Abnormalities in Cell Cycle Regulators and Their Application to Molecular Diagnosis

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In many leading hospitals and laboratory centers in Japan, tissues of gastrointestinal organs account for the majority of samples in routine daily histopathological diagnosis. Certainly, histopathological diagnosis is extremely useful for definitive diagnosis or guidance as to therapy. However, diagnosis depending only on histomorphology is limited in certain respects and is unsuitable for diagnosis of the presence of cancer.

Based on recent integrated research in molecular pathology over the past 15 years, the details of genetic and epigenetic abnormalities in the course of development and progression of gastrointestinal cancer have been clarified. Particularly in various cell cycle regulators, it has been known that cyclin and cyclin-dependent kinase (CDK) (positive regulators), and CDK inhibitor (negative regulator) are related to gastrointestinal cancer. In addition, various diagnostic markers such as telomerase activity, genetic instability, tumor suppressor gene, and oncogenes (growth factor receptor type) were reported to be effective for the diagnosis of gastrointestinal cancer.

In this year 2000, it was announced that nearly the entire base sequence of the human genome had been determined. Following this achievement, genetic analysis by means of DNA microarray may become mainstream in the diagnosis of gastrointestinal tissues in the future. Genetic analysis may make clear the characteristics of each type of cancer; that is, commonality and specificity in the development/progression of each cancer can be found. When this occurs, diagnosis that is directly useful for genetic therapy or molecule-targeted therapy can be conducted through pathological examination. The comparison of morphological changes with the abnormalities of genes/molecules is the main benefit to the genetic analysis/diagnosis of histopathological samples. The ultimate objective of the field of pathology is that morphological abnormalities of all diseases described in pathology literature can be traced to gene abnormalities or molecular abnormalities.

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Key Words Cell Cycle Regulator, Molecular Pathological Diagnosis, Gastrointestinal Cancer

INTRODUCTION

With the expansion and advancement of endoscopic diagnosis and treatment of gastrointestinal lesions, pathologists' opportunities for histopathological examination of gastrointestinal tissue samples are markedly increasing. The Hiroshima Prefectural Tumor Registration Committee is registering tumors that were diagnosed by pathologists, whether benign or malignant, together with histopathological reports and tissue preparations. Over the last 20 years, the numbers of cases registered by the Committee have increased 3 times for gastric cancer, 6 times for colon cancer, 20 times for adenoma of the stomach, and, surprisingly, 64 times for adenoma of the colon. These findings indicate that the many lesions have been biopsied or dissected endoscopically, although tissue was formerly collected only by surgical operation. Recently, in leading hospitals and laboratory centers, the gastrointestinal tissues account for the majority of samples in routine daily histopathological diagnosis.

Although histopathological diagnosis is extremely important for obtaining definitive diagnosis or guidance of treatment, diagnosis depending only on histomorphology is limited in certain respects. Many lesions have morphology that is borderline between benign and malignancy, and there are interpathologist variations in the diagnosis criteria for tumors. Furthermore, information from morphology is of limited use in determining degree of malignancy and prognosis, and histopathological diagnosis is unsuitable for diagnosis of the presence of cancer. These are weak points for pathological diagnosis.

Based on recent integrated research in molecular pathology over the past 15 years, the details of genetic and epigenetic abnormalities in the course of development and progression of gastrointestinal cancer have been clarified. As to excessive proliferation, which is one of the major characteristics of cancer, various results have been obtained using growth factor receptor, cell cycle regulator, and apoptotic approaches. In particular, various positive and negative regulators of the cell cycle and underlying gene control mechanisms have gradually been clarified. The abnormalities associated with and significance of these gastrointestinal cancers have also been clarified. It is possible to analyze these findings in tissue samples and apply them to diagnosis together with the results of morphology.

In this paper, abnormalities in cell cycle regulator are outlined in cases of gastrointestinal cancer, which serves the most part of samples for pathlogical diagnosis. In addition, as an approach to genetic diagnosis, our molecular pathological diagnosis of digestive tract lesions is introduced, and future prospects of genetic diagnosis are discussed.

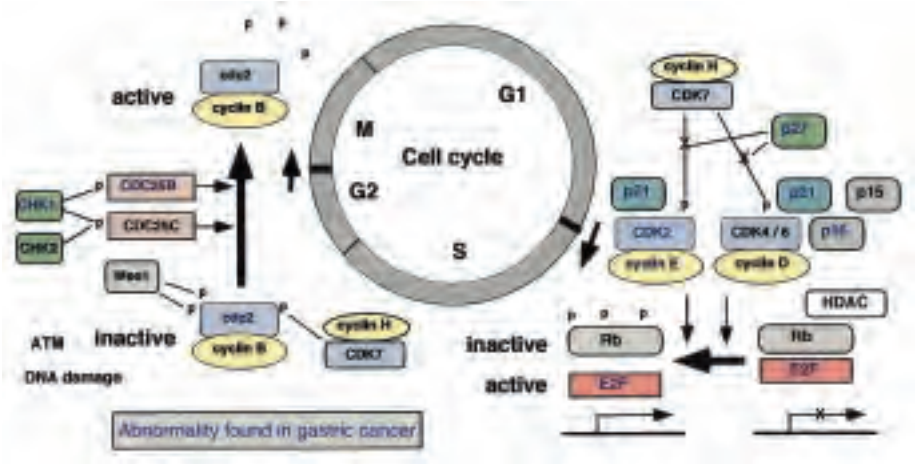

Fig. 1 *Mechanism of control of cell-cycle progression and abnormalities in gastric cancer (character in blue)*

ABNORMALITIES OF CELL CYCLE REGULATORS

Cyclin (positive regulator), cyclin-dependent kinase (CDK) and CDK inhibitor (negative regulator) act as monitoring mechanisms (***Fig. 1***)[1] in the process of the cell cycle from the G1 phase to S phase, or from the G2 to M phase. At upstream of the cyclin/CDK complex, Chk kinase and CDC25 phosphatase regulate the activity of the cyclin/CDK complex, and, Rb protein and transcription factor E2F are involved "downstream". The balance between positive and negative regulators involved in a machinary cell cycle is disturbed due to abnormalities of the genes and their expression, thereby inducing the development and progression of cancer via excessive cell proliferation.

Cyclin and CDK

Cyclin forms a complex with its partner CDK, and phosphorylates Rb protein and other target molecules, controlling the cell cycle to be positive. Cyclin D1 gene exists close to oncogenes *hst-1* and *int-2*, where locate on chromosome 11q13 and amplification of the genes in this region is found in 40-50% of the genes in the primary focus of esophageal cancer and in the majority in metastatic foci[2]. In patients with amplification or excess expression of cyclin D1 genes, the frequency of postoperative recurrence is higher and prognosis is quite poor. Although, amplification of cyclin D1 genes is not observed in gastric cancer and colon cancer, patients with colon cancer tend to show high level expression of cyclin D1. On the other hand, amplification of cyclin E genes is observed in 15 - 20% and 10% of patients with gastric cancer and with colon cancer, respectively, but is not found in patients with esophageal carcinoma[3,4]. Over expression of cyclin E is observed in 8% and 27% of patients with gastric adenoma and adenocarcinoma, respectively. Therefore, the incidence of over expression of cyclin E is significantly higher in patients with cancer. In addition, this over expression of cyclin E shows a significant correlation with the degree of malignancy including invasion into deeper tissues, progress of stage, and metastasis. Even in the colon, over expression of cyclin E is found in 5% of adenomas, and 20% of cancers, and its incidence is significantly higher in patients with cancer, particularly cancer which invade into submucosal tissue[5]. In many patients with colon cancer who have amplified cyclin E gene, simultaneous amplification of CDK2 is observed[4].

The expression of CDK1 (cdc2) and kinase activity, which regulate mainly the process of G2/M phase transition, are markedly accelerated in tissues of almost gastric and colon cancers compared with normal mucosal tissue (***Fig. 2***)[6]. The acceleration level is closely correlated with PCNA expression as the indicator of the proliferation activity. Furthermore, the kinase activity tends to be higher in patients having p53 gene mutation.

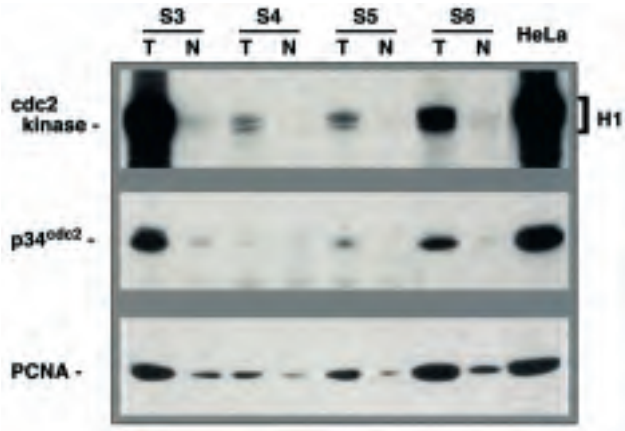

Fig. 2 *Expression of cdc2 (CDK1) in gastric cancer and kinase activity and PCNA expression*
T: Tissues of gastric cancer
N: Non-cancerous gastric mucosal tissues

CDK inhibitor

The CDK inhibitor $p21^{WAF1/CIP1}$, a negative regulator of the cell cycle, binds to CDK2/cyclin E complex, and inhibits the cell cycle progress of G1/S transition. Although neither deletion or mutation of the genes has been found in gastric cancer, decrease in the gene expression at the mRNA and protein levels has been observed in 40 - 50% of patients with gastric cancer, which might be related to inhibition of the gene transcription, such as methylation of CpG[7]. One of the transcription factors for p21 expression is p53. Expression of p21 is maintained in the gastric cancer cell lines, MKN-45 and MKN-74, and in the colon cancer cell line Lovo, which contain wild type p53, by contrast, the level of p21 expression is low in the p53 mutant. In colon cancer, abnormality of p21 genes is not found, but attenuation of expression of p21 genes is reported to be correlated with degree of malignancy. In a stage 3 or 4 patient whose cancer has invaded to the muscularis propria or deeper, and patients with lymph node metastasis, p21 expression is always attenuated[8]. A new cancer related gene *p73* which is a homologue of *p53* gene also acts as a transcription factor similarly to p53, and accelerates the expression of p21[9]. Deletion of *p73* genes has been mainly found in well cancer, and particularly in well differentiated gastric adenocarcinoma[9].

Attenuation of the expression of the CDK inhibitor $p27^{KIP1}$ is also correlated with the degree of progression and malignancy of gastric cancer and colon cancer (***Table 1***)[10,11]. The expression of p27 is relatively well maintained in gastric mucosal cancer. However, the expression of p27 is attenuated in advanced cancer and deeply invading cancer, and is significantly correlated with lymph node metastasis. When p27 expression in primary focus is compared with that in the metastatic focus of the lymph node, p27 expression in the primary focus is not uniform, and almost no expression is observed in the metastatic focus, indicating that cancer cells where p27 expression is attenuated might selectively metastasize to other sites. Furthermore, the prognosis of patients with low in p27 expression is generally poor. On the other hand, p27 expression is attenuated in approximately 10% of adenomas of the stomach, many of which progress to cancer. In adenoma of the colon, p27 expression is relatively well maintained, and attenuation or elimination of p27 expression is correlated with deeper invasion or lymph node metastasis. Thus, the attenuation of p27 expression might be related to both development and progression of gastric cancer and colon cancer. The attenuation of p27 gene has been reported to be due to acceleration of proteolysis of after translation but not due to the mutation of the gene.

In the $p16^{MTS1/INK4A}$ and $p15^{MTS2/INK4B}$, which mainly inhibit CDK4/6, complete deletion of the genes was observed in various cancer cell strains including esophageal cancers, and deletion and mutation of the genes have been reported even in primary esophageal cancer[12]. In esophageal cancer cell lines, excessive expression of cyclin D1 and CDK4 has been observed in cells with deletion of *p16/p15* genes[13]. Although deletion or mutation of *p16* genes is not found in gastric cancer, attenuation of expression of p16 protein due to methylation of the promoter is found in approximately 20% of gastric cancer.

Table 1 *Expression of CDK inhibitor p27 in colorectal cancer*

		Expression of p27*1			p-value*2
		Preserved	Reduced	Negative	
Cases	109	67(61%)	28(26%)	14(13%)	
Stage*3					
0	39	30(77%)	6(15%)	3(8%)	0.1706
1	27	19(70%)	6(22%)	2(7%)	
2	19	9(47%)	9(47%)	1(5%)	
3	18	7(39%)	6(33%)	5(28%)	
4	6	2(33%)	1(17%)	3(50%)	
Depth of invasion*3					
m	39	30(77%)	6(15%)	3(8%)	0.0443
sm, mp	29	19(66%)	6(21%)	4(14%)	
≥mp	41	18(44%)	16(39%)	7(17%)	
Lymph node metastasis					
No	87	59(68%)	21(24%)	7(8%)	0.0042
Yes	22	8(36%)	7(32%)	7(32%)	

*1: Expression of p27:
"Preserved": only 5% or less of tumor cells are slightly positive.
"Reduced" : 5-30% of tumor cells are positive.
"Negative": 30% or more of tumor cells are strongly positive.
*2: Significant if $p < 0.05$ (Fisher's exact test).
*3: According to the criteria of the Japanese Society for Cancer of the Colon and Rectum.

CDC25 and Chk families

Cyclin/CDK complex is inactivated by the phosphorylation at 14-thr and 15-tyr. There are CDC25A, CDC25B and CDC25C. CDC25A is suggested to act in G1/S phase, and CDC25B and CDC25C in G2/M phase. Excessive expression of CDC25A and CDC25B is found in 40% and 70% of gastric cancers, respectively, and in particular excessive expression of CDC25B is significantly correlated with progress of stage, degree of invasion, and lymph node metastasis[14].

Chk1 exists at the upstream of CDC25, and phosphorylates CDC25 to inhibit the phosphatase activity[15]. Expression of mRNA in Chk1 is clearly decreased in most gastric cancers. Inactivation of Chk1 might release negative control of the cell cycle, resulting in carcinogenesis. Chk2 is inactivated by phosphorylation of serine at the 216-position of CDC25C[16]. Chk2 is activated through ATM (ataxia telangiectasia mutated) by various DNA damage, and plays a role to terminate the cell cycle at G2/M phase. The germ line mutation of Chk2 has been found in some families with multiple cancers similar to Li-Fraumeni syndromes, and occurrence of colorectal cancer and gastric cancer has been reported in these families[17]. Involvement to somatic mutation of Chk2 genes should be further investigated in cases with sporadic gastrointestinal cancer.

Transcription factor E2F

CDK, which is activated at the G1/S checkpoint, changes to its high-phosphorylate form of Rb protein, and releases arrest of the cell cycle[1]. Transcription factor E2F is the target of Rb, and controls the expression of genes responsible for DNA synthesis and the cell cycle, including cyclin E, DNA polymerase α, and dihydrofolate reductase. At least 6 types of E2F, from E2F-1 to E2F-6, are known, and the role of each appears to differ depending on the kind of cancer or target organ. For example, E2F-4 has transforming activity, while E2F-1 inhibits or accelerates the generation of cancer depending on the organs (in the knockout mouse). In gastric cancer, gene amplification of E2F-1 is observed in only 4% of patients, whereas excessive expression at the mRNA level exists in 40% of the patients[18]. On the contrary, expression of E2F-3 is attenuated in 70% of patients with gastric cancer. In colorectal cancer, gene amplification of E2F-1 is observed in 25% of patients, and excessive expression of E2F-1 mRNA is the observed in 60% of patients. However, there is no correlation of excess expression of E2F-1 mRNA with advancement of stage, degree of invasion, and potencial for metastasis[18]. It has been reported that excessive expression of E2F-1 increases the drug resistance to 5-FU, which inhibits excessive expression of E2F-1 through expression/activation of thymidylate synthase. A relationship between the level of expression of E2F-1 and drug sensitivity is attracting attention in gastrointestinal cancer[19]. In E2F-4 genes, there are 13 repeats of the AGC sequences encoding serine residue. Genetic replication error at this site is observed in 30% of gastric cancers and 40% of colorectal cancers which exhibit genetic instability[20].

Two factors are involved in inhibition of the transcription activity of E2F by Rb: 1) low phosphorylated-type Rb forms a complex with E2F, and inhibits binding of DP (which is a patner of E2F), to DNA, and 2) the structure of nucleosome is made strong through activation of the histone deacetylase. Furthermore, it has been found that DNA binding ability is inhibited if E2F itself is deacetylated, and that E2F-1 and Rb bind to DNA methylase (DNMT1) and methylate the region of transcription regulation[21, 22].

APPROACH TO MOLECULAR PATHOLOGICAL DIAGNOSIS

In addition to the abnormalities of the cell cycle regulators described above, other abnormalities of various genes/molecules involved in carcinogenesis and progression of gastrointestinal cancer have been clarified, and can be used as genetic markers for molecular pathological diagnosis. This section starts with a review of abnormalities of individual gene/molecules and their significance as markers for diagnosis.

Significance of abnormalities of genes/molecules as diagnostic markers

In gastrointestinal cancers, genetic or epigenetic abnormalities accumulate through multiple steps, and many of these abnormalities develop into precancerous lesions and finally to cancers[23]. There are abnormalities of genes/molecules commonly observed in esophageal cancer, gastric cancer, and colorectal cancer, and abnormalities specific to each of them. As the significance of each abnormality differs in these cancers, the roles of individual abnormalities should be sufficiently understood before their use in diagnosis.

Maintaining telomere DNA[24] (repeating sequence of TTAGGG) by telomerase activation induces immortalization of cells through the stability of chromosomes, and is involved in the early stages of carcinogenesis[25, 26]. Strong telomerase activity is commonly observed in esophageal cancer, gastric cancer, and colorectal cancer, regardless of tissue type and degree of advancement. Furthermore, telomerase activity is found even in dysplasia of esophagus, which is considered precancerous lesion, intestinal metaplasia and adenoma of the stomach, adenomas of colon at frequencies of 30 - 50%, though it being low percentage[27]. The catalytic subunit (TERT) also excessively expresses in most gastrointestinal cancers. However, in precancerous lesions, expression of TERT is observed prior to telomerase activation, thus the expression of TERT is a useful marker for detecting “true precancerous lesion”.

Genetic instability[28] causes accumulation of genetic abnormalities, and is involved in carcinogenesis in its early stages. Cases where 2 or more of 5 regions in microsatellite show replication error are “high-frequency of microsatellite instability” (MSI-H), and those with only 1 region are considered “low-frequency of microsatellite instability” (MSI-L). MSI-H is usually observed only in 3% of patients with gastric cancer, but MSI-L is usually observed in 30 - 60% of

patients with gastric and colorectal cancer. Some intestinal metaplasias and adenomas also have genetic instability, and these should be considered "true precancerous lesions"[29, 30]. MSI-H can be used as an indicator of hereditary non-polyposis colorectal cancer (HNPCC), which is caused by the germ line mutation of mismatch repair genes such as *hMSH2* and *hMLH1*[31]. Decreased expression of the *hMLH1* gene can be detected by immuno staining using a specific antibody, but non-genetic inpairment of expression is often caused by methylation of the transcription regulating region[32]. The frequency of MSI is approximately 10% in single cancers, but approximately 90% in multiple cancers, and clearly much more frequently in multiple cancers, and thus useful for estimating the multiplicity of a cancer[33].

The deletion or mutation of the tumor suppressor gene *p53* is observed at a frequency of 50-60% in esophageal cancer, gastric cancer and colorectal cancer, and the frequency of inactivation of tumor suppressor gene *p53* increase relating to dysplasia level in metaplasia and adenoma[23]. Deletion/mutation of *APC* genes is observed at high frequency from adenoma of both colon and stomach.

Details of abnormalities of cell cycle regulators in gastrointestinal cancer have been described above. Decrease in the expression of the CDK inhibitor $p21^{WAF1/CIP1}$ and $p27^{KIP1}$ can be used as a marker to determine the degree of malignancy of colorectal and gastric cancer/colorectal cancer[8, 10, 11] respectively. Attenuation of $p27^{KIP1}$ expression can be used as an indicator of advancement to cancer from adenoma in the stomach. On the other hand, gene amplification/excessive expression of cyclin E and cyclin D1 are good genetic markers for diagnosis of the degree of malignancy of gastric/colorectal cancers and esophageal cancer, respectively[2, 3, 5].

Amplification of the c-*erb*B2 and K-*sam* genes, which are oncogene of the type of a growth factor receptor is selectively observed in the well differentiated adenocarcinoma and the scirrhous cancer in the stomach, respectively, and amplification of the c-*met* genes is specifically observed in the gastric cancer[23]. Amplification and excessive expression of these genes is well-correlated with degree of malignancy. Amplification/excessive expression of EGFR genes is also a good indicator of degree of malignancy of esophageal and gastric cancer. In addition, excessive expression of various growth factors and cytokines has been observed in malignant cancers[23]. For angiogenic factors such as VEGF and IL-8, relationships with metastasis have been reported[34, 35].

Gene diagnosis in gastrointestinal histopathological samples (molecular pathological diagnosis)

Based on the molecular pathological findings mentioned above, we developed a gene diagnosis system for histopathological samples of gastrointestinal tract in cooperation with Hiroshima City Medical Association Clinical Laboratory, and have performed diagnosis with it[23, 36]. This system is designed for differential diagnosis of benignancy or malignancy, detection of the true precancerous lesion, diagnosis of the degree of malignancy of cancer, diagnosis of multiple cancers, and identification of HNPCC.

1) Procedures and methods of molecular pathological diagnosis

Fig. 3 illustrates the processes used for sample receipt to the final diagnosis in the pathology laboratory. The samples are formalin-fixed tissue materials obtained by biopsy or dissection (endoscopic mucosal resection or surgical dissection), and have been submitted for the purpose of histopathological diagnosis. (They have not been obtained for genetic diagnosis.) Routine paraffin slices of the tissues are prepared, stained, and subjected to

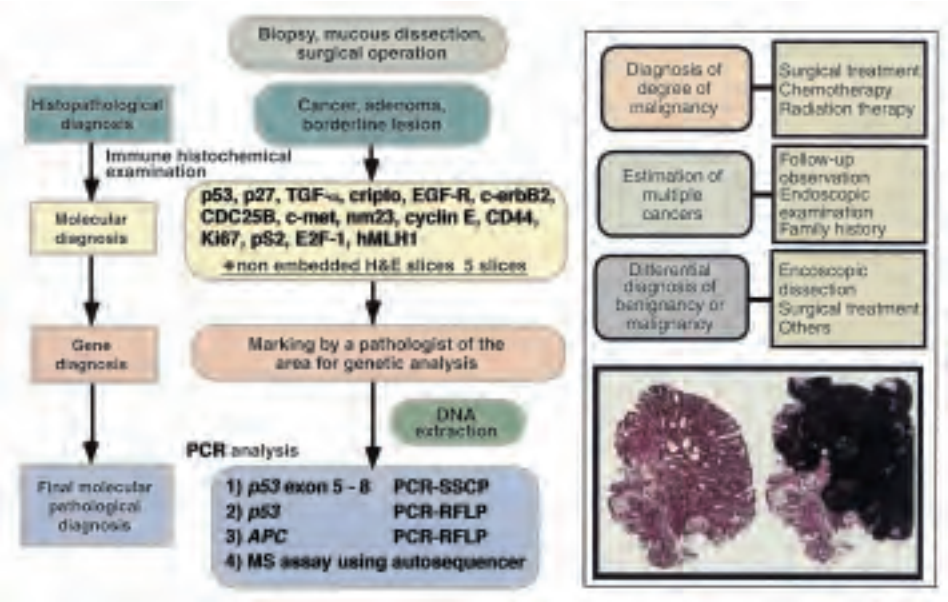

Fig. 3 *Procedures of gene diagnosis for histopathological samples of the stomach*

Table 2 *Molecular pathological diagnosis markers in the gastrointestinal cancer and the results of diagnosis of the degree of malignancy*

	Markers	Purpose	Highly malignant cancer
Esophageal cancer	p53 EGF, TGFα, EGFR, Ki-67 cyclin D1	Differential diagnosis Diagnosis of malignancy Estimation of metastatic ability	66/214 (31%)
Gastric cancer	p53, APC TGFα, EGFR, cripto, c-met, c-erbB2, cyclin E, p27, CDC25B, E2F-1, Ki-67 nm23, CD44 Transcription error, MLH1, MSH2	Differential diagnosis Diagnosis of malignancy Estimation of metastatic ability Estimation of multiple cancers	357/2969 (12%)
Colorectal cancer	p53, APC, CD44 EGF,TGFα, cripto, EGFR cyclin E, p27, p21, Ki-67 nm23, SLX Transcription error, MLH1, MSH2	Differential diagnosis Diagnosis of malignancy Estimation of metastatic ability HNPCC estimation	148/2011 (7%)

histopathological diagnosis. A pathologist indicates the tissue sample to be examined for genetic analysis (cancer, atypical adenoma dysplasia, or borderline lesion) at his microscopic examination. A laboratory technician slices the paraffin block indicated by the pathologist; immuno staining is conducted for below-stated molecule/gene markers, and 5 slices of HE-stained tissue are prepared for gene analysis. The pathologist combines the results of immuno staining and histopathological findings, adds the molecular pathological findings and their significance if new information is obtained, and prepares the molecular pathological report for the attending physician.

In genetic analysis, in order to assure that only the histologicaly doubted location is examined accurately, the position that a pathologist examines microscopically is marked with a felt pen on the slice stained with HE without inclusion. A laboratory technician collects the tissues on the area marked with a felt pen into an Eppendorf tube using the tip of a needle, and extracts DNA using routine procedures. For this extracted DNA, a laboratory technician examines mutation or deletion of *p53* gene and *APC* gene by non-isotopic polymerase chain reaction-single-strand conformational polymorphism (PCR-SSCP) and -restriction fragment length polymorphism (PCR-RFLP)[37]. In addition, for detection of genetic replication error by microsatellite methods[28], the microsatellite region is amplified with PCR using fluorescent stain-labeled primers, and is analyzed with the PRISM310 automatic sequencer (ABI Company). It is an advantage of these procedures that multiple loci can be detected simultaneously by combinations of fluorescent stain. The final molecular pathological diagnosis (genetic diagnosis) is performed by combining the results of the genetic analysis, immuno staining, and histopathological findings.

Among these investigations, immuno staining, DNA extraction, PCR-SSCP, and PCR-RFLP have been conducted by a laboratory technician in the department of pathology and cytology in the laboratory center, and microsatellite analysis is conducted in our laboratory. The expense required for analysis is not collected. The majority of expenses are covered by a donation from the Hiroshima City Medical Association and research funds of First Department of Pathology, Hiroshima University. A molecular pathologist who is in charge of gene diagnosis is working voluntarily.

2) Parameters for assessment

There are 2 types of abnormalities of the genes/molecules, that is, commonly observed and specifically observed abnormalities in esophageal cancer, gastric cancer or colon cancer. Therefore, a set of genitic markers is provided for each cancer by organ (***Table 2***). In gastric cancer, *p53*, *APC*, and CD44 have been used as markers for differential diagnosis, and EGFR, c-*met*, c-*erb*B2, cyclin E, p27, and CDC25B for degree of malignancy. Furthermore, as markers of degree of malignancy, cyclin D1 for esophageal cancers, and p21 and SLX for colorectal cancer have been added. For screening of genetic instability, abnormalities of *hMLH*1 expression are examined by immuno staining methods. With PCR-SSCP and PCR-RFLP, deletion/mutation of the *APC* gene and *p53* gene is examined. In the microsatellite methods, 4 regions such as D1S191 and D17S855 (BRCA I region) (sequence of repeating CA), BATR II (TGF β II type receptor), and BAT40 (polyadenine sequence) are examined, and if 2 or more regions with abnormalities are detected, it is judged as MSI-H. For MSI-H, presence or absence of mutation of *hMLH1* and *hMSH2* is determined by PCR-SSCP methods. The assessment parameters is reviewed periodically, and new markers are always studied retrospectively to verify their usefulness for diagnosis prior to use in clinical practice.

3) Results and points at issue

In 1993, molecular pathological diagnosis was begun with immuno staining methods alone, and PCR-SSCP and PCR-RFLP were introduced in 1995 and microsatellite methods in 1996. More than 10,000 lesions of

histopathological samples of gastrointestinal organs have been analyzed. A total of 31% of esophageal cancers, 12% of gastric cancers, and 7% of colorectal cancers were diagnosed as highly malignant cancers. The prognosis of patients with these diagnoses tended to be poor on follow-up observation. Cancer was identified in 20% or more borderline lesions of the stomach. Of adenomas of the stomach, 10% were judged to have a high probability of carcinogenesis (true precancerous lesions) based on abnormalities of p53, p27, or cyclin E. About 3% of gastric cancers exhibited MSI-H. In half of the cases showing MSI-H, clinically synchronous or asynchronous multiple cancers were confirmed.

One of the problems with our diagnosis system is that the area for genetic analysis (position for extraction of DNA) is marked with a felt pen on the sliced speciment, and the tissue sample in the marked area is collected quickly and accurately. Consequently, accurate sample collection is difficult and genetic analysis cannot be done in cases where a small number of cancer cells are distributed in the normal tubular interstitium (such as signet ring cell carcinoma), or when cancer cells infiltrate and proliferate diffusely in the interstitial connective tissue such as scirrhous cancer. To deal with these problems, we introduced laser microdissection[38], and are now developing it for routine use. With use of this technique, tumor tissues can be accurately separated from control nomal mucosal epithelium. (***Fig. 4***)

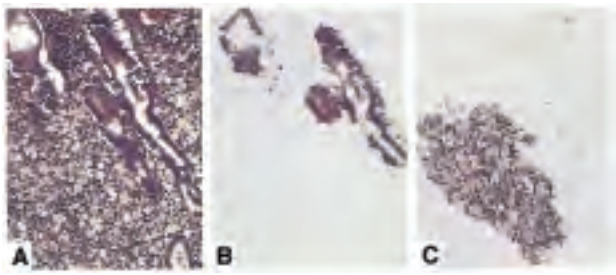

Fig. 4 *Sampling by laser microdissection*
Signet ring cells are observed in the interstitium (A). With IR laser irradiation of the target region, normal tubular tissues (B) and cancer tissues (C) can be collected separately without contamination.

Prospects of gene diagnosis in the field of pathology

June 26, 2000, President Clinton of the United States and Prime Minister Blair of Great Britain announced that nearly the entire base sequence of the human genome had been determined. The announcement continued that this is the greatest scientific achievement of this century, and this information should be a common property in all over the world. About 100,000 genes were identified by this achievement. It is a start of the post-sequence era. DNA chip and DNA microarray technology[39] have been established, thereby information of many kinds of gene mutations and expressions can be quickly detected, filed and analyzed. There is also a genetic diagnosis evolution in the field of pathology corresponding to this new scientific age. The findings which have been accumulated on molecular mechanisms of development/proliferation/advancement of gastrointestinal cancer, and findings which will be systematically clarified in the future will be combined, and genetic analysis by means of DNA microarray will surely become a mainstream in the diagnosis of gastrointestinal tissues. For construction of a routine system, a chip to detect gene abnormalities whose significance has already been known should be prepared for each organ cancer, the results of analysis of these abnormalities should be pooled. If essential abnormalities and secondary changes could be clarified in many gene abnormalities and epigenetic abnormalities identified in cancers, examinations of a hundred to several hundreds of genes are estimated to be enough for diagnosis of cancer. The important point is that the characteristics of each cancer, that is, communality and specificity of generation/development of each cancer become clear and the diagnosis directly useful so that genetic therapy or molecule-targeted therapy can be conducted by a pathological examinations.

Moreover, the relationship between single nucleotide polymorphism (SNP) occurred by one base replacement and the drug sensitivity or the carcinogenesis sensitivity has been noticed[40,41]. With progress in the SNP project, the association of detailed pathological conditions or sensitivities with various SNP will be clarified, and in the near future information on pharmacotherapy and prophylaxis of disease may be obtained from histopathological samples.

Comparison of morphological changes with abnormality in genes/molecules is the main benefit of genetic analysis diagnosis of histopathological samples. Therefore, this comparison will improve the accuracy of pathological diagnosis. "Pathology" has discovered minute abnormalities of shape, and has closely classified pathological conditions by accumulating knowledge obtained from the morphological observation (including pathological anatomy). Another important mission for gene diagnosis in the field of pathology is to clarify how abnormalities in gene/molecule/functions are reflected in morphology. The ultimate objective of the field of pathology is that morphological abnormalities of all diseases described in the literature of pathology correspond to gene abnormalities or molecule abnormalities.

CONCLUSION

The significance of the cell cycle regulators in gastrointestinal cancer, and molecular pathological diagnosis by examination of abnormalities of genes/molecules were described. Histopathological samples yield important information on prognosis and inheritance in addition to the diagnosis of histological benignancy or malignancy and degree of differentiation. In particular, for gastrointestinal cancer, tissue collection is endoscopically easy. In addition to formalin-fixed materials for histopathological diagnosis, fresh samples collected for genetic analysis will provide much important information for us, as stated in the section 3, prospects of gene diagnosis.

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