Alterations of the p53 Tumor Suppressor Gene and Its Association with Activation of the c-K-ras-2 Protooncogene in Premalignant and Malignant Lesions of the Human Uterine Endometrium

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ABSTRACT

We previously reported (T. Enomoto et al., Cancer Res., 50: 6139-6145, 1990; T. Enomoto et al., Cancer Res., 51: 5308-5314, 1991) a significant frequency of activating point mutations in codon 12 of the c-K-ras-2 protooncogene in endometrial adenocarcinoma and its premalignant precursor lesions (series 1 and 2). To reveal the role of the p53 tumor suppressor gene in the development of endometrial adenocarcinoma and to study the association of p53 alterations with K-ras activation, an additional 28 endometrial adenocarcinomas and an additional 11 premalignant atypical uterine hyperplasias (series 3), as well as 12 cases of endometrial adenocarcinoma (10 having K- or N-ras activation) and 2 cases of atypical hyperplasia from series 1 and 2, were screened for the presence of p53 alterations. Allelic loss, recognized at the polymorphic site in codon 72 of the p53 gene, was detected in 6 of 19 (32%) informative cases of endometrial adenocarcinoma and 1 of 4 (25%) informative cases of endometrial atypical hyperplasia by restriction fragment length polymorphism analysis. Mutations in the highly conserved regions of the p53 gene were detected by single-strand conformation polymorphism analysis of PCR-amplified DNA fragments. Mutations in codons 12 and 13 of the c-K-ras-2 protooncogene were observed in 7 of 28 adenocarcinomas and none were found in exons 1 and 2 (codons 59-63). The spectrum of point mutations in p53 in endometrial adenocarcinomas was almost identical to what we found in K-ras in series 1 and 2, suggesting the possible role of a mutagen that might be responsible for mutations in both K-ras and p53. However, there was no correlation between the presence of p53 gene mutations and K-ras activation in these tumors. It appears that inactivation of K-ras, as well as K-ras activation, plays a significant role in the development of endometrial adenocarcinoma. In contrast to K-ras activation, which commonly occurs as an early event, inactivation of p53 usually occurs as a later event in endometrial carcinogenesis, independently of K-ras activation.

INTRODUCTION

Endometrial carcinomas are common, representing 13% of all malignancies in women in the United States (1), where the incidence appears now to have leveled off after an abrupt increase during the late 1960s to early 1970s (2-4). In Japan, they are the second most common malignancy of the female urogenital tract, and their incidence is increasing (5). However, the pathogenesis of endometrial carcinoma is not yet fully understood.

While ras gene mutation is observed in some endometrial adenocarcinomas, more than 60% of these tumors do not contain any detectable ras gene mutations. This indicates that there are alternative genetic alterations which contribute to carcinogenesis of the human endometrium. However, little is known about other genetic changes that contribute to endometrial carcinogenesis. In colorectal carcinomas, deletions of chromosomes 5q, 17p, and 18q are frequently found in addition to ras gene mutations. These observations suggest that multiple additive genetic changes are involved in the development of colorectal carcinomas (8) and, by inference, possibly in other carcinomas as well that, like the majority of colonic carcinomas, evolve by progression from premalignant precursor lesions.

Alteration of the p53 tumor suppressor gene by base substitution, deletion or insertion mutations, or allelic loss or rearrangements is observed in a wide variety of human cancers and is currently the most commonly found alteration associated with human cancers (9, 10). Alteration of both p53 alleles, one through deletion and the other through a base substitution mutation, occurs frequently in colorectal (11), lung (12), breast (13), brain (10), and other human cancers (14). We therefore attempted to detect various possible alterations in p53 in malignant and premalignant lesions of human uterine endometrium. We detected loss of heterozygosity by restriction fragment length polymorphism analysis and SSCP analysis of PCR-amplified DNA fragments (15) and screened for mutations by PCR-SSCP (16). We established the identity of these mutations by genomic DNA sequencing.

MATERIALS AND METHODS

Tissues. Samples used in this study were from patients who had been admitted to the Department of Obstetrics and Gynecology at the Osaka University Hospital in Osaka, Japan. No initial chemotherapy or hormonal therapy was performed prior to tumor excision. Surgically removed tissues were sampled for histopathological diagnosis, and remainders were frozen for extraction of DNA. In some cases, especially those in which normal stromal cells or infiltrative cells comprised more than 30% of cells in histological sections of...
a lesion, DNA was extracted from selected areas of formalin-fixed paraffin-embedded tissue sections. The clinical stage of each carcinoma was established according to the International Federation of Gynecology and Obstetrics staging system (17). Peripheral blood samples from the patients were also collected for extraction of DNA prior to tumor removal. The current series included 28 new cases of endometrial adenocarcinomas and 11 new cases of atypical hyperplasia (series 3). Ten cases of endometrial adenocarcinomas which were shown to contain K- or N-ras gene mutations in series 1 and 2 (6, 7), two cases from series 1 and 2 in which no ras gene mutations could be detected, and 2 atypical hyperplasias in series 2 (7) from which DNA from WBC was available were also analyzed for the presence of p53 alterations.

Detection of Point Mutations in the K-ras Gene by Direct Sequencing. All 28 cases of endometrial carcinoma and all 11 cases of atypical endometrial hyperplasia in series 3 were analyzed for the presence of point mutations in K-ras. PCR was performed to generate amplified fragments of exon 1 or exon 2 segments of the K-ras gene, which were subsequently sequenced by the dideoxy termination method as previously reported (7, 18).

Detection of Loss of Heterozygosity in the p53 Gene. The second position of codon 72 of p53 is known to be naturally polymorphic; CGC (Arg) as well as CCC (Pro) is frequently observed (19). This polymorphism can be detected by the restriction enzyme BstUI in cases where its restriction site (CGCG) is present, i.e., those in which codon 72 is CGC (15). PCR was performed to generate an amplified DNA fragment of 247 base pairs surrounding codon 72 of p53. Primers used were 5’-GATGCTGTCCCGGA-3’ (upstream) and 5’-CGTGCAAGTCACAGACTTGGC-3’ (downstream) (15). One base in this upstream primer was altered to create an artificial restriction site for BstUI digestion (236 and 11 base pairs) when no restriction site was present in that codon.

BstUI digestion (236 and 11 base pairs) when no restriction site was present in codon 72, whereas three fragments (160, 76, and 11 base pairs) resulted when the restriction site was present in that codon.

Loss of heterozygosity in the p53 gene was also detected by an alternative PCR-SSCP analysis in all cases. PCR amplification was performed to generate 66-base pair DNA fragments surrounding codon 72. Primers used were 5’-CAGATGAAGCTCCTCAGAA-3’ (upstream) and 5’-GTGTAGGAGCTCGGTTGGT-3’ (downstream) (15). The PCR reaction mixture (total 3 μl) contained genomic DNA (0.1 μg), deoxyribonucleotide triphosphates (60 μM), 32P-end-labeled primer (0.1 μM each), MgCl2 (1.5 mM), Tris (pH 8.3) (10 mM), KCl (50 mM), and Taq polymerase (0.1 unit; Perkin Elmer Cetus Corp., Norwalk, CT). One cycle of PCR consisted of 95°C for 1 min, 57°C for 30 s, and 72°C for 30 s, and a total of 30 cycles of PCR amplification were performed. SSCP analysis was conducted following the procedures previously published (20).

Detection of Point Mutation in p53 by PCR-SSCP Analysis. Exons 5, 6, 7, and 8 were amplified by PCR using primers, the sequences of which were provided by Dr. U. Schlegel* and published previously (20). Conditions of PCR amplification and SSCP analysis were the same as previously published (20).

DNA Direct Sequencing Analysis. PCR-amplified DNA fragments of p53 which showed bands with altered mobilities by SSCP were subsequently digested by the dideoxy termination method as previously described (20).

Statistics. The significance of differences in the frequency with which mutations occurred in different categories of lesions was estimated using Fisher’s exact test (21).

RESULTS

Point Mutations in K-ras. Point mutational activation of K-ras was detected by direct sequencing. Of 28 endometrial adenocarcinomas in series 3, 7 tumors (25%) contained demonstrable point mutations in exon 1 of K-ras, 5 cases in exon 2, and 2 cases in exon 13 (Table 1). As was previously the case for series 1 and 2, no mutations were detected in exon 2 (codons 59–63) of K-ras. The incidence of point mutations in K-ras detected by direct sequencing was 28% (8 of 29) in series 1 and 2 (7), which was consistent with the incidence in series 3 but slightly underestimates the frequency identifiable by the more sensitive oligonucleotide hybridization method (38% for K-ras plus N-ras in series 1 plus 2).

Point mutations in K-ras in atypical hyperplasias were also examined. Of 11 atypical hyperplasias in series 3, one case contained a GGT→GTT transversion in codon 12 and one case contained a GCC→GAC transition in codon 13 (Table 2).

Loss of Heterozygosity in p53. Loss of heterozygosity in the p53 gene was initially surveyed by restriction fragment length polymorphism analysis of the PCR-amplified 247-base pair DNA fragment. Loss of heterozygosity was further analyzed by SSCP analysis of a
There was no discrepancy between observations by restriction fragment length polymorphism analysis and by SSCP analysis in those cases in which loss of heterozygosity could be detected by both methods. There was no significant association between loss of heterozygosity and any clinical parameters of the tumors such as stage, grade, presence of distant metastases, or depth of myometrial invasion.

Loss of heterozygosity in the p53 gene in atypical hyperplasias in series 2 and 3 was also detected. Of 9 cases of atypical hyperplasia in which DNA derived from WBC was available, 4 cases (44%) showed heterozygosity in codon 72 of the p53 gene. Loss of heterozygosity was observed in 6 of 19 (32%) informative cases of adenocarcinoma (Tables 1 and 3). There was no discrepancy between observations by restriction fragment length polymorphism analysis and by SSCP analysis in those cases in which loss of heterozygosity could be detected by both methods. There was no significant association between loss of heterozygosity and any clinical parameters of the tumors such as stage, grade, presence of distant metastases, or depth of myometrial invasion.

Loss of heterozygosity in the p53 gene in atypical hyperplasias in series 2 and 3 was also detected. Of 9 cases of atypical hyperplasia in which DNA derived from WBC was available, 4 cases (44%) showed heterozygosity in codon 72 of the p53 gene. Loss of heterozygosity was observed in one of these 4 informative cases (25%) (Table 2).

**Mutations in p53 in Endometrial Adenocarcinomas.** A total of 40 adenocarcinomas, including 28 in series 3 and 12 from series 1 and 2, were screened for p53 mutations in exons 5 through 8, where the majority of the point mutations are reported in human neoplasms, by PCR-SSCP analysis of each exon. Altered mobilities of PCR products, corresponding to the normal allele, were observed in these 9 cases, whereas only the normal allele bands were seen in the remaining 31 cases. In 8 of 9 cases which showed altered mobilities, signals of 2 bands corresponding to the normal allele were significantly reduced or not detectable. We concluded that the normal allele of p53 was lost in such cases.

Mutations in p53 were defined by direct sequencing. Missense point mutations were found in 5 cases, and a nonsense mutation was found in one case (Fig. 1). A G:C->T:A transversion resulted in Glu->new stop codon (GAG->TAG) in the 476% of cases, and a G:C->A:C transition (CAT-GAT) in codon 193 resulted in His->Asp substitution in one case. The A:T transitions were found in four cases: CGG->CAG in codon 248 in case 69, resulting in Arg->Gln substitution; CGC->TGC in codon 248 in case 69, resulting in Arg->Gln substitution; CGC->TGC in codon 181 in case 70, and in codon 175 in case 72, resulting in Arg->Cys substitution; and CGG->TGG in codon 248 in case 15, resulting in Arg->Trp substitution. A single base pair deletion in codon 149 in exon 5 (TCC->ACC) resulting in a new stop codon (TGA) in codon 169 was observed in case 64 (Fig. 1), and a 2-base pair deletion in codon 293 in exon 8 (GGG->AAG) resulting in a new stop codon (TAA) in codon 304 was observed in case 6. A single base pair insertion in codon 233 (CAT->CACC) in exon 7 resulting in a new stop codon (TAA) in codon 304 was detected in case 55.

### Table 2: Alterations of p53 and K-ras in atypical endometrial hyperplasias of the human uterus (series 2 and 3)

<table>
<thead>
<tr>
<th>Case</th>
<th>Grade</th>
<th>Exon</th>
<th>Codon</th>
<th>Mutation Codon</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td></td>
<td>7</td>
<td>248</td>
<td>GCG-&gt;CAG</td>
</tr>
<tr>
<td>74</td>
<td></td>
<td>7</td>
<td>248</td>
<td>GCG-&gt;CAG</td>
</tr>
<tr>
<td>75</td>
<td></td>
<td>7</td>
<td>248</td>
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<td>7</td>
<td>248</td>
<td>GCG-&gt;CAG</td>
</tr>
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<td>79</td>
<td></td>
<td>7</td>
<td>248</td>
<td>GCG-&gt;CAG</td>
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<td>GCG-&gt;CAG</td>
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<td>7</td>
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</tr>
<tr>
<td>82</td>
<td></td>
<td>7</td>
<td>248</td>
<td>GCG-&gt;CAG</td>
</tr>
</tbody>
</table>

### Table 3: Alterations of p53 and K-ras in endometrial adenocarcinomas of the human uterus (series 1 and 2)

<table>
<thead>
<tr>
<th>Case</th>
<th>Grade</th>
<th>Exon</th>
<th>Codon</th>
<th>Mutation Codon</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td></td>
<td>12</td>
<td>GGT-GTT</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>12</td>
<td>GGT-GTT</td>
<td></td>
</tr>
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<td>18</td>
<td></td>
<td>12</td>
<td>GGT-GTT</td>
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<td>20</td>
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<td>GGT-GTT</td>
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<td>25</td>
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<td>12</td>
<td>GGT-GTT</td>
<td></td>
</tr>
<tr>
<td>293</td>
<td></td>
<td>12</td>
<td>GGT-GTT</td>
<td></td>
</tr>
<tr>
<td>293</td>
<td></td>
<td>12</td>
<td>GGT-GTT</td>
<td></td>
</tr>
<tr>
<td>293</td>
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<td>12</td>
<td>GGT-GTT</td>
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<tr>
<td>293</td>
<td></td>
<td>12</td>
<td>GGT-GTT</td>
<td></td>
</tr>
</tbody>
</table>

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**PCR-amplified 66-base pair DNA fragment.** The second step was necessary since the 247-base pair DNA fragments were not successfully amplified in some cases in which DNA was extracted from formalin-fixed paraffin-embedded sections. Of 37 cases of endometrial adenocarcinomas from series 1, 2, and 3 in which DNA derived from WBC was available, 19 cases (52%) showed heterozygosity in codon 72 of the p53 gene. Loss of heterozygosity was observed in 6 of 19 (32%) informative cases of adenocarcinoma (Tables 1 and 3).
A mutation in the p53 gene in one premalignant lesion of uterine endometrium was also identified. The existence of a mutation in exon 7 was suggested in one of 13 atypical hyperplasias studied by PCR-SSCP analysis (Fig. 2). Point mutation was subsequently confirmed by direct sequencing in this single case from series 2, a CGG→CAG transition in codon 248 resulting in Arg→Gln substitution (Table 3, for an overall incidence of one in 13 (8%).

DISCUSSION

The combined results of our previous studies and the present investigation are summarized in Table 4. We previously reported that 11 of 29 endometrial adenocarcinomas of series 1 and 2 (38%) contained ras gene mutations detectable by dot-blot hybridization; 10 were in K-ras (34%) and one in N-ras (4%) (6). We subsequently performed direct sequencing, which is less sensitive than dot-blot hybridization, to confirm ras mutations. We could not detect mutated alleles by this method when they comprised 12% or less of all K-ras gene copies; only 8 of 29 tumors (28%) revealed mutated K-ras sequences by this method (6). In the present study, we found that 7 of 28 tumors (25%) in series 3 contained K-ras mutations detectable by direct sequencing, which was consistent with the incidence in series 1 and 2. When the 29 endometrial adenocarcinomas of our series 1 and 2 were added, at least 17 of 57 cases (30%) contained activating point mutations in the K-ras gene, and 15 of 57 (26%) contained one or more mutated K-ras alleles comprising more than 12% of K-ras gene copies. Recently Mizuuchi et al. (22) confirmed the existence of activated K-ras in endometrial adenocarcinoma in Japanese women, although they reported that only 6 of 49 tumors in their series (12%) contained mutations. These authors extracted DNA from frozen tissue samples which were estimated to consist of more than 80% tumor cells, but they did not microdissect the sections to obtain tissue enriched in tumor cells. In our experience, in even the most carefully prepared samples, it was rare to obtain tissue in which tumor cells constituted more than 80% of total cells, since most endometrial cancer tissues contain abundant stromal cells and infiltrative cells. If microdissection from paraffin-embedded sections was not performed, wild-type K-ras alleles from normal cells usually diluted the mutated allele below the level of detection, and significant underestimation of the true incidence would result (18).

All of the activating mutations we found in K-ras occurred in either codon 12 (15 tumors) or codon 13 (2 tumors); no mutations were found in the exon 2, codon 59–63 region. If three double mutations we detected by dot-blot hybridization in series 1 and 2 are counted separately, we found 20 point mutations in G:C pairs: G:C→A:T transversions in 10 cases; G:C→T:A transversions in 7 cases; and G:C→C:G transversions in 3 cases. No mutations were observed in A:T pairs.

We also studied K-ras activation in 11 cases of atypical hyperplasia in series 3 by direct sequencing. A codon 12 GTG→GTG transversion was detected in one case and a codon 13 GCC→GAC transition in a second case. When 16 atypical hyperplasias in series 1 and 2 were added, point mutations in K-ras were detected in a total of 4 of 27 atypical hyperplasias (15%). The incidence of point mutations in K-ras in G1 cancers (10 of 33; 30%) was higher than that in atypical hyperplasias, but not to a statistically significant extent (P = 0.13), and there was only a limited correlation between histological grade and the presence of point mutations in K-ras in endometrial adenocarcinomas. This suggests that activation of K-ras more commonly occurs in atypical hyperplasias that progress to cancer, rather than during progression of G1 cancer to more highly malignant grades. K-ras gene activation apparently does occur as a later event in some cases, from the previous observations that some carcinomas (but, so far, no atypical hyperplasias) contain two different mutations differing in relative abundance. Also, in one case, a K-ras codon 12 mutation was predominantly found in a region of a carcinoma which showed a more aggressive histological pattern (7). More such cases are needed to assess the significance of these observations.

Both K-ras activation and incidence of allelic loss and mutations in p53 were much more frequent in endometrial adenocarcinoma of the
p53 AND K-ras IN HUMAN UTERINE LESIONS

Table 4 Summary of p53 and K-ras gene alterations in primary human uterine lesions (series 1, 2, and 3)

<table>
<thead>
<tr>
<th>Lesion</th>
<th>p53 LOH</th>
<th>p53 Mutation</th>
<th>Activated K-ras genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corpus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premalignant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atypical hyperplasia</td>
<td>1/4 (25%)</td>
<td>1/13 (8%)</td>
<td>4/27 (15%)</td>
</tr>
<tr>
<td>Malignant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endometrial adenocarcinoma</td>
<td>6/19 (32%)</td>
<td>9/40 (23%)</td>
<td>17/57 (31%)</td>
</tr>
<tr>
<td>G1</td>
<td>3/9 (33%)</td>
<td>2/19 (11%)</td>
<td>10/33 (30%)*</td>
</tr>
<tr>
<td>G2</td>
<td>1/4 (25%)</td>
<td>1/7 (14%)</td>
<td>3/8 (38%)</td>
</tr>
<tr>
<td>G3</td>
<td>2/6 (33%)</td>
<td>6/14 (43%)</td>
<td>4/16 (25%)</td>
</tr>
<tr>
<td>Cervix</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malignant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervical carcinoma</td>
<td>0/10 (0%)*</td>
<td>2/36 (6%)</td>
<td>1/23 (4%)*</td>
</tr>
</tbody>
</table>

* Data included from series 1 (6) and series 2 (7).
* Significantly higher than G1 cancers (P = 0.042) or than G1—G2 cancers combined (P = 0.033).
* Data from Refs. 7 and 20.
* Lower than in endometrial adenocarcinoma but of borderline significance (P = 0.057). Data from Ref. 20.
* Significantly lower than in endometrial adenocarcinoma (P = 0.0035). Data from Ref. 7.

Table 4: Summary of p53 and K-ras gene alterations in primary human uterine lesions (series 1, 2, and 3).

In cervical carcinoma, K-ras activation was found in only one of 23 tumors (4%, P = 0.0099) (7), loss of heterozygosity in p53 was found in none of the informative cases (0%, P = 0.057), and mutations in p53 were detected in only 2 of 36 tumors (6%, P = 0.035). This indicates a significant difference in oncogenic pathway between endometrial carcinoma and cervical carcinoma. Cervical carcinoma is known to be strongly associated with specific strains of HPV (23), and E6 and E7 proteins of certain oncogenic HPVs (HPV-16 and -18) form stable complexes with p53 and pRB, respectively (24). Loss of normal tumor suppressor functions of these two genes may play key roles in the etiology of cervical carcinomas positive for HPV infection. In fact, we detected HPV DNA by Southern blot analysis and found that 19 of 36 cervical carcinomas (53%) were HPV-positive (20), whereas only 2 of 27 endometrial carcinomas in series 3 (7%) were HPV-positive (P = 0.00012). No p53 mutations were detected in any HPV-positive cervical carcinomas. In these tumors, p53 may be inactivated by complexing with E6 protein. Therefore, inactivation of p53 by allelic loss or inactivation may not be obligatory. Since no such viruses that can inactivate p53 have been identified in endometrial carcinoma, inactivation of p53 by allelic loss or mutations may play a significant role in these tumors. However, p53 mutations were detected in only 2 of 10 cervical carcinomas which contained low copy numbers of HPV DNA that was detectable only by PCR (1 copy/10^-10^ cells). Moreover, none of 9 cervical carcinomas without detectable HPV contained p53 mutations. Therefore, inactivation of the p53 gene by allelic loss or mutations is infrequent in cervical carcinoma, irrespective of the presence or absence of HPV infection. In cervical carcinomas, especially those without HPV infection or with infection but at low HPV copy number, there should exist an alternative oncogenic pathway which is independent of p53 inactivation.

Association between the presence of K-ras activation and the presence of p53 mutation in endometrial carcinoma was evaluated. Incidence of p53 mutations in the tumors with ras activation (2 of 17, 11.8%) was lower (but not significantly so) than in the tumors without ras activation (7 of 23, 30%; P = 0.16). This indicates that ras and p53 gene mutations occur independently in endometrial adenocarcinoma, which is consistent with the findings in non-small-cell lung cancer (25). In non-small-cell lung cancer, however, p53 mutations which coexist with ras mutations tend to cluster in exon 8 (26). We did not observe any such tendency in the only two endometrial adenocarcinomas with both ras and p53 mutations, both of which had p53 mutations in exon 7.

Correlations between the presence of p53 mutations and histological grade of endometrial carcinomas were attempted. Mutations were significantly more frequently found in G3 cancers (6 of 14, 43%) than in G1 cancers (2 of 19, 11%; P = 0.042) or in G1 and G2 cancers combined (3 of 26, 12%; P = 0.033). No correlations were established between the presence of p53 mutations and other clinical parameters, such as clinical stage, presence of lymph node or distant metastases, or depth of myometrial invasion.

Association between allelic loss and the presence of mutations in p53 was evaluated. The frequency of allelic loss, based on the codon 72 polymorphism (6 of 19, 32%), was slightly higher (but not significantly so) than the frequency of mutations in p53 (9 of 40, 23%; P = 0.33). Of 9 tumors with mutations in p53, 8 were also analyzed for allelic loss in p53 detectable by the codon 72 polymorphism (Tables 1 and 3). Four of these 8 cases were informative, and allelic loss was observed in all 4 cases. Moreover, in 4 of 4 carcinomas with mutations in p53 in which allelic loss could not be evaluated by codon 72 polymorphism, loss of a normal allele was suggested by the absence of two bands which represent the wild-type sequences in PCR-SSCP analysis. Of 31 tumors that did not contain mutations in highly conserved regions of the p53 gene, 15 cases were informative (48%) and one showed allelic loss in p53 by the codon 72 polymorphism criterion (case 57). It is possible that mutations exist in exons that were not evaluated in this case. However, studies that included outlying exons suggest mutations outside exons 5–8 are rare (14).

Although mutations in p53 were more frequently detected in G3 cancers than in G1—G2 cancers, and mutations in p53 may thus occur as a later event in tumor progression, it is important to note that alterations in p53 were detected in one case of atypical hyperplasia. This shows that p53 alteration can also occur in earlier stages of tumor development. Atypical hyperplasia is considered to have the potential to develop into carcinoma. Although the risk of invasive cancer for women with atypical hyperplasia is considered to be 5–12% (26), little is known about prognostic indicators for individual cases of atypical hyperplasia. Evaluation of oncogene activation and tumor suppressor gene alteration, including c-K-ras-2 and p53, may be a useful indicator of prognosis for atypical endometrial hyperplasia.

Of 7 point mutations in p53 which were detected in endometrial carcinoma or in atypical hyperplasia, 5 were G:C—>A:T transitions, one was a G:C—>T:A transversion, and one was a G:C—>C:G transversion. Like the mutations in K-ras, none were observed at any A:T base pairs. All G:C—>A:T transversions were at CpG sites, and 3 of them were in codon 248, which was the most common site for mutation in our combined series of endometrial lesions. When 10 missense point mutations reported from other groups are included (27, 28, 29), 13 G:C—>A:T transitions, 3 G:C—>T:A transversions, and one G:C—>C:G transversion have been reported in endometrial lesions. Of the 13 transitions, 10 were at CpG sites, and 5 of 10 were in codon 248. G:C—>A:T transversions in CpG sites are thought to occur in part through deamination of methylated cytosine to thymidine (30). Certain toxic agents may catalyze this deamination reaction, such as nitric oxide (NO), which is a cigarette smoke constituent, air pollutant, and endogenous bioregulatory agent, resulting in G:C—>A:T transition (31). G:C—>A:T transversions in CpG sites therefore are not necessarily “spontaneous” in origin. The fact that the spectrum of missense point mutations in p53 is similar to that in K-ras in endometrial lesions may suggest a role of exogenous or endogenous chemical agent(s) that may cause mutations in both genes.

2 M. Fujita and T. Enomoto, unpublished data.

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In the present study, we found that 13 of 28 endometrial adenocarcinomas of series 3 contained either K-ras and/or p53 mutations. However, 15 of these 28 tumors contained neither K-ras mutations detectable by direct sequencing nor p53 mutations in the highly conserved regions. This may indicate that there should exist other oncogenes or tumor suppressor genes that are involved in endometrial carcinogenesis. Further study will be necessary to identify such genes.

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