcription factor involved in TGFβ signaling, is a candidate tumor-suppressor gene localized in 1p36, a region commonly deleted in a variety of human cancers, including gastric cancer. *RUNX* gene family is composed of three members, *RUNX1/AML1*, *RUNX2* and *RUNX3*, and encodes the DNA binding(α) subunits of the Runt domain transcription factor polyomavirus enhancer-binding protein 2 (PEBP2)/core-binding factor (CBF), which is a heterodimeric transcription factor. All three *RUNXs* play important roles in both normal developmental processes and carcinogenesis. *RUNX1*, which is required for definitive hematopoiesis, is the target of chromosome translocations in leukemia. *RUNX2*, which is essential for osteogenesis, is mutated in the human disease cleidocranial dysplasia. *RUNX3* is necessary for the suppression of cell proliferation of gastric epithelium, neurogenesis of the dorsal root ganglia, and T-cell differentiation. The gastric epithelium of RUNX3 knockout mice shows hyperplasia, reduced rate of apoptosis, and reduced sensitivity to TGFβ1, suggesting that the tumor-suppressor activity of RUNX3 operates downstream of the TGFβ signaling pathway.

Recent studies on RUNX3 methylation in human cancers demonstrated that loss of RUNX3 by hypermethylation of the promoter CpG island was detected not only in 64% of gastric cancer but also in 73% of hepatocellular carcinoma, 70% of bile duct cancer, 75% of pancreatic cancer, 62% of laryngeal cancer, 46% of lung cancer, 25% of breast cancer, 23% of prostate cancer, 12% of endometrial cancer, 2.5% of uterine cervical cancer, and 5% of colon cancer. The RUNX3 methylation is especially frequent in cancers from tissues of a foregut origin. Interestingly, the RUNX3 methylation is found in 8% of chronic gastritis, 28% of intestinal metaplasia, and 27% of gastric adenoma, but not in chronic hepatitis B. These findings suggest that RUNX3 is a target for epigenetic gene silencing in gastric carcinogenesis.

Cell adhesion molecules may also work as tumor suppressors. Mutations in the E-cadherin gene occur preferentially in 50% of diffuse type gastric carcinoma. E-cadherin mutations affecting exons 8 or 9 induce the scattered morphology, decreased cellular adhesion and increased

cellular motility of diffuse gastric cancers. The mutations are even detected in intramucosal carcinoma. E-cadherin germline mutations responsible for hereditary diffuse gastric cancer (HDGC) have been reported since 1998, but their frequency is extremely rare. The β- and γ-catenin mutations but not E-cadherin mutations bring about constitutive Tcf transcriptional activity in gastric and pancreatic cancer cells. As mentioned in esophageal SCC, cross-talk between β-catenin and receptor tyrosine kinases including c-met, EGFR and c-*erb*B2 is found in gastric cancer cells, leading to diffuse spreading or scattering of gastric cancer cells through enhanced Wnt signaling pathway. These results indicate that genetic and epigenetic alterations in E-cadherin and catenins are involved in the development and progression of diffuse and scirrhous-type gastric cancer.

As to alterations in cell-cycle regulators, the cyclin E gene is amplified in 15 to 20% of gastric carcinomas that are associated with overexpression. Gene amplification or overexpression of cycline E, or both cause aggressiveness and lymph node metastasis. Cyclin D1 gene amplification, on the other hand, is exceptional in gastric cancer but frequently occurs in esophageal SCC. p27, a member of the cip/kip family of CDK inhibitors, binds to a wide variety of cyclin/CDK complexes and inhibits kinase activity. We have found that growth suppression of interferon-β is associated with the induction of p27 in the gastric cancer cell line TMK-1. More importantly, reduction in p27 expression is frequently observed in advanced gastric cancers while it is well preserved in 90% of gastric adenomas and 85% of early cancers. Reduced expression of p27 significantly correlates with tumor invasion and nodal metastasis. The expression of p27 in gastric cancer is inversely correlated with the expression of cyclin E. Loss of p27 expression and gain of cyclin E promotes progression and metastasis of gastric cancer.

Reduction in p27 occurs at posttranslational levels, resulting from ubiquitin mediated proteosomal degradation rather than genetic abnormalities.

An important downstream target of cyclin/CDKs at G1/S transition is a family of E2F transcription factors. Gene amplification of E1F-1 is seen in 4% of gastric cancers and in 25% of colorectal cancers. Overexpression of *E2F* is detected in 40% of primary gastric carcinomas. In addition, E2F and cyclin E tend to be coexpressed in gastric cancer, whereas 70% of gastric cancers show lower levels of E2G-3 expression than corresponding normal mucosa. These results overall suggest that gene amplification and abnormal expression of the *E2F* gene may permit the development of gastric cancer.

3.2 Factors Associated with Increased Incidence of Gastric Cancer

Three major factors, including environmental factors, host factors and genetic factors, cooperatively affect the genesis of gastric cancer. Of these, environmental factors are the most important, as diet and cigarette smoking are primary offenders; in particular, the presence of carcinogens such as *N*-nitroso compounds and benzo[α]pyrene is directly linked to carcinogenesis. The mutation spectrum of the *p53* gene is different between intestinal type and diffuse type gastric cancers, as p53 mutation at A:T sites are common in intestinal type carcinoma whereas GC → AT transitions are predominant in diffuse type carcinoma. Carcinogenic *N*-nitrosoamines, which cause mainly GC → AT base substitutions, are found in many

foods and can also be produced from amines and nitrates in the acidic environment of the stomach.

As for host factors, meta-analysis of relationship between *H. pylori* infection and gastric cancer has indicated that *H. pylori* infection is associated with a twofold increased risk of gastric cancer. Younger *H. pylori* infected patients have a higher relative risk of gastric cancer than older patients. *H. pylori* infection is equally associated with intestinal-type and diffuse-type gastric cancers. In fact, the observations in a Mongolian gerbil model of stomach carcinogenesis show that *H. pylori* infection promotes stomach carcinogenesis induced by chemical carcinogens, and that histological types of gastric carcinoma may depend on the concentration of chemical carcinogens rather than *H. pylori* infection. Eradication of the bacteria evidently decreases the incidence of gastric cancer in the Mongolia gerbil model.

H. pylori infection produces reactive oxygen and nitrogen species that cause DNA damage, followed by chronic gastritis and intestinal metaplasia. Goto et al. reported that the expression of inducible nitric oxide synthase (iNOS) and nitrotyrosine in the gastric mucosa was significantly high in *H. pylori* infected patients who developed gastric cancer at least two years after the initial biopsies. These findings suggest that high production of iNOS and nitrotyrosine may participate in gastric carcinogenesis.

Not only can *H. pylori* activate NF-κB in gastric epithelial cells, but activated NF-κB activates the transcription of IL-1, IL-6, IL-8, TNF-α, and cyclooxygenase-2 (Cox-2). Successful eradication of *H. pylori* leads to downregulation of Cox-2 in the epithelial and stromal cells. High expression of Cox-2 mRNA, protein, and enzymatic activity is observed in the tumor cells of intestinal type gastric cancer. Loss of Cox-2 promoter methylation may enhance Cox-2 expression and promote gastric carcinogenesis associated with *H. pylori*.

Strain of *H. pylori* and genetic factors play a critical role in susceptibility to stomach carcinogenesis. Prinz et al. reported that CagA+/VacAs1+ strains of *H. pylori* that are blood-group antigen-binding adhesion (BabA2)-positive are associated with activity or chronicity of gastritis. Adherence of *H. pylori* via BabA2 may play a key role for efficient delivery of VacA and CagA. Moreover, Hatakeyama's group has recently shown that CagA binds an Src homology 2 (SH2)-containing tyrosine phosphatase SHP-2 in a tyrosine phosphorylation-dependent manner and stimulates the phosphatase activity of SHP-2. In addition, they found that prevalent CagA protein in East Asian countries are significantly more potent in binding SHP-2 and in inducing cellular morphological changes than are CagA proteins of Western isolates. Differences in the biological activity of Western and East Asian CagA protein, which are determined by variation in the tyrosine phosphorylation sites, may underlie the different incidences of gastric cancer in these two geographic areas. Regarding the development of gastric mucosa-associated lymphoid tissue (MALT) lymphoma, heat-shock protein 60 kDa (hsp60) of *H. pylori* is an important antigen in the pathogenesis of MALT lymphoma.

In addition to *H. pylori* strains, DNA polymorphism including HLA, MUC1, T-cell helper 1 and IL-1β has been reported to be associated with an increased risk of both atrophic gastritis induced by *H. pylori* and gastric cancer. In addition, El-Omar et al. reported that proinflammatory genotypes of tumor necrosis factor α and IL-10 are associated with increased risk

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

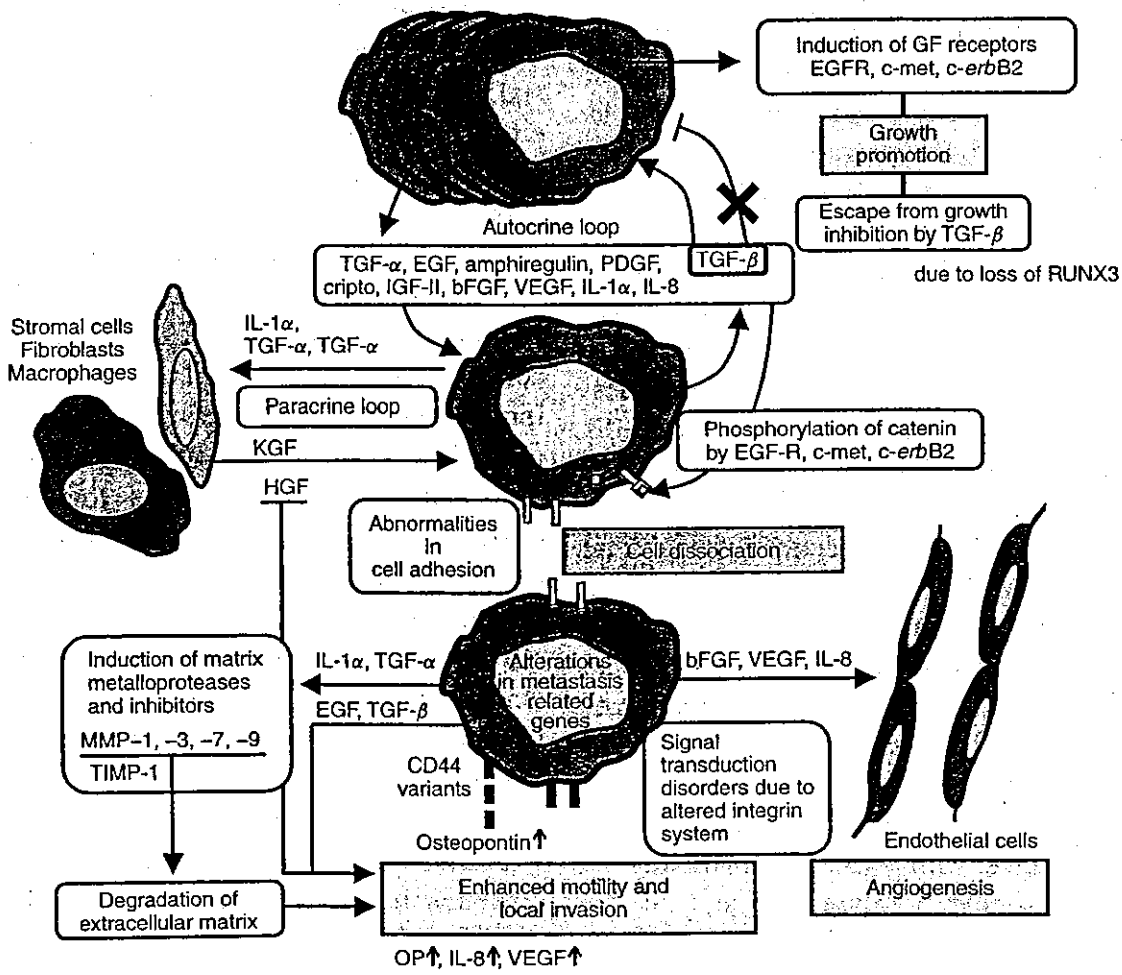

Fig. 4 Cancer-stromal interaction in gastric cancer through growth factors and cytokines.

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

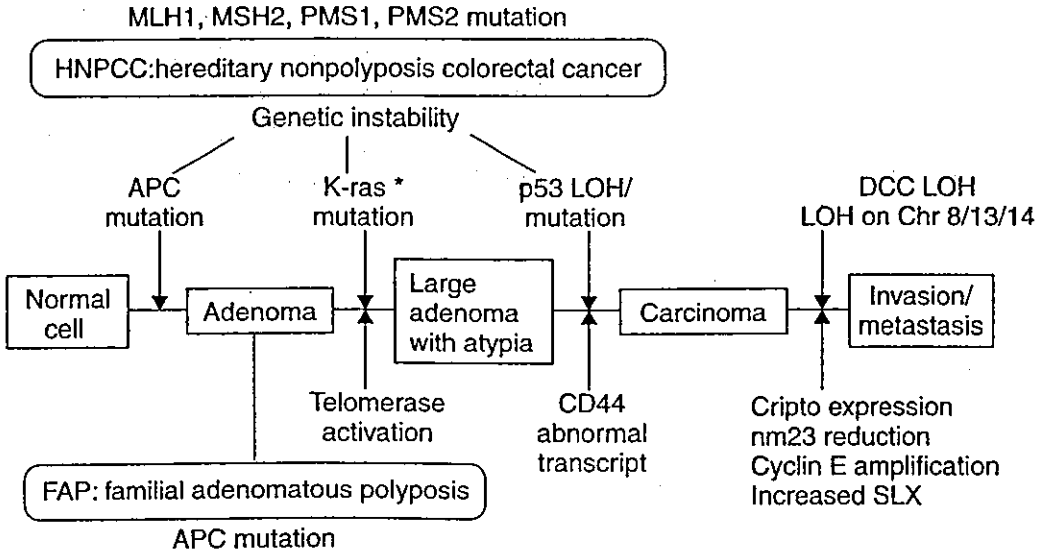

Fig. 5 A major genetic pathway for colorectal carcinogenesis. (*K-ras mutation is infrequent in flat adenoma)

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.

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