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Encyclopedia of Molecular Cell Biology and Molecular Medicine

Edited by Robert A. Meyers

Second Edition

Volume 6

**Growth Factors and Oncogenes in Gastrointestinal Cancers to
Informatics (Computational Biology)**



**WILEY-
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Encyclopedia of Molecular Cell Biology and Molecular Medicine

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Growth Factors and Oncogenes in Gastrointestinal Cancers

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Encyclopedia of Molecular Cell Biology and Molecular Medicine, 2nd Edition. Volume 6
 Edited by Robert A. Meyers.
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 ISBN: 3-527-30548-3

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 chromatin-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 oncogenes.

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- κ B 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)-1 α , 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

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

Genetic and epigenetic alterations	Incidence [%]
<i>Tumor suppressors</i>	
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
RAR β loss	40–50
<i>Cell cycle regulators</i>	
p16 loss, mutation	45–76
Cyclin D ₁ amplification	40–50
<i>Oncogenes</i>	
EGFR amplification	10–15
Telomerase activity	86
TERT expression	96

(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

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 *RAR* β has three isoforms ($\beta 1$, $\beta 2$, $\beta 4$). Overexpression of *RAR* $\beta 2$ induces inhibition of tumor cell growth and apoptosis in human cancer cell lines including esophageal cancer cells. Moreover, induction of *RAR* $\beta 2$ suppresses cyclooxygenase-2 (*COX2*) expression in esophageal cancer cells. More importantly, DNA methylation of *RAR* $\beta 2$ promoter CpG sites has been reported to cause the loss of *RAR* $\beta 2$ expression in many human cancers including lung, breast, prostate, stomach, head and neck, and esophageal cancers. *RAR* β 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 *RAR* β , or more specifically, the isoform $\beta 2$, 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-erbB2* 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 member 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 1B) 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. *RAR β 2*) 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 D1 and *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 (TGF β), interleukin (IL)-1 α and IL-6. Among them, the EGF/TGF α 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 TGF α act as autocrine growth factors through EGFR which is encoded by the proto-oncogene *c-erbB1*.

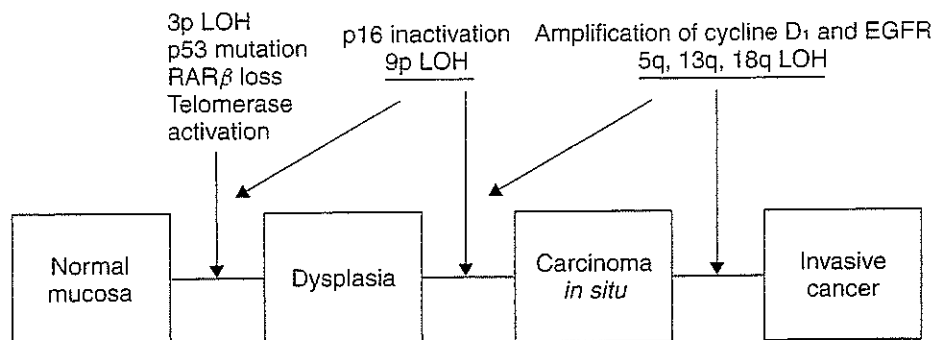


Fig. 1 Genetic progression model of esophageal SCC.

Membrane-bound TGF α 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 TGF α induce the expression of TGF α and EGFR, thus indicating the presence of an autocrine loop of EGF/TGF α /receptor system. We have found that EGF is expressed in 30% of esophageal SCC while TGF α and EGFR are expressed in 77 and 89% of esophageal SCC, respectively. Coexpression of TGF α 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 TGF α increase the expression of PDGF A- and B-chain in esophageal SCC cell lines.

Grb7, a ligand for both EGFR and *c-erbB2*, is also implicated in esophageal SCC. Tanaka et al. found overexpression of Grb7 in 44 % of the tumors and

coexpression of Grb7 with EGFR or *c-erbB-2* in 32% of the advanced cases, suggesting that Grb7 functions as an extracellular stimulus for progression of esophageal SCC.

EGF or TGF α produced by the tumor cells can make not only an autocrine loop of EGF/TGF α 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 up-regulation 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
<i>Tumor suppressors</i>		
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
<i>Cell cycle regulators</i>		
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

^aPreferentially found in foveolar-type adenocarcinoma.

^bPreferentially 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.

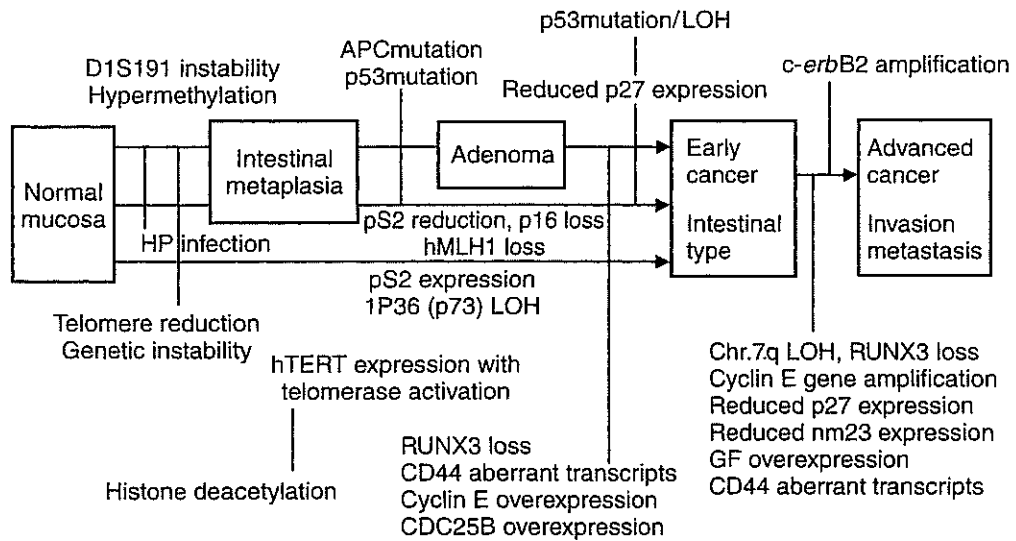


Fig. 2 Multiple genetic and epigenetic alterations during human gastric carcinogenesis (intestinal type).

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
<i>Oncogenes</i>		
K-ras mutation	10	0
c-met amplification	19	39
K-sam amplification	0	33
c-erbB2 amplification	20	0
<i>Adhesion molecules</i>		
E-cadherin, mutation	0	50
Loss of CDH1	55	79 ^b
CD44 aberrant transcript	100	100
<i>Microsatellite instability (hMLH1 methylation)</i>		
	20–40 (5–20)	20–70 ^a (0)
<i>Histone deacetylation</i>		
	61	82
<i>Telomere/Telomerase</i>		
Telomere reduction	62	53
Telomerase activity	100	90
TERT expression	100	86

^aPreferentially found in patients younger than 35 years of age.

^b*p* = 0.0175; Fisher's exact test.

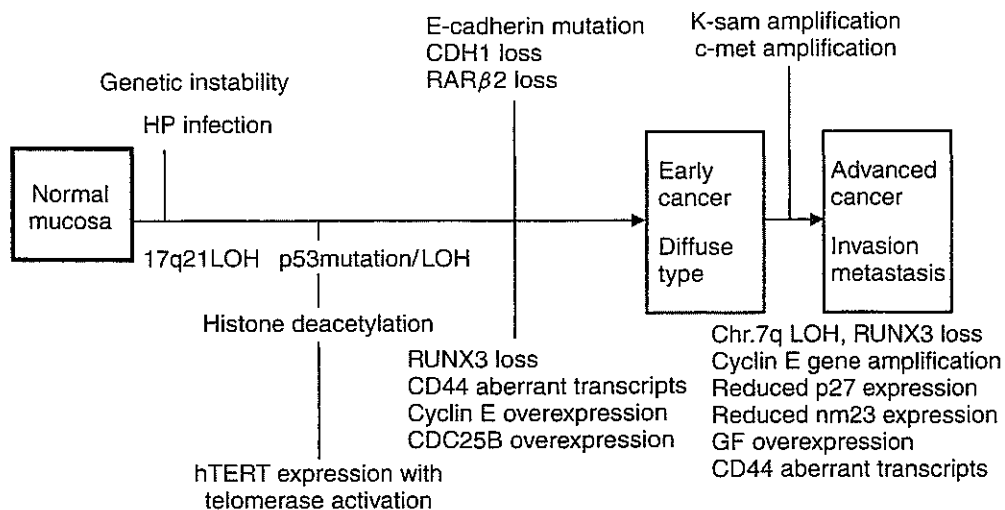


Fig. 3 Multiple genetic and epigenetic alterations during human gastric carcinogenesis (diffuse type).

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 TGF β 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 *RUNX*s 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 TGF β 1, suggesting that the tumor-suppressor activity of *RUNX3* operates downstream of the TGF β 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-erbB2* 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 cyclin 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 \rightarrow AT transitions are predominant in diffuse type carcinoma. Carcinogenic *N*-nitrosoamines, which cause mainly GC \rightarrow 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- κ B in gastric epithelial cells, but activated NF- κ B 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-1 β 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