TABLE 4
Regression Coefficients for Variables Related to the Percentages of CD28-Negative and CD57-Positive CD8 T-Cell Subpopulations and their Correlations to the Percentages of CD45RO$^+$/CD62L$^+$ and CD45RO$^+$/CD62L$^-$ CD8 T Cells in PBL among A-bomb Survivors[a]

	Effects			
T-cell subpopulation	Intercept α	Age (10 years)[b] β_1	Gender[c] β_2	Dose (Gy) β_3
CD28$^-$	1.23	0.177	−0.072	−0.016
		$P = 0.0001$**	$P = 0.25$	$P = 0.70$
CD57$^+$	1.14	0.103	−0.149	−0.033
		$P = 0.0048$**	$P = 0.027$*	$P = 0.48$

Correlation between	Correlation coefficients (r)
CD28$^-$ and CD45RO$^+$/CD62L$^+$	0.18
	$P = 0.0001$**
CD28$^-$ and CD45RO$^+$/CD62L$^-$	0.62
	$P = 0.0001$**
CD57$^+$ and CD45RO$^+$/CD62L$^+$	0.22
	$P = 0.0001$**
CD57$^+$ and CD45RO$^+$/CD62L$^-$	0.60
	$P = 0.0001$**
CD28$^-$ and CD57$^+$	0.84
	$P = 0.0001$**

[a] Regression coefficients of percentage T cells for age, gender and dose were obtained using the following formula: *Percentage T cells* = $\alpha + \beta_1 \times age + \beta_2 \times gender + \beta_3 \times dose$.
[b] Effects of age were estimated for 10-year intervals.
[c] Gender = 0 for male and = 1 for female.
* $P < 0.05$, ** $P < 0.01$.

of naïve CD8 T cells into the memory T-cell pool is enhanced in A-bomb survivors. Repeated exposure of naïve CD8 T cells to various antigens could have led to accumulation of both central and effector memory T cells. Impaired immunity to persistent infections with viruses such as EBV (*37, 38*), HBV (*5, 39*) and HCV (*40, 41*) in A-bomb survivors might be involved in this accumulation process. Homeostatic proliferation that takes place in the absence of antigen under lymphopenic conditions such as radiation-induced lymphopenia may also have contributed to the enhanced entry of naïve T cells into memory T-cell pools. However, such a possibility may contradict a previous finding, in which a population of mutant stem cells of one A-bomb survivor did not contributed greatly to the maintenance of the survivor's memory T-cell pool after A-bomb exposure (*42*).

Another interpretation is that clonal expansions of a population of memory CD8 T cells have frequently occurred in A-bomb survivors. A recent cytogenetic study on A-bomb survivors has shown that the frequency of clonal chromosome aberrations increased with increasing radiation dose and suggested that about half of the clonal chromosome aberrations may have been derived from clonal expansions of memory T cells (*43*). It has also been found that skewed TCR Vβ repertoires that are possibly associated with clonal expansions can frequently be observed in CD45RA$^-$ memory CD4 T-cell populations of A-bomb survivors, especially in those of older survivors (*44*). Although we have not yet determined the TCR Vβ repertoires in CD8 T-cell populations of the survivors, similar expansions of memory CD8 T cells can be expected among radiation-exposed persons. Nevertheless, the dose-dependent increases in the percentages of CD45RO$^+$/CD62L$^+$ and CD45RO$^+$/CD62L$^-$ memory T cells were apparent for CD8 T-cell populations but not for CD4 T-cell populations, suggesting that clonal expansions frequently occurring in the exposed individuals might not accompany the accumulation of memory T cells.

In contrast to the dose-dependent increases in the percentages of CD45RO$^+$/CD62L$^+$ and CD45RO$^+$/CD62L$^-$ CD8 T cells, there was no significant effect of radiation on these subsets of memory CD4 T cells in the present study. Although it has been established that no antigenic stimulation is required for either the CD4 or CD8 T-cell populations to maintain immunological memory, the ways in which the memory T-cell pools maintain their sizes are likely to be somewhat different for CD4 and CD8 T-cell populations. Thus, for example, homeostatic proliferation of memory CD8 T cells is accomplished by IL7 and IL15, whereas unknown factors other than those cytokines are probably involved in that of memory CD4 T cells (*45, 46*). Memory CD8 T cells are likely to persist *in vivo* for much longer than memory CD4 T cells (*47*). Furthermore, age-associated reductions in the diversity of TCR repertoire were found to be much more pronounced in memory CD8 populations than memory CD4 populations (*48*). Such differences in the CD4 and CD8 T-cell populations may be associated with the different radiation effects we observed in the CD45RO$^+$/CD62L$^+$ and CD45RO$^+$/CD62L$^-$ subsets of these T-cell populations among A-bomb survivors. The mechanisms by which radiation exposure impairs the ability of humans to maintain T-cell memory remain to be clarified.

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Rapid detection of micrometastasis by one step nucleic acid amplification

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Introduction Lymph node metastasis is one of important prognostic factors of caner patients. We examined the efficacy of one step nucleic acid amplification (OSNA) for rapid detection of lymph node metastasis with GD-100 (SYSMEX).

Materials and methods Five colon cell lines (Lovo, DLD1, WiDr, Colo201, Colo320) and 11 lymph nodes of colorectal carcinoma were examined. Cytokeratin (CK) 19 mRNA of cell lines and lymph nodes were measured quantitatively by OSNA.

Results Cellular mRNA of Lovo, DLD1, WiDr and Colo201 differed from 85.7 to 113.2 copies at semiconfluent condition. CK19 mRNA tended to vary at the different cell density except for Colo320, in which no CK mRNA was detected. By using Lovo cells, 0.8 cells were calculated as the least detectable number at one reaction. In the lymph nodes analysis, OSNA was completed within 30 min and results by OSNA were equal to those by histological examination when whole lymph node was examined for both methods.

Conclusion OSNA is a newly developed useful method to detect micrometastasis rapidly.

P-23

CK19 mRNA ANALYSIS BY LAMP WITH GD-100 IS APPLICABLE TO AN INTRA-OPERATIVE DETECTION OF MICROMETASTASIS OF LYMPH NODE.

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Introduction; Micrometastasis of lymph node has been detected either by immunohistochemistry or molecular analysis. The former has a difficulty in a quantitative analysis and the latter is time-consuming. The authors applied the CK19 mRNA analysis by loop-mediated isothermal amplification (LAMP) with GD-100 (SYSMEX) to an intra-operative detection of micrometastasis of lymph nodes.
Materials and methods; Five colon cancer cell lines (Lovo, DLD1, WiDr, Colo201, Colo320) and 38 lymph nodes were examined. Cytokeratin (CK) 19 mRNA of cell lines were analyzed quantitatively by LAMP with GD-100 and conventional real-time reverse transcriptase-polymerase chain reaction (RT-PCR). Lymph nodes were examined by histology and LAMP with GD-100.
Results; Cellular CK19 mRNA levels detected with GD-100 of Lovo, DLD1, WiDr and Colo201 varied more widely than those by conventional RT-PCR. Cellular CK19 mRNA of Colo320 could not be detected with GD-100 and was in very low level by real-time RT-PCR. Three of 38 lymph nodes revealed CK19 mRNA of various levels in spite of no detectable carcinoma cells by histological examination, and CK19 mRNA analysis with GD-100 was completed within about 40 minutes.
Conclusion; CK19 mRNA analysis with GD-100 can be applicable to an intra-operative detection of micrometastasis of lymph nodes regardless of some limitations.

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Chapter 22

Molecular Biology of Gastric Cancer

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Striking advances in molecular dissection of precancerous and cancerous lesions of the stomach indicate that genetic and epigenetic alterations in oncogenes, tumor suppressor genes, DNA-repair genes, cell-cycle regulators, telomeres, and telomerase, as well as genetic instability at microsatellite foci, are involved in the multistep process of human stomach carcinogenesis.[1–3] The scenario of these changes found in gastric carcinoma differs depending on the histologic type of gastric cancer, indicating that different carcinogenetic pathways exist for well-differentiated or intestinal-type carcinomas and poorly differentiated or diffuse-type 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 very different between the two types of gastric carcinoma.[4,5] Moreover, well differentiated–type gastric carcinomas are subdivided into intestinal type and gastric or foveolar type, according to the phenotypic characteristics of cancer cells.[6] More recently, we found that alterations of the *p73* gene, including loss of heterozygosity (LOH) and abnormal expression, may be responsible for the genesis of gastric adenocarcinoma with foveolar epithelial phenotype.[6]

This chapter provides a detailed overview of the molecular machinery that underlies stomach carcinogenesis and describes a system for the molecular–pathologic diagnosis of gastrointestinal cancer. This system may provide a new approach to cancer diagnosis and novel therapeutics for the twenty-first century.[7,8]

Telomeres and Telomerase

Human telomeres comprise 2 to 20 Kb of tandem repeated sequences (TTAGGG) and telomere-repeat binding proteins (TRF1 and TRF2). Recently, Griffith et al.[9] showed that telomeres in mammalian cells end as large terminal loops. In normal somatic cells without telomerase activity, the telomeres shorten with cell division and increased age, leading to senescence and eventually to crisis. However, in a few types of normal cells, including germ cells and hematopoietic or epithelial stem cells, and in most cancer cells, activation of telomerase allows the telomeres to be regenerated indefinitely, immortalizing these cells.[10,11] Human telomerase consists of several components including human telomerase RNA (hTR), telomerase-associated protein 1 (TEP1), and human telomerase reverse transcriptase (hTERT), which is a catalytic subunit component.[12] The expression of hTERT is closely associated with activation of telomerase in vitro and in vivo.[13,14] 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.[15]

Ninety percent of gastric carcinomas exhibit higher expression of hTERT and higher telomerase activity than does the corresponding non-neoplastic mucosa.[16] Most well-differentiated or intestinal-type carcinomas contain remarkably shortened telomere length, associated with high levels of telomerase activity. More importantly, over 50% of intestinal metaplasias share reduction in telomere length, and 35% of intestinal metaplasias, as well as gastric adenoma, express low levels of telomerase activity equivalent to about one-tenth of the activity in gastric carcinoma.[17] As for mechanisms of telomerase activation, results of a recent study suggest that Akt protein kinase is involved in activation of telomerase through phosphorylation of hTERT.[18] In fact, Akt protein kinase is expressed in gastric cancer tissue at high levels.

Immunohistochemical analysis shows that the hTERT protein is strongly expressed in the nuclei of the tumor cells of all carcinomas but weakly expressed in the nuclei of epithelial cells of intestinal metaplasia and gastric adenoma and in normal fundic

mucosa.[16] Thus, hTERT-positive epithelial cells in the above precancerous lesions and normal gastric mucosa may be viewed as epithelial stem cells. Moreover, the prevalence of *Helicobacter pylori* infection in gastric mucosa correlates well with the grade of intestinal metaplasia and the levels of hTR and telomerase activity, the latter of which is frequently associated with hyperplasia of hTERT-positive epithelial cells.[17,19] These observations indicate that *H. pylori* infection may be a strong trigger for hyperplasia of hTERT-positive cells in intestinal metaplasia, followed by increased telomerase activity and telomere reduction. Hyperplasia of hTERT-positive cells caused by *H. pylori* may induce chronic mitogenesis, which can facilitate increased mutagenesis. In fact, DNA hypermethylation at the D17S5 locus, pS2 loss, abnormal CD44 transcripts, CA repeat instability at the D1S19 locus, and *APC* and *p53* mutations, all of which are commonly seen in well-differentiated or intestinal-type gastric cancer, take place in over 30% of incomplete intestinal metaplasias.[20] These data all indicate that telomere reduction and hTERT overexpression due to stem cell hyperplasia are very early events in the multistep carcinogenesis of well-differentiated gastric cancer, followed by the epigenetic and genetic alterations described above. The frequent development of well-differentiated gastric cancer in aged patients with *H. pylori* infection indicates that this type of gastric cancer is a disease of a chronically afflicted genome rather than a genetic disease.

The hTERT gene promoter contains myc protein-binding sites and multiple consensus motifs for transcription factors, including SP1 and AP2.[21] These ubiquitous transcription factors may maintain the expression of hTERT in normal tissues. In fact, normal gastric mucosa expresses SP1, although at a much lower level than does gastric carcinoma.[22] Overexpression of hTERT in gastric carcinoma may be associated with increased levels of regulatory factors such as SP1 and c-myc protein or with low levels or loss of inhibitory factors for hTERT expression.

Recently, a new partner for telomerase, tankyrase, has been identified, a protein with homology to ankyrin and to the catalytic domain of poly (ADP-ribose) polymerase (PARP).[23] Moreover, lack of PARP by gene targeting is associated with severe chromosomal instability.[24] Tankyrase binds to TRF1, a negative regulator of telomere-length maintenance. By ADP ribosylation of TRF1, tankyrase may enable telomerase to replace lost DNA on the chromosome ends. We have found overexpression of TRF1 in most gastric cancer tissues. Of great interest is whether gene alterations or abnormal expression of tankyrase is linked to chromosomal instability implicated in stomach carcinogenesis.

Genetic Instability

Two types of genetic instability involve microsatellite instability (MSI) and chromosomal instability. Microsatellite instability is caused by altered DNA mismatch repair and has been found in 15% to 39% of sporadic gastric carcinomas worldwide.[25,26] Gastric carcinomas with a high frequency of MSI (MSI-H) can be divided into two subtypes: well-differentiated and poorly differentiated carcinomas, each of which has specific clinicopathologic characteristics. Well differentiated–type gastric cancers with MSI-H are often seen in patients over 73 years of age and often occur in the antrum pylori. They are frequently associated with abundant lymphoid infiltration, a putative favorable prognosis, and cancer multiplicity.[27,28] Hypermethylation of the *hMLH1* gene promoter occurs in over 70% of cases with this type of gastric cancer and is often associated with down-regulation or loss of *hMLH1*.[28,29] This evidence indicates that MSI-H in well-differentiated or intestinal type gastric cancer is mostly due to epigenetic inactivation of the *hMLH1* gene.

Poorly differentiated or diffuse-type gastric cancers with MSI-H occur mostly in patients under 35 years of age, and are often accompanied by scirrhous-type carcinoma with diffusely productive fibrosis.[30] However, poorly differentiated or diffuse-type gastric canceres harbor no germline mutation of *hMLH*1 and *hMSH2* and no alteration at BAT-RII. This type of gastric cancer is frequently associated with LOH on chromosome 17q21, including the *BRCA1* gene, although we have found no mutation of the *BRCA1* gene. There are two possibile explanations for this: *(1)* chromosome 17q12–21, including the *BRCA1* locus, may contain a candidate tumor suppressor gene, and *(2)* allelic loss of the *BRCA1* gene may be linked to frequent genetic instability in young patients with gastric cancer.

Microsatellite instability at the locus D1S191 (chromosome 1q) is found in 46% of well-differentiated or intestinal-type gastric cancers but not in any poorly differentiated–type gastric cancer. Microsatellite alteration at the same locus is also seen in 26% of incomplete-type intestinal metaplasias adjacent to primary gastric cancers. Moreover, an identical pattern of microsatellite alteration at the locus D1S191 is detected in both well-differentiated adenocarcinoma and the adjacent intestinal metaplasia, which suggests the sequential development of well-differentiated adenocarcinoma from incomplete intestinal metaplasia.[31] The results described above indicate that MSI at the D1S191 locus is one of the early events in the multistep process of stomach carcinogenesis.

Chromosomal instability (CIN) leading to DNA aneuploidy also underlies stomach carcinogenesis. Telomere length is necessary for maintaining chromosomal stability as described above. Mutations in the *p53* gene are also linked to CIN.

Tumor Suppressor Genes

Alterations in the structure and function of tumor suppressor genes, including *p53, p73, APC* and *DCC* and *FHIT,* are involved in stomach carcinogenesis. Among them, inactivation of the *p53* tumor suppressor gene by LOH and mutation is the most frequent genetic event in gastric cancer, occring in over 60% of gastric carcinoma regardless of histologic types.[1,32,33] Alterations in the *p53* gene are also found in 13% to 37% of intestinal metaplasias and 33% to 58% of gastric adenomas or dysplasias,[34–37] indicating that *p53* gene mutation is an early event in stomach carcinigenesis. The mutation spectrum of this gene can serve as a marker of the effect of putative carcinogens.[38] The mutation spectrum of the *p53* gene in gastric cancer patients in Hiroshima displays an intermediate pattern between that of colon cancer and esophagus cancer.[39–41] *p53* mutations at A:T are common in well-differentiated or intestinal-type carcinomas; G:C-to-A:T transitions are predominant in poorly differentiated–type carcinomas.[32] Carcinogenic *N*-nitrosamines, which cause mainly G:C-to-A:T base substitutions, are found in many foods and can also be produced from the amines with nitrates in the acidic environment of the stomach.[42,43]

Loss of heterozygosity of the *p73* gene, a newly discovered *p53*-related tumor suppressor gene, is detected in 38% of gastric cancers, especially well-differentiated adenocarcinomas that exhibit papillary structure–like foveolar epithelium and express the pS2 trefoil factor.[6] This type of gastric cancer with *p73* LOH shows allele-specific expression of *p73* but no gene mutation in the remaining allele. In addition, the incidence of *p53* abnormalities is low (25%). These observations indicate that LOH and

abnormal expression of the *p73* gene may play a large role in the genesis of foveolar-type gastric adenocarcinoma, although this is not in line with Knudson's classic two-hit model of carcinogenesis. We have already reported that 25% of well-differentiated gastric cancers show LOH on chromosome 1p by restriction fragment length polymorphism (RFLP) analysis using the MS1 (1p33–p35) probe.[1] However, these loci are rather centromeric when compared with the mapped region of the *p73* gene (1p36.33).

APC is a susceptible tumor suppressor gene for familial polyposis coli.[44] Mutations in the *APC* gene also take place in gastric cancers and sporadic colorectal cancers.[45] Interestingly, more than 50% of well-differentiated or intestinal-type gastric cancers harbor *APC* mutations, whereas poorly differentiated gastric cancers harbor no such mutations. Moreover, there is a distinct difference in the nature of *APC* mutations between gastric and colorectal cancers; namely, missense mutation is dominant in gastric cancer while nonsense mutation and frameshift mutation are common in colorectal cancers.[45] Somatic mutations of the *APC* gene are also seen in 20% to 40% of gastric adenomas and 6% of incomplete intestinal metaplsias.[46,47] *APC* alteration is viewed as an early genetic event in the pathogenesis of well -differentiated gastric cancers.[48] Loss of heterozygosity at the *DCC* locus is also one of the characteristics of well-differentiated gastric cancer and is seen in 50% to 60% of primary gastric cancers.[1,49]

The hypothesis that *FHIT* gene alterations are involved in the development of primary gastric cancer remains controversial. Huebner's group reported the rearrangement of the *FHIT* gene, aberrant transcripts, or both in 53% of primary gastric cancers and loss of FHIT protein in 67%.[50,51] Chen and colleagues demonstrated that aberrant transcripts were found not only in 46% of gastric cancers but also in 30% of noncancerous gastric mucosas.[52] Other studies showed that 13% to 16% of primary gastric cancers shared LOH of the *FIHT* gene and no abnormal transcripts,[53,54] although four of seven gastric cancer cell lines exhibited LOH of the *FHIT* gene.[53] *FHIT* gene alterations and loss of FHIT protein should be evaluated in series involving many cases of gastric cancer and precancerous lesions to determine whether environmental factors or putative carcinogens are associated with differences in frequency of FHIT abnormalities between countries.

Several distinct chromosomal loci are deleted in gastric cancers. Loss of heterozygosity at 1q and 7q are frequently associated with well-differentiated gastric cancer, while loss of 1p is relatively common in advanced poorly differentiated gastric cancer.[1] Moreover, LOH at the *bcl-2* gene locus is seen in many well-differentiated gastric cancers and colorectal cancers.[55] Our deletion mapping study on 7q shows that LOH at the D7S95 locus correlates well with peritoneal dissemination.[56] Recently, investigators in a study on allelic loss in xenografted human gastric carcinomas reported a high degree of allelic loss on several chromosomal arms (3p [81%], 4p [64%], 5q [69%], 8p [57%], 13q [59%], 17p [80%], and 18q [61%]) in 18 xenografted gastric adenocarcinomas.[57] From these assigned loci, candidates for the tumor suppressor gene responsible for stomach carcinogenesis may be identified in the future.

pS2, a gastric-specific trefoil factor normally expressed in the gastric feveolar epithelial cells, may function as a gastric-specific tumor suppressor. Recently, we found that the reduction or loss of the *pS2* gene by DNA methylation at the promoter region occurs in intestinal metaplasias and gastric adenomas.[58] Conversely, 32% of gastric cancers display strong expression of the *pS2* gene and 40% of gastric cancers, especially the well-differentiated type, show no expression.[60] Reduced expression or loss of the *pS2* gene due to promoter methylation may play a role in early stages of intestinal-type stomach carcinogenesis. Recent in vivo and in vitro studies suggest that the nuclear retinoid acid receptor β2 (RAR β2) and RUNX3 functions as tumor suppressors and that loss of the RAR β2 and RUNX3 by the promoter CpG hypermethylation is associated with gastric tumorigenesis.[59,60]

Oncogenes

Several protoncogenes, including c-*met*, K-*sam*, and c-*erbB2*, are frequently activated in gastric carcinomas. The amplification of the c-*met* gene encoding a receptor for hepatocyte growth factor/scatter factor (HGF/SF) is found in 19% of well-differentiated and 39% of poorly differentiated gastric cancers, frequently accompanied by diffusely productive fibrosis of the scirrhous type.[61] Most gastric carcinomas express two different c-*met* transcripts, one 7.0 kb and the other 6.0 kb. Expression of the 6.0 kb c-*met* transcript, which is expressed preferentially in cancer cells, correlates well with tumor staging, lymphnode metastasis, and depth of tumor invasion.[62] Soman and co-workers[63] reported that the *tpr-met* rearrangement is expressed in gastric carcinomas and gastric precancerous lesions. However, we have not detected *tpr-met* rearrangement in any gastric cancer or intestinal metaplasia.

The K-*sam* (KATO-III cell-derived stomach cancer amplified) gene has at least four transcriptional variants.[64] Type II encodes a receptor for keratinocyte growth factor.[64] Type II transcript is expressed only in carcinoma cells but not in cell lines from sarcomas. K-*sam* is preferentially amplified in 33% of advanced poorly differentiated or scirrhous-type gastric carcinomas but not in well-differentiated gastric carcinomas.[65] Moreover, K-*sam* is never seen in esophageal and colorectal carcinomas. Gastric cancers that overexpress K-sam protein are associated with a less favorable prognosis.

In contrast to K-*sam*, c-*erb*B2 is preferentially amplified in 20% of well-differentiated gastric cancers but not in poorly differentiated gastric cancer.[66,67] Overexpression of c-*erb*B2 associated with gene amplification is closely correlated with a poor prognosis and liver metastasis.[68,69] The amplification of c-*erb*B1 and c-*erb*B3 is found in 3%[67] and 0%,[70] respectively, of gastric cancers.

K-*ras* mutation is found in gastric intestinal metaplasias, adenomas, and well-differentiated adenocarcinomas,[1,71,72] although its incidence is low (10% to18%). However, K-*ras* mutation is not seen in poorly differentiated gastric cancer. The *hst*-1 gene, isolated from a surgical specimen of human gastric cancer by NIH/3T3 transformation assay, is rarely amplified in gastric cancer (2% of cases).[73]

Cell-Cycle Regulators

Genetic and epigenetic abnormalities in cell-cycle regulators are involved in the development and progression of gastric cancer by causing unbridled proliferation. Most gastric cancers are commonly associated with overexpression of positive regulators and reduction or loss of negative regulators, both of which cooperate to drive normal cells into malignancy.

The cyclin E gene is amplified in 15% to 20% of gastric carcinomas that are associated with overexpression.[74] The gene amplification, overexpression of cyclin E, or both cause aggressiveness and lymph node metastasis.[74] Cyclin D1 gene amplification, by contrast, is exceptional in gastric carcinomas but frequently occurs in esophageal carcinoma.[75]

CDC25 phosphatases dephosphorylate the threonine and tryosine residues at positions 14 and 15 in the cyclin-dependant kinases (CDKs) and then activate them.[76] Three types of CDC25 have been identified: CDC25A, CDC25B, and CDC25C.[77] CDC25A is expressed early in the G_1 phase of the cell cyle, CDC25B is expressed in both the G_1/S and G_2 phases.[78] CDC25C is predominantly expressed in the G_2 phase. CDC25B is overexpressed in more than 70% of gastric cancers regardless of histologic type and closely correlates with tumor invasion and nodal metastasis.[79] Only 2% of gastric adenomas overexpress CDC25B, however, and no gene amplification of CDC25B has been found in any gastric cancers. In 38% of gastric cancers, CDC25A is overexpressed but CDC25C is at very low or undetectable levels. Thus, the overexpression of CDC25B in tumor cells may stimulate progression of gastric cancer.

In addition to the binding of cyclin/CDK complexes to CDK-inhibitory proteins, CDKs interact with regulatory protein that is the product of the *suc*1 (suppressor of Cdc temperature-sensitive mutations) gene or *Cks1* (Cdc28 kinase subunit) gene in *Saccharomyces*.[80] Cks proteins are necessary for cell-cycle progression in vivo and are physically associated with active forms of CDKs.[80] CKshs1 is a human homolog of the yeast cell-cycle regulatory proteins Cks1 and suc1.[81] We have already found that more than 60% of gastric cancers show higher levels of CKshs1 than do the corresponding normal mucosa.[82] CKshs1 overexpression is less frequent in well-differentiated adenocarcinomas than in poorly differentiated adenocarcinomas. There is no obvious relationship between CKshs1 overexpression and either tumor progression or *p53* mutations. No gene amplification has been found in any gastric cancer cell lines. These findings suggest that overexpression of CKshs1 plays a role in the development of well-differentiated type gastric cancer.

As for negative cell-cycle regulators, the p53-inducible cyclin-dependent kinase inhibitor p21 is associated with the senescence of non-neoplastic gastric epithelial cells.[83] In the neoplastic lesions, the expression of p21 is seen in 78% of gastric adenomas and 76% of gastric adenocarcinomas regardless of *p53* gene mutation. This finding suggests that a *p53*-independent pathway is substantially involved in the induction of p21 in gastric tumors.[84] In fact, the growth inhibition of transforming growth factor β (TGF-β) or retinoic acid is associated with *p53*-independent induction of p21 in a gastric cancer cell line.[85] Moreover, the strong expression of p21 in cancer cells is frequently observed in advanced cancers and nodal metastasis, whereas there is no inverse correlation between p21 expression and proliferative activity measured by Ki-67. These findings overall indicate that the proliferative activity of gastric cancer is not solely dependent on control of the cell cycle by p21.[86] In addition, mutation of the p21 gene is exceptional in gastric cancer[86] and a codon 31 polymorphism does not affect the expression levels of p21.[87]

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-γ is associated with the induction of p27 in a gastric cancer cell TMK-1.[88] More important, reduction in p27 expression is frequently seen in advanced gastric cancers, whereas p27 is well preserved in 90% of gastric adenomas and 85% of early cancers.[89] Gastric adenomas with reduction or loss of p27 are capable of developing into malignacies. Reduced expression of p27 significantly correlates with depth of tumor invasion and nodal metastasis. Moreover, metastatic tumor cells in lymph nodes express p27 at weaker levels than those in primary tumors, which suggests that tumor cells with reduction or loss of p27 may selectively metastasize to lymph nodes or distant organs.[90] The expression of p27 in gastric cancer is inversely correlated with the expression of cyclin E.[91] Loss of p27 function and gain of cyclin E evidently stimulate progression and metastasis of gastric carcinomas. Reduction in p27 expression occurs at post-translational levels, resulting from ubiquitin-mediated proteosomal degradation rather than genetic abnormalities.[90]

Deletion or mutations of the *p16* gene are uncommon in primary gastric carcinomas,[91–93] while homozygous deletion of the *p16* gene has been found in two of eight gastric cancer cell lines, and lack of p16 protein expression has been found in five of eight gastric cancer cell lines.[94] Another mechanism of *p16* gene silencing is hypermethylation of the 5 CpG island.[95] In particular, loss of p16 protein is often seen in advanced cancers with nodal metastasis. Loss of p16 and p27 proteins may be associated with the progression of gastric carcinoma. Recently, Chen and co-workers reported that aberrant RNA transcripts of the *p16* gene were noted in 30% to 45% of primary gastric cancers.[96]

Major alterations in the *Rb* gene are also infrequent in primary gastric cancers.[97] All primary tumors and all gastric cancer cell lines express *pRb*.[94]

An important downsteam target of cyclin/Cdks at the G_1/S transition is a family of E2F transcription factors. Gene amplification of *E2F-1* is seen in 4% of gastric cancers and 25% of colorectal cancers.[98] Overexpression of E2F is found in 40% of primary gastric carcinomas.[98] Moreover, E2F and cyclin E tend to be coexpressed in gastric cancer. In contrast, 70% of gastric cancers exhibit lower levels of E2F-3 expression than do corresponding non-neoplastic mucosas. These results suggest that gene amplification and anomalous expression of the *E2F* gene may permit the development of gastric cancer.

Growth Factors and Cytokines

Gastric cancer cells express a broad spectrum of growth factors, cytokines, or both, including TGF-α, epidermal growth factor (EGF), amphiregulin (AR), cripto, heparin binding (HB)-EGF, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF) II, TGF-β_1, basic fibroblast growth factor (bFGF), interleukin (IL)-1α, IL-6, IL-8, and osteopontin (OPN).[2,4,99,100] These growth factors and cytokines function as autocrine, paracrine, and juxtacrine modulators of growth of cancer cells, and they organize the complex interaction between cancer cells and stromal cells that plays a key role in morphogenesis, invasion, neovascularization, and metastasis. Interestingly, the expression of these growth factors, cytokines, or both by cancer cells differs between the two histologic types of gastric carcinoma. The EGF family, including EGF, TGF-α, and cripto, is commonly overexpressed in well-differentiated gastric carcinoma; TGF-β, IGF-II, and bFGF are predominantly overexpressed in poorly differentiated gastric carcinoma.[101] Coexpression of EGF/TGF-α, EGF receptor, and cripto correlates well with the biological malignancy of gastric cancer, because these factors induce metalloproteinases.[102–104] Overexpression of cripto is frequently associated with intestinal metaplasia and gastric adenoma.[105]

In the EGF family, AR, which is overexpressed in more than 60% of gastric carcinomas regardless of histologic type,[106] works as an autocrine growth factor and induces the expression of AR itself, TGF-α, and EGF receptors by gastric cancer cells.[107] 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 (GCF) may regulate gene expression of these growth factors and receptors.[108]

IL-1α is a cytokine mainly produced by activated macrophages and mediates many of the local and systemic responses to infection and inflammation.[109] IL-1α is also produced by gastric cancer cells.[110] We have found that IL-1α evidently acts as an atuocrine growth factor for gastric carcinoma cells and plays a pivotal role as a trigger for induction of EGF and EGF receptor expression.[110] The expression of IL-1α by tumor 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 tumor cells themselves. This finding indicates that an intimate interplay between IL-1α and the EGF/EGF receptor system stimulates the growth of gastric cancer.

In addition to IL-1α, IL-6 is an autocrine growth stimulator for gastric cancer cells.[82] The expression of IL-1α by tumor cells is induced by IL-6, whereas IL-1α increases the expression of IL-6 by tumor cells themselves.[111]

Our recent findings suggest that interaction between c-*met* overexpressed on tumor cells and HGF/SF from stromal cells is related to the morphogenesis and progression of gastric cancer in vivo. Stromal cells, especially fibroblasts stimulated by growth factors or cytokines such as IL-α, TGF-α, and TGF-β, secrete HGF, which can function in a paracrine manner as morphogen or motogen of tumor cells. For example, when a clone maintains expression of cell-adhesion molecules, tubular formation of tumor cells is promoted, resulting in well-differentiated gastric cancer. Conversely, when a clone has reduced expression of cell-adhesion molecules, HGF/SF can act as a motogen and induce scattering of tumor cells, resulting in poorly differentiated gastric cancer.[2,48]

The negative growth factor TGF-β_1 is commonly overexpressed in gastric carcinoma, particularly in poorly differentiated carcinoma with diffusely productive fibrosis.[112] However, most human gastric cancer cells escape from TGF-β–induced growth inhibition at the receptor or postreceptor levels. TGF-β inhibits 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 CDK activity, reduced phosphorylation of *Rb*, and a decrease in cylin A.[113,114] Various mutations in the TGF-β receptor type II (RII) gene have been reported in gastric cancer. One type of mutation in the TGF-β RII gene is mutation in the polyA tract (i.e., 1- or 2-base deletion or insertion) that frequently occurs in the hereditary nonpolyposis colon cancer syndrome (HNPCC)[115] and in gastric carcinoma with MSI-H.[26] Another type of mutation in the TGF-β RII gene involves abnormal amplification and truncation of the TGF-β RII gene.[116] However, we have not seen genetic alterations of the TGF-β RII gene in any gastric carcinoma cell lines. Moreover, results of a recent study on expression of TGF-β RI in TGF-β–resistant gastric cancer cell lines that contain no discernible alteration in the TGF-β RII gene suggest that hypermethylation of a CpG island in the 5′ region of the TGF-β RI gene is involved in another potentially important mechanism of escape from negative growth control by TGF-β.[117] We have already found that most gastric carcinomas show reduced levels of TGF-β RI and that this correlates well with the depth of tumor invasion.[118]

More recently, Ito et al.[119] reported that mice lacking PEBP2αC had hyperplasia of gastric epithelial cells in the glandular stomach and that four of nine human gastric cancer cell lines showed no expression of PEBP2αC. These observations suggest that loss of PEBP2αC, which binds directly to Smad 1, 2, 3, and 5, provides a novel mechanism of escape from TGF-β–mediated control. Additionally, we have found no mutation of *Smad2* in any gastric carcinomas or cell lines.[120]

A large number of angiogenic factors in human malignancy have been identified. Among them are VEGF, bFGF, and IL-8, which are derived from tumor cells and participate mainly in neovascularization within gastric carcinoma tissues. We have shown that all eight gastric cancer cell lines secrete VEGF into the condition medium.[121] EGF or IL-1α up-regulates VEGF expression by tumor cells, whereas interferon-γ down-regulates it by tumor cells. Moreover, VEGF promotes angiogenesis and the progression of gastric carcinomas, especially the well-differentiated type.[122] By contrast, bFGF produced by tumor cells is frequently associated with angiogenesis and extensive fibrosis in poorly differentiated gastric carcinoma, particularly of the scirrhous type.[123]

IL-8, a member of the CXC chemokine family, induces haptotatic migration and proliferation of melanoma cells and angiogenesis.[124,125] More important, gastric carcinoma cell lines express mRNA and protein for IL-8 and IL-8 receptors (IL-8RA and IL-8RB).[124,125] More than 80% of gastric carcinomas coexpress IL-8 and IL-8 receptors; this coexpression directly correlates with tumor vascularity and disease progression. IL-8 enhances the expression of EGF receptor, type IV collagenese (MMP-9), VEGF, and IL-8 itself by gastric cancer cells, whereas IL-8 decreases expression of E-cadherin mRNA. In addition, IL-8 also increases MMP-9 activity and invasion through matrigel of gastric cancer cells.[125] Taken together, IL-8 may play an important role in the growth and progression of gastric carcinoma by autocrine and paracrine mechanisms.

Cell Adhesion Molecules, Metastasis-Related Genes, and Mucin

Cell adhesion molecules may also work as tumor suppressors. Mutations in the E-cadherin gene have been reported to occur preferentially in 50% of poorly differentiated gastric carcinomas.[126] E-cadherin gene mutation is found in the diffuse component of mixed gastric carcinomas composed of the intestinal type and diffuse type.[127] Results of a 1999 study indicate that E-cadherin mutations affecting exons 8 or 9 induce the scattered morphology, decrease cellular adhesion, and increase cellular motility of poorly differentiated or diffuse-type gastric cancers.[128] The mutations are detected even in intramucosal carcinoma.[129] E-cadherin germline mutations in familial gastric cancer have been reported since 1998 but their frequency is extremely rare.[130–133] Kawanishi and co-workers[134] found that the poorly differentiated gastric carcinoma cell line HSC-39 contained a mutation of the β-catenin gene. Moreover, Caca and co-workers[135] reported that β- and γ-catenin mutations but not E-cadherin inactivation brought about constitutive Tcf transcriptional activity in gastric and pancreatic cancer cells. In addition to genetic alterations in E-cadherin and β-catenin, crosstalk between β-catenin and receptor tyrosine kinases including c-*met*, EGF receptor, and c-*erb*B2 takes place in gastric cancer cells in vitro and in vivo, leading to diffuse spreading or scattering of gastric cancer cells.[136,137] These results indicate that genetic and epigenetic alterations in E-cadherin and catenins are involved in the development and progression of poorly differentiated and scirrhous-type gastric cancers.

The *CD44* gene contains at least 20 exons, 12 of which can be alternatively spliced to make up a wide variety of molecular variants.[138,139] We have found that expression of abnormal CD44 transcripts, including exon 11, is frequently associated with primary gastric carcinomas and metastatic tumors.[140] Moreover, the pattern of abnormal CD44 transcripts in the tumors differs between well-differentiated and poorly differentiated gastric cancers. More important, all gastric cancer tissues and gastric cancer cell lines show overexpression of abnormal CD44 transcripts containing the intron 9 sequence,[141] which suggests that the abnormal CD44 transcript containing the intron 9 sequence is presumably an effective biomarker for early detection of gastric cancers. Sixty percent of gastric intestinal metaplasias express CD44 variants containing an intron 9 sequence; normal gastric mucosa does not express these variants.[142]

Osteopontin, also termed Eta-1 (early T-lymphocyte activation 1), a reported protein ligand of CD44, is overexpressed in 73% of gastric carcinomas.[143] The coexpression of OPN and CD44v9 in tumor cells correlates with the degree of lymphatic vessel invasion or distant lymph node metastasis in poorly differentiated gastric cancer.[144] In particular, clustering of the tumor cells in lymphatic vessels shows strong coexpression of OPN and Cd44v9. Therefore, mutual interactions between OPN and CD44v9 on the tumor cells may be used by CD44-bearing, poorly differentiated gastric carcinomas to promote lymphogenous metastasis.

A candidate suppressor gene related to metastasis, *nm23*, encodes nucleotide diphosphate kinase and c-*myc* transcription factor. Although LOH of the *nm23* gene in gastric cancer is rare, the reduced expression of *nm23*, presumably due to epigenetic mechanisms, is frequently associated with metastasis of gastric cancer.[145] In addition to *nm23*, galectin-3, which belongs to a family of galactoside-binding proteins, is frequently overexpressed in primary tumors and liver metastases of well-differentiated gastric cancer.[146] This higher expression of galectin-3 in gastric cancers and metastases implicates this lectin in the metastatic phenotype.

Amplification of c-*met* or K-*sam* in gastric cancer evidently contributes to progression and peritoneal invasion of poorly differentiated gastric carcinoma. In addition, peritoneal dissemination requires LOH of 7q. Our study on deletion mapping of 7q has already demonstrated that LOH at the D7S95 locus is frequently associated with peritoneal dissemination.[147] The D7S95 locus may contain a candidate suppressor gene for the progression and metastasis of gastric cancer.

To date, nine human mucin genes have been identified: *MUC1, MUC2, MUC3, MUC4, MUC5ac, MUC5B, MUC6, MUC7,* and *MUC8*.[148] Among them, The *MUC1* gene encodes a high-molecular-weight transmembrane glycoprotein with a tandem repeat region (VNTR) in the extracellular domain. *MUC1* mucin is expressed in normal gastric mucosa and the aberrant expression of its underglycosylated form has been described in a variety of malignancies.[149] Results of a study published in 1998 showed that expression of an underglycosylated form of *MUC1* is significantly associated with aggressiveness and lymphatic invasion of gastric cancer.[149] More important, *MUC1* gene polymorphism may have implications for susceptibility to gastric carcinogenesis.[124] Small *MUC1* alleles and small *MUC1* genotypes are significantly more

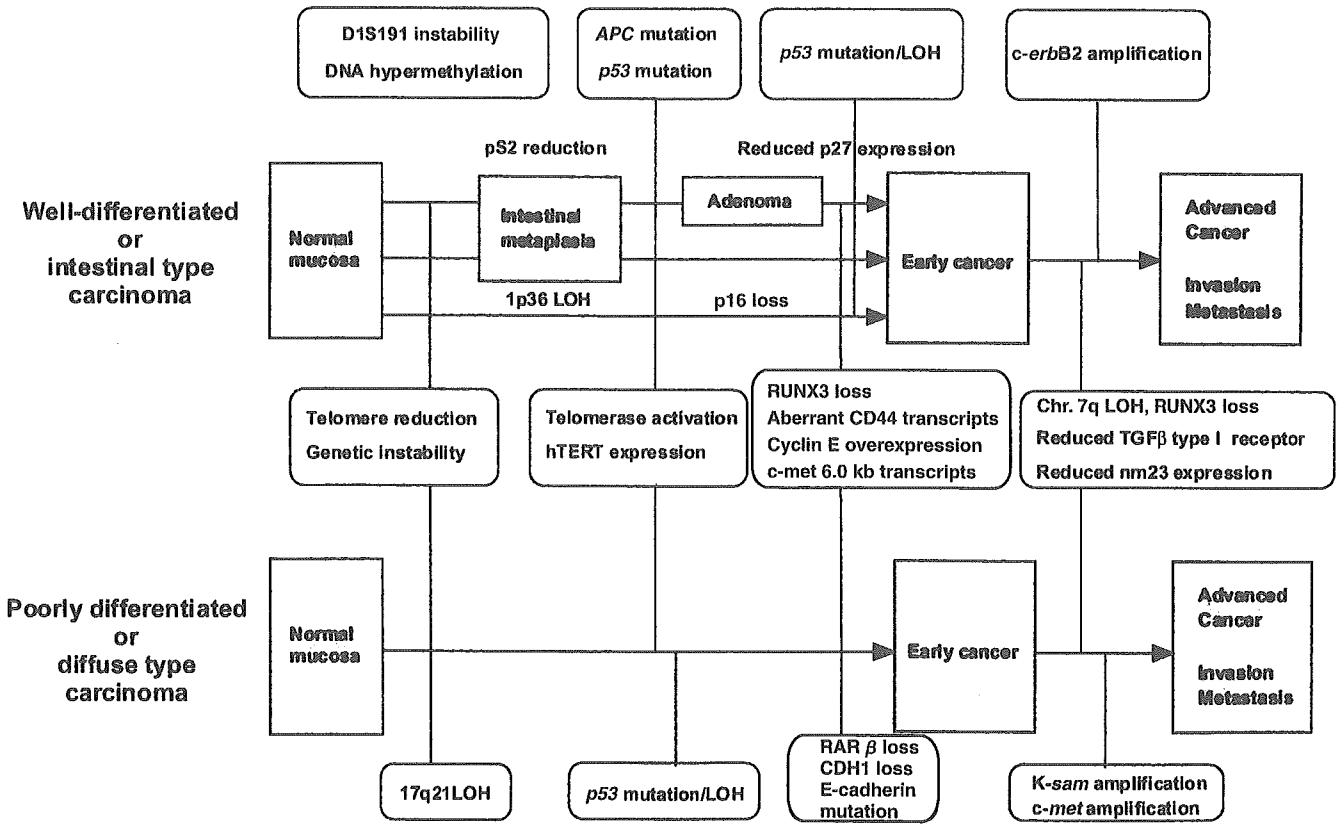

Figure 22-1 Genetic pathway of two types of gastric cancer.

frequent in patients with gastric carcinoma than in controls, and a significant association has been observed between small *MUC1* genotypes and glandular atrophy due to *H. pylori*.[150] These observations indicate that individuals with small *MUC1* genotypes, especially persons infected with *H. pylori*, are at increased risk for development of gastric carcinoma. It would be interesting to note whether the Japanese population has high frequency of small *MUC1* genotypes, because the highest incidence of gastric cancer is still observed in Japan.

Genetic Pathway of Two Types of Gastric Cancer

The overall observations on molecular events of gastric cancer may provide supporting evidence for our working hypothesis that there are two distinctive major genetic pathways of stomach carcinogenesis (Fig. 22–1). Genetic and epigenetic alterations found in two types of gastric cancer are summarized in Table 22–1. The scenario of these epigenetic alterations found gastric cancer differs, depending on the two types of gastric cancer, indicating that there are at least two types of CpG island methylator phenotype responsible for the development of intestinal- and diffuse-types gastric cancer. Genetic instability, including microsatellite instability 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 well differentiated–type or intestinal-type gastric carcinogenesis, infection with *H. pylori* may be a strong trigger for hyperlasia of *hTERT*-positive stem cells in intestinal metaplasia. Genetic instability and hyperplasia of *hTERT*-positive stem cells may pre-

Table 22–1 Genetic and epigenetic alterations found in two types of gastric cancer of the stomach

	Incidence of Cases with Indicated Alterations (%)	
Genetic and Epigenetic Alterations	*Well Differentiated*[a]	*Poorly Differentiated*[a]
Tumor Suppressor Genes		
p53 LOH, mutation	60	75
p73 LOH	53[b]	24
APC LOH, mutation	40–60	0
DCC LOH	50	0
LOH of Chr.1a	44	0
LOH of Chr.7q	53	33
LOH of Chr.17q	0	40[c]
Loss of *pS2* expression	49	31
Loss of RAR β	50	73
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	10
Loss of *p27* expression	46	69
Oncogenes		
K-*ras* mutation	10	0
c-*met* amplification	19	39
K-*sam* amplification	0	33
c-*erbB2* amplification	20	0
Adhesion Molecules		
E-cadherin mutation	0	50
Loss of CDH1	55	79
CD44 aberrant transcript	100	100
Growth Factors and Cytokines		
VEGF overexpression	46	9
IL-8 overexpression	75	85
TGF-β overexpression	33	71
Microsatellite instability	20–40	20–70
(h MLH1 methylation)	(5–20)	(0)
Telomere/telomerase		
Telomere reduction	62	53
Telomerase activity	100	90
TERT expression	100	86

IL-8, interleukin-8; TERT, telomerase reverse transcriptase; TGF-β, transforming growth factor β; VEGF, vascular endothelial growth factor.

[a]According to the criteria of the Japanese Classification of Gastric Carcinoma.

[b]Preferentially found in foveolar-type adenocarcinoma.

[c]Preferentially found in patients younger than 35 years of age.

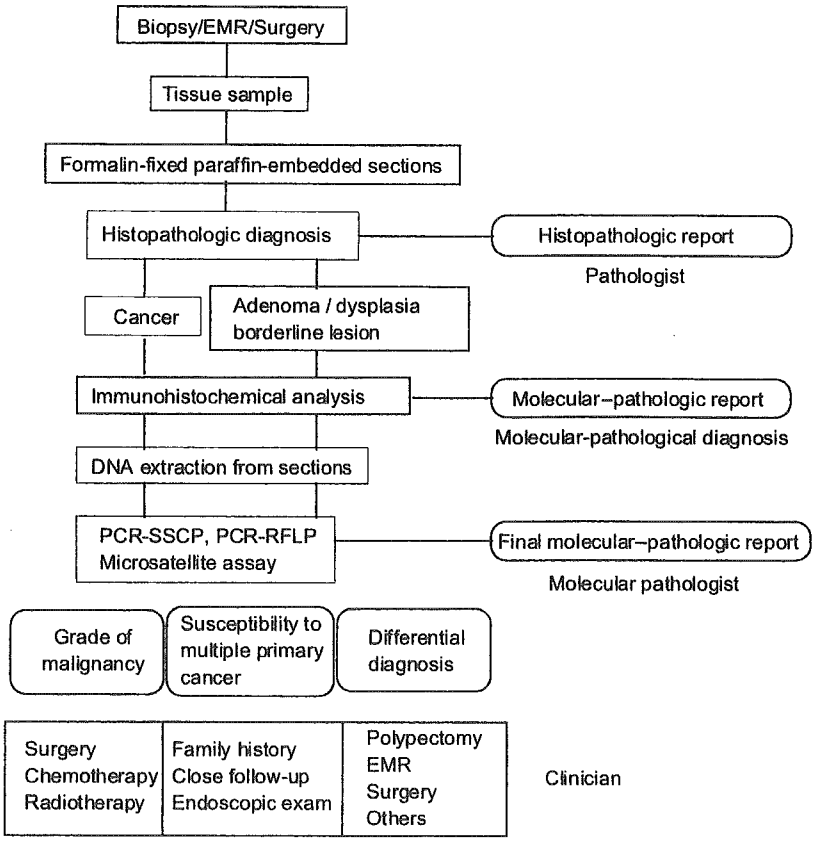

Figure 22-2 Strategy of molecular–pathological diagnosis of gastero-intestinal lesions. EMR, electromagnetic radiation; PCR-RFLP, polymerase chain reaction–restriction fragment length polymorphism; PCR-SSCP, PCR–single-strand conformation polymorphism.

cede replication error at the D1S191 locus, DNA hypermethylation at the D17S5 locus, pS2 loss, p16 loss, CD44 abnormal transcripts, and *p53* mutation, all of which accumulate in at least 30% of in complete intestinal metaplasias. All of these epigentic and genetic alterations are common events in intestinal-type gastric cancer. Incomplete intestinal metaplasia that contains an accumulation of the above multiple molecular events, i.e., 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, RUNX3 loss, *p53* mutation, and LOH, reduced *p27* expression, cyclin E expression, and presence of c-*met* 6.0 kb transcripts allow malignant transformation from the above precancerous lesions to intestinal-type gastric cancer. *DCC* loss, *APC* mutations, 1q LOH, *p27* loss, reduced TGF-β type I receptor expression, reduced nm23 expression, and c-*erb*B gene amplification, as well as RUNX3 loss, are implicated in the progression and metastasis of intestinal-type gastric cancer. Another pathway for carcinogenesis of well-differentiated gastric cancer involves LOH and abnormal expression of the *p73* gene, which may be responsible for the development of foveolar-type gastric cancers with *pS2* expression.

Loss of heterozygosity at chromosome 17p, mutation or LOH of *p53*, RAR β 2 loss, and mutation or loss of E-cadherin, however, are preferentially involved in the development of poorly differentiated gastric cancers. Interestingly, hypermethylation of the RAR β 2 and CDH1 promoters occurs concordantly. In addition to these changes, gene amplification of K-*sam* and c-*met*, *p27* loss, and RUNX3 loss as well as reduced *nm23* obviously confer progression, metastasis, and diffusely productive fibrosis. One point should be added here: mixed gastric carcinomas composed of well-differentiated and poorly differentiated components exhibit some, but not all, of the molecular events described so far for each of the two constituent types of gastric cancer.

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

Molecular-Pathologic Diagnosis

By analyzing genetic and epigenetic alterations in pathology specimens, we can facilitate and improve cancer diagnosis, predict the grade of malignancy or patient prognosis, identify patients at high risk for developing multiple primary cancers, and discover novel therapeutic approaches. Since 1993, a system of molecular–pathologic diagnosis of gastrointestinal cancer based on the findings discussed in this chapter has been routinely provided by the First Department of Pathology, Hiroshima University School of Medicine, in colllaboration with the Hiroshima City Medical Association Clinical Laboratory.[7,8,150]

The strategy of molecular–pathological diagnosis of gastrointestinal lesions is illustrated in Fig. 22–2. The cases of cancer, adenoma (dysplasia), and borderline lesion are selected for immunohistochemical analysis by using a set of biomarkers

Table 22–2 Biological markers used for molecular–pathologic diagnosis of gastrointestinal cancer

Tumor	*Biological Marker*	*Purpose*
Esophagus	*p53*	Diagnose disease
Esophagus	EGF, TGF-α, EGFR, Ki-67	Determine malignancy grade
Esophagus	Cyclin D1	Predict metastasis
Stomach	*p53, APC*	Diagnose disease
Stomach	TGF-α, EGFR, cripto, c-*met*, c-*erbB2*, CyclinE, CDC25B, *p27*, Ki-67	Determine malignancy grade Predict metastasis
Stomach	*nm23, CD44*	Predict susceptibility to multiple cancers
Colon	*p53, APC, CD44*	Diagnose disease
Colon	EGF, TGF-α, cripto, EGFR, Cyclin E, *p27, p21,* Ki-67	Determine malignancy grade
Colon	*nm23, SLX*	Predict metastasis
Colon	Replication error/mismatch repair genes	Predict NHPCC

NHPCC, hereditary nonpolyposis colon cancer.

(Table 22–2). Genetic analyses are performed using several primers for detecting *p53* and *APC* mutations, LOH, and microsatellite instabilility at five loci of two CA repeats (D1S191, D17S855, BRCA1) and two polyA tracts (BATRII, BAT40).

As reported recently,[150] 10,419 lesions (from 9241 cases) were examined from August 1993 to November 1998. These consisted of 221 esophageal lesions (216 cases), 4647 gastric lesions (4435 cases), and 5551 lesions colorectal lesions (4590). Their histologic diagnoses were adenoma, dysplasia, borderline lesion, carcinoma, and suspicious lesion for carcinoma. The results of molecular–pathologic diagnosis on gastric lesions are summarized in Table 22–3. Of 1154 adenomas, 10% were diagnosed as adenoma with malignant potential, and 2% were suspected of being adenocarcinoma on the basis of an abnormal accumulation of *p53* and mutation or LOH of the *p53* gene as well as of the *APC* gene. Of the borderline lesions, 22% were judged to be carcinomas. Of 2969 adenocarcinomas, more than 80% of which were early cancers, 12% were regarded as high-grade malignancies, based on overexpression of c-*erb*B2, EGF receptor, and cyclin E and loss of *p27*. By microsatellite analysis of about 700 gastric cancer cases, MSI-H was found in approximately 4% of gastric cancers, 50% of which were confirmed as having multiple primary cancers. Follow-up study is necessary to prove the priority of the molecular–pathologic diagnosis. The 2-year survival rates of the patients after surgical removal of gastric and colorectal carcinomas show that the patients with high-grade malignancies had poorer prognoses.

Although the evaluation of our strategy requires continuous assessment and follow-up studies, the evidence cited above indicates that it provides new opportunities for early cancer diagnosis and more accurate evaluation of prognosis. Although DNA-, RNA-, and protein-chip technology will greatly promote new directions in cancer diagnosis and therapeutics in the future, pathologic analysis of precancerous and cancerous lesions remains the first step in DNA, RNA, and protein analyses.

Table 22–3 Molecular–Pathologic Diagnosis of Gastric Lesions

Histologic Diagnosis	*Lesions*	*Molecular Diagnosis*[a]	n *(%)*
Adenoma	1154	Adenoma with malignant potential	119 (10)
		Suspected adenocarcinoma	19 (2)
Borderline	469	Adenoma with malignant potential	6 (1)
		Suspected adenocarcinoma	45 (10)
		Adenocarcinoma	53 (11)
Suspected adenocarcinoma	250	Adenocarcinoma	63 (25)
		High-grade malignancy	3 (1)
Adenocarcinoma	2969	High-grade malignancy	357 (12)

[a]Molecular diagnosis is made according to both molecular and histologic findings.

Definitions of molecular diagnosis are as follows. *Adenoma with malignant potential* is an adenoma with a high probability of becoming carcinoma. Tumors contain some molecular and genetic abnormalities, including abnormality of the *p53* gene, but morphologic aberration is within the range for benign tumor. Suspected adenocarcinoma is a tumor that exhibits genetic abnormalities of *p53* and/or *APC* as well as abnormal expression of many cancer-related molecules such as c-*erb*B2, cyclin E, EGFR, c-*met*, and so on. Biological atypia is not severe enough to be regarded as carcinoma. Adenocarcinoma is a tumor whose genetic and molecular abnormalities are the same degree as for suspected adenocarcinoma and that can be regarded as malignant histologically if such molecular finding are available.

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Histone Acetylation and Gastrointestinal Carcinogenesis

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ABSTRACT: **The importance of altered histone acetylation in gastrointestinal carcinogenesis, especially in relation to invasion and metastasis, is described. Histone acetylation and chromatin remodeling linked with CpG island methylation play a major role in epigenetic regulation of gene expression. Acetylation of histones through an imbalance of histone acetyltransferases and deacetylases disrupts nucleosome structure, which leads to DNA relaxation and subsequent increase in accessibility to transcription factors. The expression of acetylated histone H4 is reduced in a majority of gastric and colorectal cancers, indicating the low level of global histone acetylation in tumor cells. Moreover, reduced histone acetylation is significantly associated with depth of tumor invasion and nodal metastasis of gastrointestinal cancers. A histone deacetylase inhibitor, trichostatin A (TSA), induces growth arrest and apoptosis and suppresses invasion of cancer cells. Treatment with TSA, which is followed by increased histone acetylation in the promoters, induces the expression of many genes that are suppressors of invasion and metastasis, including tissue inhibitors of metalloproteinase and nm23H1/H2, in addition to negative cell cycle regulators and apoptosis-related molecules. Our approach, serial analysis of gene expression (SAGE), enabled us to identify a gene that is a novel candidate for a metastasis suppressor, whose expression is induced by histone acetylation. These findings suggest that, by modifying gene expression, histone deacetylation may participate not only in tumorigenesis but also in invasion and metastasis. Therefore, histone acetylation should be a promising target for cancer therapy, especially against invasive and metastatic disease, but also for cancer prevention.**

KEYWORDS: **histone acetylation; gastric cancer; colorectal cancer; metastasis; invasion; serial analysis of gene expression**

INTRODUCTION

Among the various epigenetic alterations that lead to modified gene expression, the most important are believed to be DNA methylation and chromatin remodeling by histone modification.[1–4] As shown in FIGURE 1, the nucleosome is composed of core histones, which are wrapped by turns of double-stranded DNA. The amino-

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