et al., 1994b). Overexpression of cripto is frequently associated with intestinal metaplasia and gastric adenoma (Kuniyasu *et al.*, 1991).

AR, a member of the EGF family which is overexpressed in more than 60% of gastric carcinomas regardless of histological type (Kitadai *et al.*, 1993a), works as an autocrine growth factor and induces the expression of AR itself, TGF-α and EGF receptors by gastric cancer cells (Akagi *et al.*, 1995). Overexpression of the EGF family in gastric cancer usually does not accompany gene amplifications. The relative expression levels of positive transcription factor, Sp-1, and negative transcription factor, GC factor, may regulate gene expression of these growth factors and receptors (Kitadai *et al.*, 1993b).

IL-1α is a cytokine mainly produced by activated macrophages and mediates many of the local and systemic responses to infection and inflammation (Dinarello, 1992). It is also produced by gastric cancer cells. We have found that IL-1α evidently acts as an autocrine growth factor for gastric carcinoma cells and plays a pivotal role as a trigger for induction of EGF and EGF receptor expression (Ito *et al.*, 1993). The expression of IL-1α by tumour cells is induced by either IL-1α, EGF or TGF-α, while IL-1α up-regulates the expression of TGF-α and EGF receptor by tumour cells themselves, indicating that an intimate interplay between IL-1α and the EGF/receptor system stimulates the growth of gastric cancer.

In addition to IL-1α, IL-6 is also an autocrine growth stimulator for gastric cancer cells. The expression of IL-1α by tumour cells is induced by IL-6, while IL-1α increases the expression of IL-6 by tumour cells themselves (Ito *et al.*, 1997).

Stromal cells, especially fibroblasts stimulated by growth factors or cytokines such as IL-α, TGF-α and TGF-β, secrete HGF/SF (hepatocyte growth factor/scatter factor), which can function in a paracrine manner as a morphogen or motogen of tumour cells. For example, in the case of a clone maintaining expression of cell-adhesion molecules, HGF/SF promotes tubular formation of tumour 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 motogen and induce scattering of tumour cells, resulting in diffuse gastric cancer (Tahara, 1993; Yokozaki *et al.*, 1997). Our recent findings suggest that interaction between c-*met* overexpressed in tumour cells and HGF/SF from stromal cells is related to the morphogenesis and progression of gastric cancer *in vivo*.

The negative growth factor TGF-β1 is commonly overexpressed in gastric carcinoma, particularly in diffuse-type carcinoma with diffusely productive fibrosis (Yoshida *et al.*, 1989). However, most human gastric cancer cells have escaped from TGF-β-induced growth inhibition at the receptor or post-receptor levels. TGF-β inhibited the growth of only one (TMK-1) of seven gastric carcinoma cell lines; this inhibition is associated with p53-independent induction of p21 which induces suppression of cyclin-dependent kinase activity, reduced phosphorylation of Rb and a decrease in cyclin A (Ito *et al.*, 1992a; Akagi *et al.*, 1996). Various mutations in the *TGF-β* receptor type II (RII) gene have been reported in gastric cancer. One type of mutation in the *TGF-βRII* gene is mutation in the polyA tract (i.e. deletion or insertion of 1–2 bases) that frequently occurs in the hereditary non-polyposis colon cancer syndrome (Markowitz *et al.*, 1995) and in gastric carcinoma with MSI-H (Yokozaki *et al.*, 1999b). Another type of mutation in the *TGF-βRII* gene involves abnormal amplification and truncation of the gene (Yang *et al.*, 1999). However, we have not seen genetic alterations of the *TGF-βRII* gene in any gastric carcinoma cell lines. Moreover, results of a study on expression of TGF-βRI in TGF-β-resistant gastric cancer cell lines that contain no discernible alteration in the *TGF-βRII* gene suggest that hypermethylation of a CpG island in the 5′ region of the *TGF-βRI* gene is involved in another potentially important mechanism of escape from negative growth control by TGF-β (Kim *et al.*, 1999). We have already found that most gastric carcinomas show reduced levels of TGF-βRI and that this correlates well with the depth of tumour invasion (Ito *et al.*, 1992b).

A large number of angiogenic factors have been identified in human malignancy. Among them are vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and IL-8, which

are derived from tumour cells and participate mainly in neovascularization within gastric carcinoma tissues. We have shown that all eight gastric cancer cell lines secrete VEGF into conditioned media (Yamamoto *et al.*, 1998). EGF or IL-1α up-regulates VEGF expression by tumour cells, whereas interferon-γ down-regulates it. Moreover, VEGF promotes angiogenesis and the progression of gastric carcinomas, especially carcinomas of the intestinal type (Takahashi *et al.*, 1996). On the other hand, bFGF produced by tumour cells is frequently associated with angiogenesis and extensive fibrosis in diffuse gastric carcinomas, particularly those of the scirrhous type (Tanimoto *et al.*, 1991).

IL-8, a member of the CXC chemokine family, induces haptotactic migration and proliferation of melanoma cells and angiogenesis. More importantly, gastric carcinoma cell lines express mRNA and protein for IL-8 and IL-8 receptors (IL-8RA and IL-8RB) (Kitadai *et al.*, 1998, 2000). More than 80% of gastric carcinomas co-express IL-8 and IL-8 receptors; this co-expression correlates directly with tumour vascularity and disease progression. IL-8 enhances the expression of EGF receptor, type IV collagenase (metalloproteinase (MMP)-9), VEGF and IL-8 mRNA itself by gastric cancer cells, whereas IL-8 decreases expression of E-cadherin mRNA. In addition, IL-8 also increases MMP-9 activity and the ability of gastric cancer cells to invade through Matrigel. Altogether, IL-8 may play an important role in the growth and progression of gastric carcinoma by autocrine and paracrine mechanisms.

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 (Table 2). 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[*a*]pyrene is directly linked to carcinogenesis. As already described, the mutation spectrum of the *p53* gene differs between intestinal-type and diffuse-type gastric cancers, suggesting that different carcinogens may be implicated in the two types of gastric carcinogenesis (Yokozaki *et al.*, 1997). Palli *et al.* (2001) found that the risk of MSI-H gastric cancer was positively associated with high consumption of red meat and meat sauce, and negatively associated with consumption of white meat.

Table 2. Factors associated with increased incidence of gastric carcinoma

Environmental factors	Diet (nitrites derived from nitrates, smoked and salted food, pickled vegetables, lack of fresh fruit and vegetables) Cigarette smoking
Host factors	*H. pylori* infection (chronic gastric and intestinal metaplasia) Partial gastrectomy Barrett esophagus
Genetic factors	Hereditary diffuse gastric cancer Hereditary non-polyposis colon cancer DNA polymorphism Genetic instability

With regard to host factors, meta-analysis of the relationship between *H. pylori* infection and gastric cancer has indicated that *H. pylori* infection is associated with a twofold increased risk of gastric cancer (Huang *et al.*, 1998; Eslick *et al.*, 1999). Younger *H. pylori*-infected patients have a higher relative risk for gastric cancer than older patients. *H. pylori* infection is equally associated with intestinal-type and diffuse-type gastric cancers (Huang *et al.*, 1998). In fact, the findings in a Mongolian gerbil model of stomach carcinogenesis indicate 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 on *H. pylori* infection (Shimizu *et al.*, 1999). Eradication of the bacteria evidently decreases the incidence of gastric carcinomas in the Mongolian gerbil model (Shimizu *et al.*, 2000).

H. pylori infection produces reactive oxygen and nitrogen species that cause DNA damage, followed by chronic gastric and intestinal meta-

plasia (Correa *et al.*, 1997). Goto *et al.* (1999) 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 2 years after the initial biopsies. These findings suggest that high production of iNOS and nitrotyrosine in the gastric mucosa by *H. pylori* may contribute to gastric carcinogenesis.

Cyclooxygenase-2 (Cox-2) expression is also induced by *H. pylori* infection (Sung *et al.*, 2000). Successful eradication of *H. pylori* leads to down-regulation of Cox-2 in the epithelial and stromal cells. High expression of Cox-2 mRNA, protein and enzymatic activity is detected preferentially in the tumour cells of intestinal-type gastric cancer (Saukkonen *et al.*, 2001). Loss of Cox-2 promoter methylation may enhance Cox-2 expression and promote gastric carcinogenesis associated with *H. pylori* infection (Akhtar *et al.*, 2001).

It should not be forgotten that in Japan the annual incidence of gastric cancer is about 100 000, accounting for 0.16% of 60 million individuals with *H. pylori* infection. Moreover, analysis of chromosomal aberrations in gastric cancer shows that they do not differ between *H. pylori*-related and non-related gastric cancers (van Grieken *et al.*, 2000). Genetic factors play a critical role in susceptibility to stomach carcinogenesis (Table 3).

Prinz *et al.* (2001) 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.

In addition to *H. pylori* strains, DNA polymorphism including HLA, MUC1 (Carvalho *et al.*, 1997), 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 (El-Omar *et al.*, 2000). More excitingly, Magnusson *et al.* (2001) found that distinct HLA class II DQ 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 gastric cancer. These host genetic factors may determine why some individuals infected with *H. pylori* develop gastric cancer while others do not. However, these studies need confirmation by a large number of prospective investigations in each of the countries concerned.

Table 3. Susceptibility to disease caused by *H. pylori*

Strain of *H. pylori*	CagA+/VacAs1+strains that are BabA2-positive (Printz *et al.*, 2001)
Genetic factors	*HLA* polymorphism: *HLA*-DQA1 genetic typing *MUC1* polymorphism T-cell helper 1 phenotype IL-1β polymorphism *HLA* DR and DQ alleles: DQA1*0102 is associated with protection from infection by *H. pylori*, whereas DRB*1601 is associated with cancer development, particularly *H. pylori*-negative diffuse type (Magnusson *et al.*, 2001).

Conclusion

Overall, the observations on the molecular events of gastric cancer may provide supporting evidence for our working hypothesis that there are two distinct major genetic pathways for stomach carcinogenesis (Figure 1). Genetic and epigenetic alterations found in two types of gastric cancer are summarized in Table 1. Among them, genetic instability including MIS and telomere reduction and immortality (activation of telomerase and expression of hTERT) are implicated in an initial step of stomach carcinogenesis. In the multistep process of intestinal-type gastric carcinogenesis, infection with *H. 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 may 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. Incomplete intestinal metaplasia that contains an accumulation of the above multiple molecular events – that is, 'metaplastic dysplasia' – may be viewed as a bud of intestinal-type gastric cancer at genetic and epigenetic levels. An adenoma→carcinoma sequence is found in about 20% of gastric adenomas with *APC* mutations. In addition to these events, *p53* mutation and LOH, reduced p27 expression, cyclin E expression and presence of c-*met* 6.0-kb transcripts allow malignant transformation from the precancerous lesions to intestinal-type gastric cancer. *DCC* loss, *APC* mutations, 1q LOH, *p27* loss, reduced TGF-βRI expression, reduced nm23 expression and c-*erb*B gene amplification are implicated in the progression and metastasis of intestinal-type gastric cancer. Another pathway for carcinogenesis of intestinal-type gastric cancer involves LOH and abnormal expression of the *p73* gene that may be 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 diffuse 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 intestinal and diffuse components exhibit some but not all of the molecular events described for each of the two types of gastric cancer.

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Growth Factors and Oncogenes in Gastrointestinal Cancers

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Keywords

Cell Adhesion Molecules
Surface ligands, usually glycoproteins, that mediate cell to cell adhesion. Their functions include the assembly and interconnection of various vertebrate systems, as well as maintenance of tissue integration, wound healing, morphogenic movements, cellular migration, and metastasis.

Cell Cycle Regulators
Proteins that regulate the cell division cycle. This family of proteins involves a wide variety of classes, including cyclin-dependent kinases, mitogen-activated kinases, cyclins, and phosphoprotein phosphatases as well as their putative substrates, such as chromatine-associated proteins, cytoskeletal proteins, and transcription factors.

Cytokines
Polypeptides secreted by inflammatory leukocytes, macrophages and lymphocytes in response to microbes and other antigens that mediate and regulate immune and inflammatory reactions. They generally act locally in a paracrine or an autocrine manner rather than in an endocrine manner.

Growth Factors
Signal molecules that act to control cell growth and differentiation in the receptor-dependent fashion. The alterations of these proteins lead to transformation and the accompanying loss in growth control. Some of the growth factors and their receptors are involved in the products of oncogens.

Oncogenes
Genes that can convert cells to cancerous growth by attacking crucial cellular machinery. They encode for growth factors, growth-factor receptors, protein kinases, signal transducers, nuclear phosphoproteins, and transcription factors. These genes are constitutively expressed after structural and /or regulatory changes, resulting in uncontrolled cell proliferation. They can be classified into viral oncogenes (v-oncogenes) and cellular oncogenes (proto-oncogenes).

Telomerase
Essential ribonucleoprotein reverse transcriptase that adds telomeric DNA to the ends of eukaryotic chromosomes. Telomerase is expressed in the testis and ovary, but repressed in normal human somatic tissues. Telomerase activity is seen in more than 90% of human cancers.

Tumor-suppressor Genes
Genes inhibit expression of the tumorigenic phenotype. They are normally involved in holding cellular growth in check. When tumor-suppressor genes are inactivated or lost, a barrier to normal proliferation is removed, leading to unregulated growth.

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 in oncogenes, tumor-suppressor genes, cell adhesion molecules, DNA repair genes, and genetic instability as well as telomerase activation are observed in esophageal, gastric, and colorectal cancers. In gastric cancer, the pattern of genetic and epigenetic alterations also differs depending on the two histological types, intestinal type or well-differentiated type and diffuse type or poorly differentiated type, indicating that there are two distinct major genetic pathways for gastric carcinogenesis.

In addition to these events, gastrointestinal cancer cells express a broad spectrum of the growth factor/cytokine receptor systems that organize complex cancer-stromal interaction, which confer cell growth, apoptosis, morphogenesis, angiogenesis, progression, and metastasis. However, these abnormal growth factor/cytokine networks also are 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 network in gastrointestinal cancers.

1 Introduction

Multiple genetic and epigenetic alterations in oncogenes, tumor-suppressor genes, cell cycle regulations, cell adhesion molecules, DNA repair genes and genetic instability as well as telomerase activation are responsible for the multistep process of human gastrointestinal carcinogenesis. However, a scenario or particular combination of these alterations differs in esophageal, gastric, and colorectal cancers. Namely, common and distinct molecular events are observed in esophageal, gastric, and colorectal cancers, respectively. Moreover, two types of gastric cancer, well-differentiated or intestinal type, and poorly differentiated or diffuse-type carcinomas also exhibit a distinct pattern of genetic pathways.

Besides these genetic and epigenetic events, gastrointestinal cancer cells express a broad spectrum of growth factors, cytokine or both, including epidermal growth factor (EGF) family, transforming growth factor (TGF)-β, heparin binding (HB)-EGF, PDGF, IGF, basic fibroblast growth factor (FGF), interleukin (IL)-1α, IL-6, IL-8 and osteopontin (OPN). These growth factors and cytokines act as autocrine, paracrine, and juxtacrine modulators of the growth of cancer cells, and then organize complex interplay between cancer cells and stromal cells, which plays an important role in cell growth, apoptosis, morphogenesis, angiogenesis, progression and metastasis. Interestingly, the expression of these growth factors, cytokines or both by cancer cells is also different among esophageal, gastric, and colorectal cancers.

This article will provide an overview of the molecular machinery that underlies gastrointestinal carcinogenesis and focuses on abnormal growth factor/cytokine network in gastrointestinal cancers.

2 Genetic and Epigenetic Alterations and Abnormal Growth Factor/Cytokine Network in Esophageal Cancer

Esophageal cancer is the third most frequent gastrointestinal cancer in the world. The most recent estimates are that esophageal cancer is the sixth most common cancer in men (212 600 new cases, 4.9% of all cancers) and the ninth most common in women (103 200 new cases, 2.7% of all cancers). The two main histological types of esophageal cancer are squamous cell carcinoma (SCC) and adenocarcinoma, but SCC is the more prevalent type worldwide. The development of esophageal SCC exhibits a multistep, progressive process. An early indicator of this process is an increased proliferation of esophageal epithelial cells including basal cell hyperplasia, dysplasia, and carcinoma *in situ*. This multistep process requires the accumulation of multiple genetic and epigenetic alterations and overexpression of growth factors/cytokine receptor systems, leading to the evolution of clonal cell populations that possess growth advantages over other cells as demonstrated in the progression model of head and neck cancer. This paragraph thus will describe recent advances in molecular dissection of multistep tumorigenesis of esophageal SCC and abnormal growth factor/cytokine network that contributes to the development and progression of esophageal SCC.

2.1 Genetic and Epigenetic Alterations in Esophageal SCC

Numerous genetic and epigenetic alterations are implicated in the development and progression of esophageal SCC (Table 1). This cancer is frequently associated with loss of heterozygosity (LOH) at multiple chromosomal loci including 3p, 5q, 9p, 9q, 13q, 17p, 17q, and 18q. No significant differences have been found in the prevalence of LOH at various loci in SCC and adenocarcinoma of the esophagus.

Among these alterations, LOH and mutation of the *p53* gene at chromosome 17p13 occur at an early stage of esophageal carcinogenesis, such as dysplasia and carcinoma *in situ*. About 50% of esophageal SCC harbor mutations of the *Tp53* gene, most of which are missense mutations leading to amino acid changes within exons 5–8, which encode the entire DNA binding domain of the p53 molecule and the flanking splice sites. Considering the

Tab. 1 Genetic and epigenetic alterations found in esophageal SCC.

Genetic and epigenetic alterations	*Incidence [%]*
Tumor suppressors	
P53 LOH, mutation	40–60
APC LOH	60–70
DCC LOH	20–40
Rb LOH	40–50
BRCA1 LOH	60
3p LOH	40–100
5p LOH	62
9p LOH	45–76
9q LOH	60
13q LOH	57
14q LOH	65
17p LOH	43–65
17q LOH	62
RARβ2 loss	40–50
Cell cycle regulators	
p16 loss, mutation	45–76
Cyclin D_1 amplification	40–50
Oncogenes	
EGFR amplification	10–15
Telomerase activity	86
TERT expression	96

base substitution spectrum, G:C to T:A transversion is common in esophageal carcinoma, similar to that in carcinomas of the lung and liver. This situation is different from the finding that colorectal carcinomas frequently contain G:C to A:T transitions at CpG dinucleotides. This evidence suggests that different environmental and intrinsic factors may affect the tumorigenesis of esophageal and colorectal carcinomas. It is of interest that LOH of the *APC, DCC,* and *Rb* genes shows high frequency but these genes are very rarely or never mutated in esophageal SCC.

The retinoic acid receptor (RAR) β gene is a putative tumor-suppressor gene on chromosome 3p24, where a high frequency of LOH is found in many human cancers, including esophageal cancer. The human RARβ has three isoforms (β1, β2, β4). Overexpression of RARβ2 induces inhibition of tumor cell growth and apoptosis in human cancer cell lines including esophageal cancer cells. Moreover, Induction of RARβ2 suppresses cyclooxygenase-2 (COX2) expression in esophageal cancer cells. More importantly, DNA methylation of RARβ2 promoter CpG sites has been reported to cause the loss of RARβ2 expression in many human cancers including lung, breast, prostate, stomach, head and neck, and esophageal cancers. RARβ is expressed in 90% of normal esophageal mucosa, while it is detected in only 60% of dysplastic lesions and in 50% of SCC. These findings indicate that loss of RARβ, or more specifically, the isoformβ2, is an early event associated with esophageal carcinogenesis and the status of squamous differentiation.

p16, an inhibitor of cyclin D1/cyclin-dependent kinase, is located on chromosome 9p21. It is inactivated by 9p21LOH with *de novo* p16 promoter hypermethylation in the majority of esophageal SCC. Recent molecular analysis of precancerous laryngeal lesions suggests that loss of p16 protein is an early step toward malignant transformation in head and neck tissues. This protein forms binary complexes with CDK4 and CDK6, inhibiting their ability to phosphorylate the Rb protein. Loss of the p16 protein may bring about increased Rb phosphorylation and allow cells to enter into S-phase. In fact, we have confirmed that homozygous deletion of the *p16* gene is closely correlated with the increased expression of cyclin D1, CDK4 and phosphorylated Rb protein in esophageal SCC cell lines.

In 1989, we discovered the coamplification of *hst-1* and *int-2*, both of which are located on chromosome 11q13, in about 50% of primary tumors and in 100% of metastases of esophageal SCC. Gene amplification, however, was not accompanied by overexpression of the two genes. Subsequently, Jiang et al. found that the cyclin D1, which is located on the same locus as *hst-1* and *int-2* genes, was amplified in 32% of SCC, associated with overexpression. The amplification of the cyclin D1 is closely correlated with tumor staging, depth of tumor invasion, and metastasis. In the esophagus, 71% of SCC and 64% of adenocarcinoma are positive for increased cyclin D1 nuclear staining, indicating that overexpression of cyclin D1 is common in both types of cancer. Cyclin D1 binds to Rb protein and stimulates its phosphorylation. Hyperphosphorylation of Rb in response to overexpressed cyclin D1 may lead to uncontrolled cell cycling and increased cell proliferation.

As for oncogene activation, amplification of the EGF receptor (*EGFR*) gene occurs in 10–15% of advanced cases of

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*.