Review article

Molecular diagnosis of gastric cancer: present and future

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Abstract

Although histopathological diagnosis is extremely useful for the definitive as well as the supportive diagnosis of gastric cancer in clinical practice, it is limited in certain respects. Over the past 15 years, integrated research in molecular pathology has clarified the details of genetic and epigenetic abnormalities of cancer-related genes in the course of the development and progression of gastric cancer. These abnormalities, which include telomerase activation, genetic instability, and abnormalities in oncogenes, tumor suppressor genes, cell-cycle regulators, cell adhesion molecules, and DNA repair genes, could be effective markers in the molecular diagnosis of gastric cancer. It is possible that the molecular analysis of these alterations in histopathology specimens may overcome deficiencies in diagnoses that depend only on histomorphology, and, consequently, we may be able to improve the differential diagnosis of cancer, obtain information on the grade of malignancy, and identify patients at high risk of developing multiple primary cancers. In Hiroshima, we have established a system of molecular-pathological diagnosis as a routine service; about 5000 lesions of the stomach have been subjected to this diagnosis, and much useful information has been obtained. In the near future, genetic analysis by means of DNA microarray may become routine in the diagnosis of gastric cancer. Genetic analysis of histopathology specimens may make clear the characteristics of individual cancers; indicating the common and specific features of molecular pathogenesis that may be directly connected with gene therapy or molecular-targeted therapy. By analyzing the relationship between single-nucleotide polymorphisms and cancer susceptibility, we will be able to obtain information on cancer prevention from histopathology samples.

Key words Molecular diagnosis · Gastric cancer · Histopathology · Genetic alterations · Epigenetic alterations

Introduction

According to the Hiroshima Tumor Registration Committee, which registers tumors that have been diagnosed by pathologists, the number of cases registered has increased threefold for gastric cancer and 20-fold for gastric adenoma over the past 20 years. This suggests that, recently, many tumors have been biopsied or dissected endoscopically, while tumor tissues were collected only by surgery in earlier periods. Although histopathological diagnosis is, thus, extremely important for obtaining definitive and supportive diagnoses, diagnosis that depends only on histopathology has certain limitations. Many lesions have a morphology which is between that of benign and malignant lesions, and differential diagnosis is therefore difficult. Variations between pathologists may lead to changes in assessments of the lesion. Differences in diagnostic criteria, such as those for early gastrointestinal cancer diagnosed by Western and Japanese pathologists, lead to misunderstanding by clinicians. Furthermore, information derived from morphology is of limited use in determining the degree of malignancy and the patient's prognosis. No genetic information can be obtained in hereditary cancers, such as hereditary non-polyposis colorectal cancer (HNPCC) kindreds.

Integrated research in molecular pathology over the past 15 years has clarified details of the genetic and epigenetic abnormalities of cancer-related genes in the course of the development and progression of gastric cancer [1–3]. Because most of these alterations can be analyzed in the formalin-fixed specimens that are taken for the purpose of histopathological diagnosis, these alterations are good targets for molecular diagnosis [4–7]. It is possible to apply molecular findings in tissue samples to diagnosis, together with the results of morphology. Thus, molecular diagnosis can overcome the drawbacks of histopathological diagnosis and can make

a great contribution to the understanding of cancer histopathology.

This review outlines the procedure for molecular diagnosis in gastric specimens that has been routinely implemented in Hiroshima. An overview of the genetic and epigenetic alterations in gastric cancer is given, to show the significance of these changes as biological markers in diagnosis. We also discuss future prospects for the molecular diagnosis of gastric cancer.

Molecular basis of multistep stomach carcinogenesis

In the course of multistep carcinogenesis in the stomach, various genetic alterations of oncogenes, tumor suppressor genes, DNA repair genes, cell-cycle regulators, and cell adhesion molecules are accumulated (Fig. 1) [1–3]. Of the various genetic and epigenetic alterations, some are found in both well and poorly differentiated types of carcinoma, while others are found in only one particular histological type. Genetic instability, inactivation of tumor suppressor genes by CpG island methylation, and telomerase activation commonly participate in the initial step of stomach carcinogenesis. While *p53* gene loss and mutation occurs at an early stage of carcinogenesis, amplification of the *c-met* and cyclin E genes is frequently associated with an advanced stage. Reduced expression of *p27^{Kip1}*, a cyclin-dependent kinase (CDK) inhibitor, participates in both the development and the progression of gastric cancer. Overexpression of growth factors and cytokines participates in progression. *K-ras* mutations, amplifica-

tion of the *c-erbB2* gene, inactivation of the *APC* gene, and loss of pS2 expression preferentially occur in the well differentiated type. Precancerous lesions, such as intestinal metaplasia and adenoma, share various alterations similar to those seen in well differentiated carcinomas. Loss of heterozygosity (LOH) of the *p73* gene occurs specifically in well differentiated carcinomas with foveolar epithelial phenotype [8]. Inactivation of cadherins and amplification of the *K-sam* gene and *c-met* gene are frequently associated with poorly differentiated or scirrhous type carcinomas. A review of representative abnormalities of individual genes and molecules, and their significance as markers for molecular diagnosis, is given below.

Telomere maintenance by telomerase activation induces cellular immortalization [9]. Strong telomerase activity is detected in most gastric carcinomas, regardless of histological type and tumor stage [10]. Relatively good correlation was found between telomerase activities and levels of catalytic subunit of telomerase (TERT) expression in gastric carcinomas. Some intestinal metaplasias and gastric adenomas express telomerase activity at certain levels, suggesting participation in an early stage of stomach carcinogenesis [11]. Telomerase activity is found in approximately half of gastric adenomas, whose levels of the activity are about 10% of those in gastric carcinomas. TERT protein is expressed in the nuclei of the adenoma cells at moderate levels that are not necessarily comparable with the telomerase activity. Therefore, TERT expression may be a prerequisite for telomerase activation in the early stage of stomach carcinogenesis; thus, the expression of

Well differentiated adenocarcinoma

Fig. 1. Genetic and epigenetic alterations during multistep stomach carcinogenesis. LOH, Loss of heterozygosity; HP, *Helicobacter pylori*; TERT, catalytic subunit of telomerase; Chr., chromosome; GF, growth factors

TERT is a useful marker for detecting “true precancerous lesions”.

Genetic instability causes an accumulation of genetic alterations, and participates in the early stage of stomach carcinogenesis [3]. Cases in which two or more of five microsatellite loci show replication error are considered as showing high-frequency microsatellite instability (MSI-H), and those with only one locus showing replication error are considered to show low-frequency MSI (MSI-L) [12]. MSI-H is observed in only 4% of gastric carcinomas, in particular in the well differentiated type in aged patients. The frequency of MSI-L is about 30% in primary gastric cancers, including early cancers [3]. Some intestinal metaplasias and adenomas also show MSI-L, and these should be considered “true precancerous lesions” [13]. MSI-H is used as an indicator of HNPCC, which is caused by the germline mutation of mismatch repair genes, including *hMLH1* and *hMSH2* [14]. In sporadic gastric cancers with MSI-H, CpG island hypermethylation of *hMLH1* is associated with loss of hMLH1 protein [15,16]. Another important aspect of MSI is that multiple primary cancers, including stomach, colon, and gallbladder carcinomas, arising in a single individual frequently display replication errors at multiple microsatellite loci [17]. This indicates that the detection of MSI in a cancer may serve as a good molecular marker for the assessment of the risk of a second cancer in the same patient.

Abnormalities in cell-cycle regulators are involved in the development and progression of gastric cancers through unbridled cell proliferation [2,3]. The cyclin E gene is amplified in 15%–20% of gastric carcinomas. The overexpression of cyclin E tends to be correlated with the aggressiveness of gastric carcinomas, such as deep invasion and advanced tumor stage. On the other hand, reduction in the expression of the CDK inhibitor, p27^{Kip1}, is frequently associated with advanced gastric carcinomas [18]. Gastric adenomas with reduced p27^{Kip1} expression tend to progress to carcinomas [7]. Reduced expression of p27^{Kip1} also significantly correlates with depth of tumor invasion and the presence of lymph-node metastasis. Therefore, gene amplification and the overexpression of cyclin E, and the reduced expression of p27^{Kip1}, are good markers for the diagnosis of high-grade malignancy. E2F-1, a target of cyclins/CDKs at G1/S transition, is overexpressed in about 40% of gastric carcinomas and may participate in the development of gastric carcinomas [19].

Abnormalities in transcriptional regulation by CpG island methylation and histone deacetylation are believed to be the most important of various epigenetic alterations [20–23]. DNA hypermethylation is known to be accompanied by allelic loss at the same locus. CpG island hypermethylation of the genes *hMLH1*, *MGMT* (O⁶-methylguanine methyltransferase), and

p16^{MTS1/INK4A} is detected in 20%–30% of gastric cancers [24–26]. Reduced expression of the gene products is well correlated with CpG island hypermethylation. Hypermethylation of these three genes is more frequently found in well differentiated adenocarcinomas than in poorly differentiated adenocarcinomas. However, cases with *hMLH1* hypermethylation do not coincide with cases with *MGMT* hypermethylation, as reported in colorectal cancer. Furthermore, *hMLH1* hypermethylation occurs in intestinal metaplasia, suggesting that hMLH1 expression reduced by promoter methylation may be an initial event in the accumulation of genetic abnormalities in stomach carcinogenesis. On the other hand, CpG island methylation of *CDH-1* (E-cadherin) and consistently reduced E-cadherin expression is commonly observed in scirrhous gastric cancers. Although the enzymes DNA methyltransferase and demethylase potentially affect promoter methylation status, the tumor-specific hypermethylation of *hMLH1*, *p16^{MTS1/INK4A}*, and *CDH-1* does not simply depend on the expression levels of promethylating (DNMT1, DNMT3A, DNMT3B) and anti-methylating (MBD2) enzymes [24,26].

Nucleosomes consist of core histones, wrapped with turns of double-stranded DNA [20]. The acetylation of histones disrupts nucleosome structure, leading to DNA relaxation, and a subsequent increase in accessibility for transcription factors. On Western blotting, with anti-acetylated histone H4, the level of acetylated histone H4 expression was significantly reduced in 70% of gastric carcinomas in comparison with findings in normal mucosa, indicating a reduced acetylation status in gastric cancers. The histone deacetylase inhibitor, trichostatin A (TSA), inhibits cell growth and induces apoptosis in gastric carcinoma cell lines [27]. TSA induces the expression of p21^{WAF1/cip1}, CBP, Bak, and cyclin E, while it reduces the expression of E2F-1, E2F-4, HDAC-1, and the phosphorylated form of Rb protein [27]. These findings suggest that histone deacetylation may participate in unbridled cell proliferation by modulating the expression of cell-cycle regulators and apoptosis-related molecules. The expression of acetylated histone H4 is also reduced in 40% of gastric adenomas and some intestinal metaplasias adjacent to carcinoma, suggesting that reduced histone acetylation may occur even in precancerous cells, and may be a candidate molecular marker to indicate such cells.

Molecular diagnosis of gastric cancer on histopathology specimens

Based on the molecular pathological findings mentioned above, it is clear that we can improve the quality of histopathological diagnosis by analyzing genetic and

epigenetic alterations. We established a molecular diagnosis system for histopathological samples of gastrointestinal tract, in cooperation with Hiroshima City Medical Association Clinical Laboratory, and we have been performing this as a routine service [6,7]. This system is designed mainly for the differential diagnosis of benignancy or malignancy, diagnosis of the degree of malignancy, identification of susceptibility to multiple primary cancers, and identification of HNPCC.

Procedures for molecular diagnosis

The procedures used, from sample receipt to the final diagnosis in the pathology laboratory, are outlined in Fig. 2. The samples are formalin-fixed tissue materials obtained by biopsy or dissection (endoscopic mucosal resection or surgical removal) that are submitted for routine histopathological diagnosis. Routine paraffin sections are prepared, stained, and subjected to histopathological observation. A pathologist indicates the cases to be examined for molecular diagnosis (cancer, adenoma [dysplasia] or borderline lesion) at microscopic examination. A laboratory technician performs immunostaining with various molecular and genetic markers, described below, and at that time prepares another five sections with H&E staining that are not included by covering glass for genetic analysis. The pathologist combines the results of immunostaining and the histopathological findings, adds the molecular pathological findings and their significance if new information is obtained, and makes a molecular-pathological report for the attending clinicians.

Some cases are further analyzed using DNA extracted from paraffin sections. In order to assure that only the histologically doubtful portion is examined accurately, the position to be analyzed on the section with

H&E staining is marked with a felt pen under the microscope by the pathologist. A technician collects the tissues on the marked area into an Eppendorf tube, using the tip of the needle, and extracts DNA using a conventional method with proteinase K and sodium dodecylsulfate (SDS). For this extracted DNA, mutation or deletion of the genes of interest is examined by nonisotopic polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP) analysis and PCR restriction fragment length polymorphism (RFLP). For the detection of genetic instabilities, microsatellite loci are amplified with PCR, using fluorescent stain-labeled primers, and analyzed with a PRISM 310 autosequencer (Applied Biosystems, Foster City, CA, USA). The final molecular-pathological diagnosis, made by combining the results of genetic analysis, immunostaining, and histopathological findings, is reported to the clinicians.

The immunostaining, DNA extraction, PCR-SSCP, and PCR-RFLP are conducted by technicians in the clinical laboratory center of Hiroshima City Medical Association, and microsatellite analysis is performed at our department (First Department of Pathology, Hiroshima University School of Medicine). Most expenses are covered by a donation from the Hiroshima City Medical Association and by research funds from our department. The molecular pathologists who are in charge of the molecular pathological diagnosis system work voluntarily.

Molecular and genetic markers

As the significance of each genetic and epigenetic abnormality differs in esophageal, gastric, and colorectal cancers, the roles of individual abnormalities should be sufficiently understood before these abnormalities are used in diagnosis. In gastric cancer, p53, APC, and

Fig. 2. Procedures for the molecular-pathological diagnosis of histopathology samples of the stomach. *EMR*, Endoscopic mucosal resection; *TGF-α*, transforming growth factor alpha; *EGFR*, epidermal growth factor receptor; *PCR*, polymerase chain reaction; *SSCP*, single-strand conformation polymorphism; *RFLP*, restriction fragment length polymorphism; *MS*, microsatellite

Fig. 3. Changes in molecular and genetic markers for molecular-pathological diagnosis of the stomach during the 7 years that our system has been operating. PCNA, Proliferating cell nuclear antigen

Table 1. Molecular-pathological diagnosis of gastric lesions

Histological diagnosis	Lesions	Molecular diagnosis
Adenoma	1154	Adenoma with malignant potential 119 (10%)
		s/o Adenocarcinoma 19 (2%)
Borderline	469	Adenoma with malignant potential 6 (1%)
		s/o Adenocarcinoma 45 (10%)
		Adenocarcinoma 53 (11%)
s/o Adenocarcinoma	250	Adenocarcinoma 63 (25%)
		High grade malignancy 3 (1%)
Adenocarcinoma	2969	High-grade malignancy 357 (12%)

s/o, Suspected of

CD44 have been used as markers for differential diagnosis, and transforming growth factor alpha (TGF α), epidermal growth factor receptor (EGFR), c-met, c-erbB2, cyclin E, p27^{Kip1}, and CDC25B for degree of malignancy. For the screening of genetic instability, hMLH1 expression is examined. With PCR-SSCP and PCR-RFLP, deletions or mutations of the *APC* gene and the *p53* gene are examined. Mutations of the *p53* gene are analyzed in exons 5 to 8, and LOH is analyzed using polymorphism at the *Bam*H1 site of the 3'-untranslated region of the gene. Genetic instability is monitored by microsatellite assay. Four loci of two CA repeats (D1S191, D17S855; *BRCA1* locus) and two poly A tracts (BATRII; TGF β type II receptor and BAT40) are examined, and if two or more loci with replication errors are detected, MSI-H is considered to be present. For MSI-H, the presence or absence of mutation of *hMLH1* and *hMSH2* is determined by PCR-SSCP methods.

The assessment of biomarkers is reviewed periodically, and we always study new markers retrospectively to verify their usefulness for diagnosis prior to use in clinical practice. Figure 3 shows the history of molecular markers for stomach specimens during the 7 years that our system has been operating. In 1993, molecular diag-

nosis was begun with immunostaining alone, with only 6 markers; genetic analysis of the *p53* and *APC* genes by PCR-SSCP and PCR-RFLP was introduced in 1995; and, since 1996, microsatellite analysis has been performed with an autosequencer. At present, we analyze 14 molecular and 3 genetic markers.

Results and evaluation

Approximately 5000 lesions in histopathological samples of the stomach have been analyzed. The histopathological diagnoses are adenoma, dysplasia, borderline lesion, carcinoma, and lesion suspected of carcinoma. The results of molecular-pathological diagnosis of gastric lesions are summarized in Table 1. As mentioned above, molecular diagnosis is made by combining the results of molecular and genetic analyses and the histopathological findings. The definitions we use in molecular diagnosis are as follows: (1) "adenoma with malignant potential" is an adenoma with a high probability of becoming carcinoma. These tumors contain some molecular and genetic abnormalities, including *p53*, but morphological aberrations are within the range for benign tumor. (2) "Suspected of (s/o) adenocarcinoma" is a tumor that has genetic abnormalities of *p53*

Fig. 4. A representative case of double cancer (gastric and colon cancers) with high-frequency microsatellite instability (MSI-H). DNA was extracted and subjected to microsatellite assay, as described in the text. Three loci (D17S855, D1S191, and BAT-40) were shown in both the gastric and the colon cancers, but not in the normal counterparts

and/or *APC*, as well as abnormal expression of many cancer-related molecules, such as *c-erbB2*, cyclin E, EGFR, and *c-met*, while histological atypia is not severe enough to be regarded as carcinoma. (3) “Adenocarcinoma” is a tumor whose genetic and molecular abnormalities are the same degree as those in (2) above, that can be also regarded as malignant histopathologically if such molecular findings are available. Ten percent of histologically diagnosed adenomas were diagnosed as adenoma with malignant potential, while 2% were suspected of adenocarcinoma. Adenocarcinoma or s/o adenocarcinoma was identified in more than 20% of histologically diagnosed borderline lesions. Twelve percent of histologically diagnosed adenocarcinomas were regarded as showing high-grade malignancy because of abnormalities of growth factors or growth factor receptors, cell-cycle regulators, and so on, such as *c-erbB2*, EGFR, cyclin E, and *p27^{Kip1}*. The prognosis of patients diagnosed with these abnormalities tended to be poor on follow-up observation. About 4% of gastric cancers exhibited MSI-H by microsatellite analysis (Fig. 4). In half of the patients who showed MSI-H, clinically synchronous or asynchronous multiple primary cancers were confirmed. We found germline as well as somatic mutations of DNA mismatch repair genes in some cases, suggesting a kindred of HNPCC, but most cases showed epigenetic inactivation of *hMLH1*, possibly caused by CpG island methylation.

Points at issue

With our diagnosis system, the portion of the section used for DNA extraction is marked with a felt pen, and tissue in the marked area is collected as accurately as possible. One of the technical problems with this system is that accurate sample collection is sometimes difficult, and genetic analysis cannot be done in cases in which a small number of cancer cells are distributed in the nor-

mal epithelia, such as in signet ring cell carcinoma, or when cancer cells are infiltrated diffusely in fibrous stroma, such as in scirrhous carcinoma. To deal with these problems, we are introducing laser microdissection [28] for routine use. With this technique, tumor cells can be distinctly separated from normal mucosal epithelia.

Another problem with our system is that we do not have enough molecular markers; we need additional markers, e.g., for differential diagnosis, grade of malignancy, and drug sensitivity, to make diagnosis more efficient. The DNA microarray technique is a powerful tool to identify novel genes participating in the development and progression of gastric cancer. Figure 5 shows the representative result of a comparative gene expression profile of well differentiated adenocarcinoma and poorly differentiated scirrhous gastric carcinoma obtained using the DNA microarray technique. Strong expression of cadherin, cell-cycle regulators, and mitogen-activated protein (MAP) kinases was observed in the well differentiated type, while survivin, growth factors (TGF β , platelet-derived growth factor [PDGF], insulin-like growth factor [IGF]), and proteases (matrix metalloproteinase [MMP]-2, MMP-3, plasminogen activator [PA]) were found to be strongly expressed in scirrhous type carcinoma. Large numbers of genes are differentially expressed in various tumors, but their role in stomach carcinogenesis is not known. Further studies should be performed to verify the value of such genes in molecular diagnosis, both retrospectively and prospectively.

Another contribution of molecular analysis to clinical findings

Super-early diagnosis of gastric cancer

As described above, many genetic and epigenetic abnormalities are found in incomplete type intestinal

Fig. 5.A,B. Differential gene expression of well differentiated adenocarcinoma (**A**), detected by Cy5, and poorly differentiated scirrhous carcinoma (**B**) detected by Cy3, using cDNA microarray

metaplasia without obvious morphological aberration. Therefore, if molecular analysis is extended to intestinal metaplasia, super-early diagnosis of gastric cancer can be made. For instance, mutations of the *p53*, *APC*, or *K-ras* genes have been detected in intestinal metaplasia, although at low frequency [29]. The intestinal metaplasia in 30% of gastric cancer cases shows weak but significant telomerase activity, suggesting the existence of “stem-cell hyperplasia” or “already immortalized somatic cells” in intestinal metaplasia [3]. TERT protein is expressed in some incomplete-type intestinal metaplasias adjacent to carcinomas [11]. The expression of acetylated histone is reduced in intestinal metaplasia. Examinations of foci with genetic instability in stomach resected because of gastric cancer have shown, replication error-positive foci located both in the cancer and in the intestinal metaplasia around the cancer [13]. Some intestinal metaplasias show CpG island hypermethylation of the *hMLH1* gene and its reduced expression (Fig. 6). Foci with replication error and portions with reduced *hMLH1* expression in nonneoplastic mucosa are lesions that are susceptible to cancer progression (true precancerous lesions), and genetic instability is a powerful genetic marker to predict such a condition.

Diagnosis of presence of cancer

It is needless to state that lymph node metastasis is the most important determinant of patient outcome. However, with routine histopathological examination of representative sections of the cut surface of lymph nodes, there is a chance that micrometastasis may be overlooked. The molecular detection of mRNAs expressed in cancer cells but not in other cells in lymph nodes,

Fig. 6. CpG island hypermethylation of the *hMLH1* gene in intestinal metaplasia. The methylation status of the CpG island in the promoter of the *hMLH1* gene was examined in intestinal metaplastic mucosa (IMM) and non-metaplastic mucosa (NMM) by a methylation-specific PCR method

such as the mRNA for keratin 19 or carcinoembryonic antigen (CEA) is useful for detecting micrometastasis [30,31]. The expression of these genes can be found by reverse transcriptase (RT)-PCR in many lymph nodes that are histologically negative for metastasis. Histologically node-negative patients with micrometastasis detected by means of cytokeratin expression show worse prognosis than those without micrometastasis [32].

The detection of cancer cells in the peritoneal cavity has important prognostic implications. Routine cytological examinations, however, are not sensitive, and gastric cancer patients with negative peritoneal cytology sometimes show peritoneal recurrence. The analysis of *CEA* mRNA expression by RT-PCR is sensitive enough to detect free cancer cells in peritoneal washes that are negative for abnormal cytology, indicating that such analysis is a powerful technique for predicting the risk of peritoneal dissemination. Real-time RT-PCR is more rapid and quantitative than the conventional one, and is applicable to decision-making during surgery [33]. A similar strategy can be employed for diagnosing the presence of cancer cells in peripheral blood [34]. *CEA* mRNA from circulating cancer cells is associated with an advanced disease stage, and is a useful diagnostic modality to determine patient selection for chemotherapy after surgery and to determine the patient's prognosis.

Future prospects for molecular diagnosis

The human genome sequence, determined by both the International Human Genome Sequencing Consortium and Celera Genomics of Rockville, was published in *Nature* of 15 February 2001 [35], and in *Science* of 16 February 2001 [36], respectively. Although the project is not yet completed, approximately 3 billion base pairs were sequenced, covering 90% of the euchromatic portion of the human genome. The number of protein-coding genes is estimated to be about 30000. We believe that this determination of the human genome sequence is the greatest scientific achievement made in the past decade. Because one principle of the Human Genome

Project, reflected in the Universal Declaration of the Human Genome and Human Rights, is unlimited access to the sequence, the entire information on the human genome in *Nature* is available without restriction (<http://www.nature.com/genomics>). This is the beginning of the post-sequence era. DNA microarray technology has been established, and with this technology, information on the mutation and expression of many genes in human cancers can be quickly detected, stored, and analyzed. It is a time of evolution in that molecular diagnosis in the field of pathology must correspond to this new scientific age. The findings that have already accumulated on the molecular mechanisms of the development and progression of gastric cancer, and findings that will be systematically clarified in the future will be combined, and genetic diagnosis by means of DNA microarray will surely become routine in the diagnosis of gastric cancer. If essential abnormalities and secondary changes can be clarified in the many genetic and epigenetic alterations identified in cancers, examinations of a hundred to several hundred genes should be sufficient for the diagnosis of gastric cancer. It is important that the characteristics of each cancer, that is, the common features and specific features in the development and progression of each cancer, are clarified so that gene therapy or molecular-targeted therapy can be directly related to the results of molecular diagnosis of histopathological samples. We plan to create DNA miniarrays for the diagnosis of gastric cancer to predict the patient's susceptibility and the grade of malignancy and chemosensitivity of the cancer.

Most genetic variation between individual humans is believed to be caused by single-nucleotide polymorphisms (SNPs). The information on SNPs identified to date is publicly available. Although the average frequency of SNPs is estimated to be 1 bp per 1.2 to 1.9 kbp, fewer than 1% of all SNPs result in variations in proteins [37]. Although the tasks of determining which SNPs have a functional consequence remain, largely, to be elucidated, some evidence has been found in relation to pathological status. Genetic polymorphism of the *CYP* genes is related to cancer predisposition, including lung cancer. SNP in the *RAD51* gene modifies breast cancer risk in *BRCA2* carriers [38]. SNP in the interleukin-1- β gene is associated with an increased risk of gastric cancer [39]. SNP in the E-cadherin gene promoter alters transcriptional activity [40] that is associated with scirrhous gastric cancer. The relationship between SNPs and chemosensitivity has also been noted. With progress in the analysis of SNP, the association of specific pathological conditions or sensitivities with various SNPs will be clarified, and, in the near future, information that will lead to the pharmacotherapy and prophylaxis of cancer may be obtained from histopathological samples.

The examination of morphological changes in relation to genetic and epigenetic abnormalities is the main benefit of the genetic analysis of histopathological specimens, and this will improve the quality of histopathological diagnosis. "Histopathology" has discovered minute histological abnormalities, and has closely classified pathological conditions by accumulating knowledge obtained from morphological observations. Another important mission for genetic diagnosis in the field of pathology is to clarify how abnormalities in the functions of the genes and molecules are reflected in morphology. The ultimate objective of molecular pathology is that the morphological abnormalities in all diseases described in the pathology literature will be found to correspond to genetic and epigenetic alterations. In the field of carcinogenesis, molecular pathology must evolve into morphological genomics.

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