Genetic and Clinical Features of Human Pancreatic Ductal Adenocarcinomas with Widespread Microsatellite Instability

Hiroyuki Yamamoto, 1,2 Fumio Itoh, Hideaki Nakamura, Hiroshi Fukushima, Shigeru Sasaki, Manuel Perucho, and Kohzoh Imai

First Department of Internal Medicine [H. Y., F. I., H. N., H. F., S. S., K. I.], Sapporo Medical University, Sapporo 060-8543, Japan, and The Burnham Institute, La Jolla, California 92037 [M. P.]

ABSTRACT

The incidences of microsatellite instability (MSI) and underlying DNA mismatch repair (MMR) defects in pancreatic carcinogenesis have not been well established. We analyzed 100 sporadic and 3 hereditary pancreatic ductal adenocarcinomas for MSI, and high-frequency MSI (MSI-H) and low-frequency MSI (MSI-L) tumors were further analyzed for frameshift mutations of possible target genes and for promoter methylation and mutation of DNA MMR genes, including hMLH1, hMSH2, hMSH3, and hMSH6 genes. Among the 100 sporadic tumors, 13 (13%) were MSI-H, 13 (13%) were MSI-L, and 74 (74%) were microsatellite stable (MSS) tumors. All of the three hereditary tumors from hereditary nonpolyposis colorectal cancer (HNPCC) patients were MSI-H. MSI-H tumors were significantly associated with poor differentiation and the presence of wild-type K-RAS and p53 genes. Patients with MSI-H tumors had a significantly longer overall survival time than did those with MSI-L or MSS tumors (P = 0.0057). Frameshift mutations of hMSH3, hMLH3, BRCA-2, TGF-B type II receptor, and BAX genes were detected in MSI-H tumors. Hypermethylation of the hMLH1 promoter was observed in 6 (46%) of the 13 sporadic MSI-H tumors but not in any of the 3 hereditary MSI-H tumors or 13 MSI-L tumors. All of the 3 HNPCC cases had germ-line hMLH1 mutation accompanied by loss of heterozygosity or other mutation in the tumor. Our results suggest that pancreatic carcinomas with MSI-H represent a distinctive oncogenic pathway because they exhibit peculiar clinical, pathological, and molecular characteristics. Our results also suggest the principal involvement of epigenetic or genetic inactivation of the hMLH1 gene in the pathogenesis of pancreatic carcinoma with MSI-H.

INTRODUCTION

A novel type of genetic instability characterized by length alterations within simple repeated sequences, termed MSI, 3 occurs in the majority of HNPCC and in a subset of sporadic cancers (1–5). This phenotype is also known as a replication error or microsatellite instability. Thus far, there has been no overestimation of rare microsatellite length alterations as being MSI-H, MSI-L are indistinguishable in genotype (other than sporadic nonpolyposis colorectal cancers with MSI-H and those without MSI-H have been shown to exhibit fundamental differences in clinical, pathological, and molecular characteristics. In this regard, it is of interest that pancreatic carcinoma with MSI-H has been shown to exhibit characteristic features such as a wild-type K-RAS gene and a medullary phenotype characterized by poor differentiation, an expanding invasion, and syncytial growth (13, 16). Gastrointestinal cancers with MSI-H have a high rate of slippage-induced frameshift mutations in target genes such as TGFβRII (17), hMSH3 (18), hMSH6 (18), and BAX (19) and high frequencies of aberrant DNA hypermethylation of tumor suppressor genes, including the hMLH1 gene (20–24). As is the case of TGFβRII, target genes could be differentially mutated in MSI-H tumors from different primary sites. However, target genes of frameshift mutations have not been characterized in pancreatic carcinoma with MSI-H. Although hypermethylation of the hMLH1 promoter has been reported in two of four MSI pancreatic carcinomas (25), the number of cases is too small to draw conclusions regarding the mechanism underlying the MSI-H phenotype in this carcinoma.

In an attempt to address these issues, we analyzed 100 sporadic and 3 hereditary pancreatic ductal adenocarcinomas for MSI status, and we subsequently analyzed both MSI-H and MSI-L tumors for promoter methylation and mutation of DNA MMR genes and frameshift mutations of mononucleotide repeat sequences within possible target genes, including hMSH3 (18), hMSH6 (18), hMBD4 (26), hMLH3 (27), BRCA1 (28), BRCA2 (28), BLOOM (29), TGFβRII (17), IGFIIR (30), PTEN (31), RIZ (32), APC (33), BAX (19), FAS (34), APAF-1 (34), BCL-10 (34), CDX2 (35), CHK1 (36), CASPASE-5 (37), and TCF-4 (38) genes.

PATIENTS AND METHODS

Patients and Tissue Samples. Seventy fresh paired surgical specimens of sporadic pancreatic carcinoma and adjacent nonmalignant tissue and 30 paraffin-embedded sporadic tumor specimens were obtained from Japanese patients who had undergone surgical resection at our university hospital and related...
hospitals. The surgical procedures for 64 patients were regarded as clinico-pathologically curative according to the TNM classification of the International Union against Cancer (UICC) (39), and survival data were available for 60 patients. The other 36 procedures were noncurative operations with or without radiation or chemotherapy. Each fresh resected tumor specimen was evaluated for its tumor cell content in a H&E-stained section, and only specimens containing more than 75% of tumor cells were used for analysis. Ten-μm sections cut from 30 paraffin blocks were used for microdissection. Three paraffin-embedded hereditary tumor specimens were obtained from three patients who met the Amsterdam criteria for HNPCC (40). One of those three patients had a synchronous colon adenocarcinoma, and the other two had a history of colon carcinoma. All of the tumors were adenocarcinoma, and the histopathological and clinical features of the specimens were classified according to the guidelines of the UICC (39). Informed consent in writing was obtained from each patient, and the experiments were approved by the institutional review committee.

**Microsatellite Analysis.** Genomic DNA was extracted from fresh tumor and adjacent nontumorous tissues and from microdissected tissues using the phenol/chloroform method. Somatic microsatellite alterations were analyzed by PCR using the 5 microsatellite markers (BAT25, BAT26, D2S123, D3S346, and D17S250) proposed by the NCI workshop (41). PCR was carried out with Taq DNA polymerase (Perkin-Elmer, Branchburg, NJ) for one cycle of 94°C for 4 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s in the presence of 0.2 mCi of [32P]dCTP. PCR products were electro-phoresed in a denaturing 6% polyacrylamide gel. The gel was dried on a filter paper and subjected to autoradiography. Each PCR amplification was carried out at least twice.

**K-RAS and p53 Mutation Analyses.** K-RAS mutations at codons 12 and 13 and p53 mutations in exons 4–9 were analyzed as described previously (23, 42).

**Frameshift Mutation Analysis of Target Genes.** To examine frameshift mutations in mononucleotide repeat sequences within the possible target genes, including (A)9 of hMSH3 (18), (C)9 of hMSH6 (18), (A)9 of hMBD2 (26), (A)9 and (A)8 of hMLH1 (27), (A)6 of BRCA1 (28), (A)10 of BRCA2 (28), (A)8 of BLOOM (29), (A)10 of TGFBR1 (17), (G)8 of IGFII (30), (A)7 of PTEN (31), (A)9 and (A)6 of RIZ (32), (A)9 and (A)7 of APC (33), (G)9 of BAX (19), (T)7 of FAS (34), (A)7 of APAF-1 (34), (G)7 of BCL-10 (34), (G)7 of CDX2 (35), (A)10 of CHUK (36), (A)10 of CASPASE-5 (37), and (A)9 of TCF-4 (38). PCR amplification using published oligonucleotide sequences was carried out with Vent DNA polymerase (New England Biolabs, Beverly, MA) for one cycle of 94°C for 4 min followed by 30 cycles of 94°C for 30 s, 55–60°C for 30 s, and 72°C for 30 s in the presence of 0.2 mCi of [32P]dCTP. PCR products were electrophoresed in a denaturing 6% polyacrylamide gel. The gel was dried on a filter paper and subjected to autoradiography.

**Promoter Methylation and Immunoblot Analyses.** Methylation patterns in the promoter of hMLH1, hMSH2, hMSH3, and hMSH6 were determined using methylation-specific PCR as described previously (22, 43). In the chemical modification of cytosine to uracil by bisulfite treatment, all of the cytosines are converted to uracil, except for those that are methylated, which are resistant to this modification and remain as cytosine. One can design PCR primers to distinguish methylated from unmethylated DNA in bisulfite-modified DNA, taking advantage of the sequence differences resulting from bisulfite modification. Bisulfite-modified DNA was subjected to methylation-specific PCR, and PCR products were electrophoresed on a 3% agarose gel. For hMLH1, primer sequences were designed to amplify the region of which methylation correlates with the loss of hMLH1 expression (44). Those for the unmethylated reaction were 5'-GAAGATTGGTATATAATGATG-3' and 5'-ATCTTTCTATCCCTTCTACTTAAACA-3' and for the methylated reaction were 5'-AGCCGGAATACGTTTTTAACG-3' and 5'-CTCTGTGTCCCTCCTTTAAG-3'. Primer sequences of hMSH2, hMSH3, and hMSH6 have been described previously (22, 43). Frozen tissues were homogenized in lysis buffer [50 mM Tris-Cl (pH 7.4), 1% Triton X-100, 1 mM diethyldithiocar- bamic acid, 1 mM EDTA, 1% Tween 20, 10 μM leupeptin, and 1 mM phenylmethylsulfonyl fluoride] on ice. After sonication and centrifugation (10,000 × g at 4°C), protein content of the supernatant was measured by the method of Bradford. Solubilized proteins (100 μg) were separated by SDS-PAGE and transferred to a polyvinylidene difluoride transfer membrane. After being blocked with 5% blocking agent, the membrane was incubated with an antihuman hMLH1 monoclonal antibody (PharMingen, San Diego, CA) and developed using the enhanced chemiluminescence system (Amersham, Arlington Heights, IL). The membrane was then stripped and reincubated with a polyclonal antibasement antibody (Sigma, St. Louis, MO).

**Mutation Analysis of DNA MMR Genes.** Screening of the DNA MMR genes (hMLH1, hMSH2, hMSH3, and hMSH6) for mutations was performed by PCR-SSCP using previously published oligonucleotides sequences (45, 46). Oligonucleotides sequences for hMSH6 are available on request. Each PCR product was mixed with a sample buffer (95% formamide, 0.05% xylene cyanol, and bromphenol blue). The samples were heated at 95°C for 5 min, followed by rapid chilling on ice, and were subjected to electrophoresis on a non-denaturing polyacrylamide gel with or without glycerol at 4°C and 20°C. DNA sequencing was carried out as described previously (28).

**Statistical Analyses.** MSI status was assessed for associations with clinicopathological and genetic parameters using the following statistical tests: Student’s t test for age, the Mann-Whitney test for depth of invasion and pT-stage, and the χ2 two-tailed test or Fisher’s exact test for the remaining parameters. Survival analysis was carried out for stage II and III patients who had undergone curative surgery, except those who died of causes other than carcinoma. Cumulative survival rates were calculated by the Kaplan-Meier method. The difference between survival curves was analyzed by the log-rank test. P < 0.05 was judged to be statistically significant.

**RESULTS**

Among the 100 sporadic tumors analyzed, 13 (13%) were MSI-H, 13 (13%) were MSI-L, and 74 (74%) were MSS tumors. All three of the tumors from patients who met the Amsterdam criteria for HNPCC were MSI-H. Representative results of BAT26 alleles are shown in Fig. 1. All of the 13 MSI-H tumors demonstrated instability at BAT26, a sensitive marker for widespread MSI, whereas none of the 13 MSI-L tumors did. Associations between MSI status and clinicopathological and genotypical characteristics are summarized in Table 1. MSI-H tumors were significantly associated with poor differentiation, but MSI-L tumors were indistinguishable from MSS tumors. As regards genotype, both K-RAS and p53 mutations were less frequent in MSI-H tumors than in MSI-L or MSS tumors. The frequencies of MSI-H, MSI-L, and MSS in stage II and III tumors were almost the same (Table 1). Subsequent survival analysis for stage II and III patients revealed that patients with MSI-H tumors had a significantly longer overall survival time than did those with MSI-L or MSS tumors (Fig. 2; P = 0.0057).

In MSI tumors, frameshift mutations were detected in the hot spots within hMSH3 (4/16, 25%), hMLH3 (1/16, 6%), BRCA2 (4/16, 25%), TGFBR1 (8/16, 50%), and BAX (7/16, 44%) genes but not in any of the other genes examined (Fig. 3 and data not shown). No mutations were detected in any of the MSI-L tumors. All of the 7 cases with BAX mutations were negative for p53 mutations.

Hypermethylation of the hMLH1 promoter was observed in 6 (46%) of the 13 sporadic MSI-H tumors but not in any of the 3 hereditary MSI-H tumors or 13 MSI-L tumors (Fig. 4 and data not shown).
The characteristics of pancreatic and colon carcinomas in these three HNPCC patients did not express hMLH1.

Immunostaining for hMLH1 of two informative hereditary cases. Immunostaining for hMLH1 revealed that both pancreatic and colon tumors with MSI-H or MSI-L. Germ-line mutations of other DNA MMR genes were not detected in any of the tumors.

hMLH1 promoter hypermethylation, whereas the expression was retained in the remaining 5 tumors without hypermethylation (Fig. 4 and data not shown). None of the MSI-H or MSI-L tumors showed promoter hypermethylation of any of the hMSH2, hMSH3, and hMSH6 genes (data not shown). Somatic hMLH1 mutation (deletion of A at codon 195) was detected in 1 hereditary MSI-H tumor, but mutations of other DNA MMR genes were not detected in any of the tumors with MSI-H or MSI-L. Germ-line hMLH1 mutations (glycine to arginine (AGG) at codon 67, insertion of C at codon 497, and deletion of a lysine at codon 616) were detected in three HNPCC patients, one of whom had a somatic mutation in the tumor.

LOH at the hMSH3 locus (5q11-q13: D5S431 and D5S2107) was detected in one informative sporadic case with a hMSH3 frameshift mutation.

**DISCUSSION**

This study was carried out to gain a better understanding of the role of widespread MSI attributable to defective DNA MMR in pancreatic carcinogenesis. Recently, criteria of MSI for colorectal cancers have been proposed by the NCI workshop (41). On the basis of these criteria, 100 sporadic pancreatic carcinomas were classified as follows: 13 (13%) MSI-H, 13 (13%) MSI-L, and 74 (74%) MSS tumors. Therefore, the proportions of tumors with MSI-H, MSI-L, and MSS in pancreatic cancer appear to be similar to those in colon cancer (7, 41). MSI-H tumors were significantly associated with poor differentiation and with wild-type K-RAS and p53 genes. In contrast, MSI-L tumors were indistinguishable from MSS tumors. These results suggest that microsatellite analysis using the criteria proposed by the NCI workshop is appropriate for pancreatic carcinoma because it unveils real differences in genotype and phenotype. As regards the frequencies of MSI, comparisons with most previous studies are difficult because of the differences in definition of MSI positivity and tumor selection. Nevertheless, our results suggest that widespread MSI plays a role in a subset of pancreatic carcinogenesis. All of the 13 MSI-H tumors demonstrated instability at BAT26, whereas none of the 13 MSI-L tumors did. BAT26 can reportedly enable identification of MSI of ovarian pancreas and tumor, respectively.

---

**Table 1**: Associations between MSI status and clinicopathological and genotypical characteristics in patients with pancreatic carcinoma

<table>
<thead>
<tr>
<th>Variable + Category</th>
<th>MSI-H (n = 13)</th>
<th>MSI-L (n = 13)</th>
<th>MSS (n = 74)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender Male</td>
<td>4</td>
<td>8</td>
<td>57</td>
<td>ns*</td>
</tr>
<tr>
<td>Female</td>
<td>9</td>
<td>5</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well</td>
<td>1</td>
<td>3</td>
<td>15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Moderate</td>
<td>2</td>
<td>2</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>10</td>
<td>3</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Primary tumor (pT)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT1</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>ns</td>
</tr>
<tr>
<td>pT2</td>
<td>2</td>
<td>2</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>pT3</td>
<td>6</td>
<td>5</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>pT4</td>
<td>4</td>
<td>5</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis (pN)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pN0</td>
<td>4</td>
<td>5</td>
<td>25</td>
<td>ns</td>
</tr>
<tr>
<td>pN1</td>
<td>9</td>
<td>8</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>Distant metastasis (pM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pM0</td>
<td>11</td>
<td>11</td>
<td>60</td>
<td>ns</td>
</tr>
<tr>
<td>pM1</td>
<td>2</td>
<td>2</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>pTNM stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pl</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>ns</td>
</tr>
<tr>
<td>pII</td>
<td>4</td>
<td>3</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>pIII</td>
<td>4</td>
<td>4</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>pIV</td>
<td>3</td>
<td>4</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>pIVa</td>
<td>1</td>
<td>1</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>K-RAS mutations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>10</td>
<td>2</td>
<td>8</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Positive</td>
<td>3</td>
<td>11</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>p53 mutations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>11</td>
<td>5</td>
<td>32</td>
<td>0.0060</td>
</tr>
<tr>
<td>Positive</td>
<td>2</td>
<td>8</td>
<td>42</td>
<td></td>
</tr>
</tbody>
</table>

* ns, not significant.

![Graph showing Kaplan-Meier survival curves of patients with stage II or III pancreatic carcinoma according to the MSI status.](image)

---

**Fig. 2.** Kaplan-Meier survival curves of patients with stage II or III pancreatic carcinoma according to the MSI status.

**Fig. 3.** Analysis of frameshift mutations in hMSH3, BRCA2, BAX, and TGFβRII genes in pancreatic adenocarcinoma tissues with MSI-H. Arrowhead at right, the position of PCR products corresponding to the wild-type repeated sequence. One paired MSS cancer tissue (Lane 1) is shown as a negative control. N and T, matched DNA samples from nontumorous pancreas and tumor, respectively.

**Fig. 4.** Methylation-specific PCR of the hMLH1 gene in pancreatic adenocarcinoma tissues (top). The presence of a visible PCR product in those lanes marked "U" indicates the presence of unmethylated genes; the presence of a product in those lanes marked "M" indicates the presence of methylated genes. Immunoblot analysis of hMLH1 protein (bottom). Actin served as an internal control. N and T, matched samples from nontumorous pancreas and tumor, respectively.
tumors from various origins such as the breast, brain, stomach, prostate, esophagus, thyroid, endometrium, and cervix without the requirement for matching normal DNA, with 99.5% efficiency (47). Our results further support the notion that BAT26 is a sensitive marker for widespread MSI. Nevertheless, it should be noted that 2–4% of the population, especially of African descent, carry significantly shorter BAT26 alleles (8).

The association of MSI-H with improved prognosis in colorectal and gastric cancers has been a matter of argument (23, 48). However, results of recent large-scale studies with protocolized longer prospective follow-up periods have confirmed that the MSI-H phenotype defines a good prognostic group (49, 50). In the current study, we have shown that the MSI-H phenotype is associated with good prognosis in patients with pancreatic carcinoma. Although the number of MSI-H cases is small and the follow-up period is short to draw any definite conclusions, our results warrant further study.

Although the frequencies were relatively low, frameshift mutations were detected in hMSH3, hMLH3, BRCA2, TGFβRII, and BAX genes. Interestingly, pancreatic carcinoma is frequently seen in some cancer families with BRCA2 mutations, and germ-line BRCA2 mutations have been reported in sporadic pancreatic cancers (51, 52). Thus, impaired BRCA2 function may play a role in pancreatic carcinogenesis. Considering the presence of wild-type K-RAS and p53 genes in most tumors with MSI-H, frameshift mutations in these genes are most likely relevant for MSI-H pancreatic carcinogenesis. The negative correlation found between p53 mutations and BAX mutations further supports this notion. Although we have analyzed a number of possible target genes reported to date, no mutations were found in some pancreatic carcinomas with MSI-H. Additional analyses are required to clarify the functional roles of inactivation of target genes and to identify other important target genes in pancreatic carcinoma with MSI-H.

High incidences of hypermethylation of the hMLH1 promoter have been reported in sporadic colorectal, gastric, and endometrial cancers with MSI-H (21–24, 43). However, considering the correlation of hMLH1 promoter hypermethylation and the loss of expression, previously reported frequencies of hMLH1 hypermethylation appear to be overestimations of the relevant hypermethylation (44). We, therefore, analyzed the region in which hypermethylation is closely correlated with the loss of hMLH1 expression (44). As a result, hypermethylation of the hMLH1 promoter was detected in 46% of the 13 sporadic MSI-H tumors. In contrast, hypermethylation was not detected in any of the 3 hereditary MSI-H tumors or in any of the 13 MSI-L tumors. The possibility of underestimation of the frequency is low, because we have detected hMLH1 promoter hypermethylation in 70% of 30 sporadic colorectal cancers with MSI-H using the same method. In contrast to hypermethylation, hMLH1 somatic mutation was not detected in any of the sporadic MSI-H or MSI-L tumors. On the other hand, all of the 3 hereditary cases had germ-line hMLH1 mutation accompanied by LOH or other mutation in the tumor. Thus, in analogy with hMLH1 mutations in colorectal cancer with MSI-H, hypermethylation is characteristic of sporadic cases, whereas in hereditary cases, hypermethylation appears to be uncommon (22, 53). As for other DNA MMR genes, no promoter hypermethylation and no mutations other than the above-mentioned frameshift mutations were detected. Taken together, our results suggest the principal involvement of hMLH1 inactivation in sporadic and hereditary pancreatic cancers with MSI-H. Considering the sensitivity of SSCP, we cannot exclude the possibility that other mutations might be present. Sequencing of the entire DNA MMR genes and Southern blot analysis will be necessary. Because a combination of mutation and LOH was observed, hMSH3 could be a primary mutator. Alternatively, other inactivation mechanisms for the known DNA MMR genes or other hitherto-unidentified mutator genes may exist.

Our results suggest that pancreatic carcinoma with MSI-H represents a distinctive oncogenic pathway. Pancreatic and colon carcinomas with MSI-H have similarities, including the principal involvement of hMLH1 inactivation, association with wild-type K-RAS and p53, and association with poor differentiation and improved prognosis. It is notable that both pancreatic and colon carcinomas in the three HNPCC patients were MSI-H and that hMLH1 was not expressed in either carcinoma. The occurrence of two carcinomas with MSI-H supports the notion of an inherited basis for the development of these carcinomas. Moreover, both of the tumors were poorly differentiated carcinomas. Correlation of the MSI-H phenotype with poor differentiation has been shown in colorectal carcinoma (2, 7). Previous studies (13, 16) as well as the present study have extended this observation to pancreatic carcinoma. It is also intriguing that medullary-type poorly differentiated adenocarcinoma of the large bowel was shown to be strongly correlated with the MSI-H phenotype (54). Some of the cases in our series of poorly differentiated pancreatic adenocarcinoma with MSI-H may be defined as a medullary carcinoma of the pancreas, a recently described, histologically distinct subset of poorly differentiated pancreatic adenocarcinoma (13, 16). However, because the number of medullary carcinomas of the pancreas reported to date is too small (16), further analysis is needed to clarify these issues.

As for MSI-L tumors, the nature of MSI-L has not yet been substantiated by the characterizations of underlying DNA MMR or other defects (8). The MSI-L phenotype could simply represent a background level of genetic instability that may be present in all tumors. Given a sufficient number of markers, all tumors could exhibit low instability. Indeed, it was reported that this was the case for Barrett’s-associated esophageal adenocarcinoma (55). On the other hand, a frequent loss of imprinting of the IGFII gene has been reported in cancer tissues as well as in matched normal colonic mucosa of patients with MSI-H or MSI-L cancer (56). These results suggest that MSI-L may be caused by defective replication and imprinting fidelity. Further study is required to clarify the real differences, if any, between MSI-L and MSS pancreatic cancers.

<table>
<thead>
<tr>
<th>Case</th>
<th>MSI</th>
<th>Differentiation</th>
<th>K-RAS mutation</th>
<th>p53 mutation</th>
<th>BAX (G) mutation</th>
<th>hMLH1</th>
<th>Expression</th>
<th>LOH</th>
<th>Somatic</th>
<th>Germ-line</th>
</tr>
</thead>
<tbody>
<tr>
<td>HI</td>
<td>MS-L</td>
<td>poor</td>
<td>wt</td>
<td>wt</td>
<td>7/8</td>
<td></td>
<td>--</td>
<td>ni</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>MS-L</td>
<td>poor</td>
<td>wt</td>
<td>wt</td>
<td>7/8</td>
<td></td>
<td>--</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>H21</td>
<td>MS-L</td>
<td>poor</td>
<td>wt</td>
<td>wt</td>
<td>7/9</td>
<td></td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>H33</td>
<td>MS-L</td>
<td>poor</td>
<td>wt</td>
<td>mut</td>
<td>wt</td>
<td></td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>MS-L</td>
<td>poor</td>
<td>wt</td>
<td>wt</td>
<td>7/7</td>
<td></td>
<td>--</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* wt, wild type; mut, mutant; ni, not informative; nd, not determined; +, positive; −, negative.
REFERENCES


Genetic and Clinical Features of Human Pancreatic Ductal Adenocarcinomas with Widespread Microsatellite Instability


Cancer Res 2001;61:3139-3144.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/61/7/3139

Cited articles
This article cites 52 articles, 30 of which you can access for free at:
http://cancerres.aacrjournals.org/content/61/7/3139.full#ref-list-1

Citing articles
This article has been cited by 9 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/61/7/3139.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.