Expression of E-Cadherin and p53 Proteins in Human Soft Tissue Sarcomas

Jinyoung Yoo, MD, PhD; Sonya Park; Chang Suk Kang, MD, PhD; Seok Jin Kang, MD, PhD; Byung Kee Kim, MD, PhD

- Objective.—To investigate the expression of E-cadherin in human soft tissue sarcomas and its potential relationship to p53 alterations.

- Design.—Tissue sections of 91 soft tissue sarcomas were analyzed by immunohistochemistry for E-cadherin and p53 proteins. Sixty-one tumors were investigated further by the application of the polymerase chain reaction technique and a direct sequence analysis procedure of exons 5 through 8 in the p53 gene.

- Setting.—Tertiary-care teaching hospital.

- Patients.—Ninety-one patients with soft tissue tumors were treated surgically. Thirteen of these patients had tumors with epithelial differentiation.

- Results.—E-Cadherin was expressed at the cell-cell boundaries in 11 samples (12%): 9/13 (69%) with and 2/78 (3%) without histologic evidence of epithelial elements. Other sarcomas were completely negative for E-cadherin. Overexpression of p53 was detected in 30 cases (33%), 7 of which also demonstrated mutations in the p53 gene. The frequencies of p53 abnormalities in tumors with and without epithelial components were 8% and 37%, respectively. No association was established between E-cadherin immunoreactivity and p53 abnormalities ($P = .13$). Tumor grade strongly correlated with p53 alterations ($P = .01$), but not with E-cadherin expression ($P = .07$).

- Conclusions.—These data support the involvement of p53 alterations in the pathogenesis of soft tissue sarcomas. The lack of E-cadherin expression in these tumors, with the exception of lesions showing epithelial differentiation, indicates that E-cadherin is not an important factor involved in cell-cell adhesion in sarcomas. It is, however, suggested that E-cadherin may play a role in the development and/or maintenance of epithelial architecture in sarcomas, regardless of p53 status.

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zyme, were also collected from molecular genetic assays. After antigen retrieval in the citrate buffer. To immunolocalize p53, ABD Co, Lexington, Ky was purchased and applied.

Table 1. E-Cadherin Expression and p53 Alterations in Soft Tissue Sarcomas*

<table>
<thead>
<tr>
<th>Histopathology</th>
<th>No. of samples</th>
<th>No. E-Cadherin Positive (%)</th>
<th>No. p53 Altered (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFH</td>
<td>14</td>
<td>9 (64)</td>
<td>0</td>
</tr>
<tr>
<td>Rhabdomyosarcoma</td>
<td>19</td>
<td>0</td>
<td>6 (32)</td>
</tr>
<tr>
<td>Embryonal</td>
<td>11</td>
<td>0</td>
<td>5 (45)</td>
</tr>
<tr>
<td>Alveolar</td>
<td>6</td>
<td>0</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Undetermined</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Leiomyosarcoma</td>
<td>10</td>
<td>2 (20)</td>
<td>5 (50)</td>
</tr>
<tr>
<td>MPNST</td>
<td>13</td>
<td></td>
<td>5 (39)</td>
</tr>
<tr>
<td>Liposarcoma</td>
<td>11</td>
<td>0</td>
<td>1 (9)</td>
</tr>
<tr>
<td>Synovial sarcoma</td>
<td>7</td>
<td>1 (14)</td>
<td>0</td>
</tr>
<tr>
<td>Biphasic</td>
<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Monophasic fibrous</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Malignant mesothelioma</td>
<td>5</td>
<td>3 (60)</td>
<td>2 (40)</td>
</tr>
<tr>
<td>Epithelial</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Spindle</td>
<td>5</td>
<td>5 (100)</td>
<td>0</td>
</tr>
<tr>
<td>Biphasic</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Clear cell sarcoma</td>
<td>5</td>
<td>5 (100)</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>7</td>
<td>0</td>
<td>2 (29)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>91</strong></td>
<td><strong>11 (12)</strong></td>
<td><strong>30 (33)</strong></td>
</tr>
</tbody>
</table>

* MFH indicates malignant fibrous histiocytoma; MPNST, malignant peripheral nerve sheath tumor.

19 had rhabdomyosarcoma (11 embryonal, 6 alveolar, and 2 undetermined), 10 had leiomyosarcoma, 13 had malignant peripheral nerve sheath tumor, 11 had liposarcoma, 7 had synovial sarcoma (5 biphasic and 2 fibrous types), 5 had diffuse malignant mesothelioma (2 biphasic, 2 spindle, and 1 epithelial types), 5 had clear cell sarcoma, and 7 had undifferentiated sarcoma. All patients were surgically treated at the Catholic University St Vincent's Hospital (Kyungkido, South Korea) between 1992 and 1998. Tissue samples were fixed in 10% buffered formalin and embedded in paraffin. The tissue sections were stained with hematoxylin-eosin for routine histologic observation and also were stained immunohistochemically for identification of cell differentiation markers. For the poorly differentiated tumors, immunohistochemical markers, including vimentin, cytokeratin, epithelial membrane antigen, desmin, actin, S100, myoglobin, lysozyme, α-antitrypsin, and factor VIII were utilized along with ultrastructural studies, if necessary, to permit an accurate diagnosis. The tumors unclassifiable even after extensive studies were categorized as “other.” All cases were reviewed to confirm the diagnosis or tumor typing according to the relevant World Health Organization classifications. Only tissue sections that contained more than 90% tumor tissue without hemorrhage and necrosis were selected for the current study. Normal tissue specimens were also collected for molecular genetic assays.

**Immunohistochemistry**

Formalin-fixed, paraffin-embedded tissues were cut into 5-μm-thick sections. Immunohistochemical staining was performed by a sensitive peroxidase-streptavidin method with an LASB kit (Dako Co, Ltd, Kyoto, Japan). A monoclonal antibody against E-cadherin (ABD Co, Lexington, Ky) was purchased and applied after antigen retrieval in the citrate buffer. To immunolocalize p53, sections were stained with DO7 monoclonal antibody (mouse anti-p53 protein antibody, wild and mutant form, Novastra, Newcastle, United Kingdom). Briefly, sections were deparaffinized and hydrated with xylene and ethanol. Endogenous peroxidase was blocked by soaking in 3% hydrogen peroxide at 45°C for 4 minutes. The slides were placed in a Coplin jar containing citrate buffer (2.1 g/L, pH 6.0) and heated to 121°C in an autoclave for 15 minutes to unmask the antigen. They were treated with protein blocking reagent before the incubation at 45°C for 1 hour with primary antibodies at a 1:100 dilution. After extensive washing, the sections were incubated at 45°C for 10 minutes with biotinylated anti-mouse immunoglobulin antibodies (Dako) at a 1:20 dilution and subsequently with streptavidin-biotin peroxidase complexes at a 1:25 dilution. The peroxidase reaction was carried out by using aminoethylcarbodimide as the final chromogen. The nuclei were counterstained with Meyer hematoxylin.

All series included positive and negative controls. As a negative control, primary antibodies were substituted by phosphate-buffered saline. Normal skin from another paraffin block of the same sample, if available, and normal gastric epithelium were used as an external positive control for E-cadherin protein. Only cells with distinct plasma membrane staining were considered as E-cadherin positive. The degree of E-cadherin expression was estimated semiquantitatively as follows: +, no expression; +, less than 20% of cells positive; +, 20% to 50% of cells positive; and +++, more than 50% of cells positive. As a positive control, we also used sections of a lung tumor that was known to be positive for p53. Cases in which nuclear staining for p53 was seen in the majority of malignant cells were interpreted as overexpressing p53.

**DNA Extraction**

Genomic DNA was extracted from 5 paraffin sections (5 μm thick). The sections were deparaffinized and hydrated by washing with 100% alcohol, 95% alcohol, and 70% alcohol, followed by distilled water. Using the hematoxylin-eosin–stained section as a guide, tumor tissue was obtained by use of a needle to ensure that greater than 80% of the recovered cells were tumor cells. The procured tissue was resuspended in 100 μL of lysis buffer (50 mmol/L Tris, 1 mmol/L EDTA, and 0.5% Tween 20, containing proteinase K) and incubated at 55°C overnight. Proteinase K was then heat-inactivated. After phenol-chloroform extraction, genomic DNA was precipitated with ethanol and quantitated by UV absorption.

The DNA was then subjected to PCR amplification utilizing the primers purchased from Clontech (Palo Alto, Calif) to amplify individually exons 5 through 8 of the p53 gene. The PCR amplification was performed in 100-μL volumes containing approximately 0.2 μg of genomic DNA, 20 mmol/L Tris-HCl (pH 8.4), 50 mmol/L KCl, 2.0 mmol/L MgCl₂, 0.2 mmol/L each of deoxynucleoside triphosphate, 0.2 μmol/L each primer, and 5 units Platinum Taq DNA polymerase (Life Technologies, Gaithersburg, Md) on an MJ Research PTC-100 thermal cycler (Wertertown, Mass). Templates were denatured for 2 minutes at 94°C, followed by 35 cycles of PCR with incubations of 1 minute at 94°C, 1 minute at 55°C, and 1 minute at 72°C. The reaction was incubated at 72°C for 10 minutes on the last cycle. Negative controls without DNA template were run routinely to detect PCR contamination. The amplified products were separated by electrophoresis in 3% agarose gel containing 10 mg/mL ethidium bromide.

![Figure 1](image1.png) **Figure 1.** Immunohistochemical staining for E-Cadherin. Distinct membranous staining in normal gastric epithelium (A). Tumor cells showing E-cadherin expression in leiomyosarcoma (B), malignant mesothelioma (C), synovial sarcoma (D), and clear cell sarcoma (E), but not in malignant fibrous histiocytoma (F) (E-Cadherin, original magnification ×100).

![Figure 2](image2.png) **Figure 2.** p53 nuclear overexpression in a malignant fibrous histiocytoma (A; original magnification ×40) and direct genomic sequence analysis of the p53 gene in the same tumor showing mutation in codon 171, exon 5 (GAG to GCC), substituting alanine for glutamic acid (sequenced in the 5’ to 3’ direction) (B).
bromide in Tris-borate-EDTA buffer. Following electrophoresis, gels were examined using UV light transillumination.

**DNA Sequencing**

Eighty-five microliters of the PCR product was purified with a QIAquick PCR purification kit (Qiagen, Valencia, Calif) and then sequenced with a 373A DNA sequencer (Applied Biosystems, Foster City, Calif) using dye-primer conditions recommended by Applied Biosystems. Both strands were sequenced for each DNA analyzed, and genomic DNAs from control samples were sequenced in parallel to confirm the mutations.

**RESULTS**

Tissue samples from 91 tumors were examined for both E-cadherin and p53 proteins. The results of the analysis are summarized in Table 1.

E-Cadherin protein was identified as a membranous pattern of expression and was observed in 11 samples (12%), including 1 synovial sarcoma, 3 malignant mesotheliomas, all 5 clear cell sarcomas, and 2 leiomyosarcomas (Figure 1). Eighty tumors (88%) did not show any E-cadherin immunoreactivity. Of the 13 sarcomas with epithelial differentiation, 9 (69%) showed positive reaction exclusively in the epithelial cells. The 9 positive tumors included 1 of 5 biphasic synovial sarcomas (20%, excluding 2 monophasic fibrous-type lesions), 1 epithelial-type and 2 biphasic-type malignant mesotheliomas (100%, excluding 2 fibrous type lesions), and 5 of 5 clear cell sarcomas (100%). Other biphasic synovial sarcomas were completely negative in spite of their coexisting epithelial elements. Interestingly, 2 of 10 leiomyosarcomas showed diffuse reactivity for E-cadherin. Microscopic examination of those tumors stained with hematoxylin-eosin showed no morphologic differences from the usual type of leiomyosarcoma, but demonstrated immunoreactivity at the cell-cell boundaries of tumor cells.

A strong membranous immunostaining in more than 50% of the cells (++) was detected in 2 of the cases, in 20% to 50% (+) of the cells in 2, and in less than 20% (+) of the cells in 7. The samples expressing E-cadherin were 7 grade 2 and 4 grade 3 tumors; the association between E-cadherin expression and tumor grade was not statistically significant (P = .07) (Table 2). E-Cadherin expression established no correlation with the patients’ age, sex, tumor size, or prognosis (data not shown).

p53 alterations were detected as p53 protein accumulation and/or gene mutations. In 30 (33%) of the tumors, p53 abnormalities were identified; 23 samples showed protein accumulation only, whereas 7 had protein accumulation and a mutation (Figure 2). The incidence was high in malignant fibrous histiocytoma and leiomyosarcoma, but it was low in liposarcoma, synovial sarcoma, and clear cell sarcoma (Table 1). In rhabdomyosarcoma, alterations of p53 were observed in 6 cases (5 embryonal and 1 alveolar; 32%). One (8%) of the 13 samples with epithelial features exhibited p53 overexpression, whereas 29 (37%) of the sarcomas without epithelial differentiation demonstrated immunoreactivity. No association was noted between E-cadherin expression and altered p53 (P = .13) (Table 3). p53 alterations, however, strongly correlated with tumor grade (P = .01); 13 (24%) of 55 grade 2 tumors and 17 (53%) of 32 grade 3 lesions exhibited altered p53 (Table 2).

**COMMENT**

In the present study, only 11 (12%) of the sarcomas examined expressed E-cadherin, the major cadherin in epithelial cells. Of these, 9 had demonstrable epithelial features within the tumor, where E-cadherin immunoreactivity was observed on the membrane.

E-Cadherin was not detected in our cases of malignant fibrous histiocytoma, rhabdomyosarcoma, malignant peripheral nerve sheath tumor, and liposarcoma. This finding suggests that E-cadherin protein may not be relevant in the establishment and maintenance of cellular architecture in sarcomas, in accordance with findings in a study by Sato et al. It was of interest that 2 leiomyosarcomas unexpectedly expressed E-cadherin at the cell-to-cell boundaries. It is uncertain whether the presence of E-cadherin in these samples actually reflects epithelial differentiation. Both of the lesions were at nonvisceral locations and did not show epithelioid cytomorphology. Leiomyosarcomas may show different histologic features, including myxoid, epithelioid, and pleomorphic patterns, and some may have either mixed pattern or transitional form between 2 histologic subtypes. Development of the epithelioid-like adhesive nature of the tumor cell population that may not be appreciable on histology may have attributed to E-cadherin immunoreactivity in these tumors, regardless of the histologic phenotype. To our knowledge, however, E-cadherin reactivity in leiomyosarcomas has never before been reported, and additional studies of cell-cell adhesion molecules in these tumors will be required in association with architectural parameters.

Monophasic fibrous synovial sarcomas have been reported to be focally and weakly positive for E-cadherin. Those cells with E-cadherin expression were described to be slightly larger and more ovoid in morphology with ultrastructural evidence of epithelial differentiation, when compared to the E-cadherin-negative spindle cells. Their results are different from our findings of a lack of immunoreactivity in synovial sarcomas of monophasic fibrous type (2/2 vs 0/2). The spindle-shaped cells in our cases appeared typically slender and elongated. Different immunostaining patterns found in these 2 studies might have a relevance to the morphologic difference of the constituent cells with and without early or incomplete differentiation toward epithelial nature, but no definite conclusion can be drawn from such a small number of samples. It was reported that all synovial sarcomas, whether mono-

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**Table 2. Correlation of E-Cadherin and p53 Expression With Tumor Grade**

<table>
<thead>
<tr>
<th>E-Cadherin</th>
<th>p53</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1 (%)</td>
<td>Grade 2 (%)</td>
</tr>
<tr>
<td>0/4 (0)</td>
<td>7/55 (13)</td>
</tr>
<tr>
<td>0/4 (0)</td>
<td>13/55 (24)</td>
</tr>
</tbody>
</table>

**Table 3. Relationship Between E-Cadherin Expression and p53 Alterations**

<table>
<thead>
<tr>
<th>E-Cadherin</th>
<th>p53 overexpression and/or mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>52</td>
</tr>
<tr>
<td>Positive</td>
<td>28</td>
</tr>
</tbody>
</table>

The table shows the correlation between E-Cadherin expression and p53 alterations (Table 3).
phasic fibrous or biphasic, expressed E-cadherin, indicating E-cadherin as a marker for differentiation between synovial sarcomas and other types of spindle cell sarcomas. In contrast, staining was positive in only 20% of our biphasic-type lesions, in spite of the presence of an epithelial component within the tumor. This variation may imply that the epithelial nature of the tumor cell population is not the only factor involved in E-cadherin protein expression. There may be other mechanisms affecting E-cadherin that have not yet been identified. Therefore, the possibility of the use of E-cadherin staining in distinguishing synovial sarcomas from other types of sarcomas cannot be entertained at present.

Clear cell sarcoma is a distinct type of soft tissue sarcoma intimately associated with tendons and aponeuroses, and it possesses melanocytic features. Unlike typical forms of malignant melanoma, however, clear cell sarcoma lacks epidermal involvement and junctional changes, and displays a more uniform growth pattern characterized by pale-staining fusiform tumor cells that are arranged in nest-like aggregates. Differentiation from other sarcomas may be difficult, particularly in those cases with the absence of clear cell appearance of the tumor cells. A recent investigation of E-cadherin in clear cell sarcomas demonstrated immunoreactivity in 80% of the cases. The results are similar to our findings of 100%. These studies suggest that immunostaining for E-cadherin would assist in confirming the diagnosis of clear cell sarcoma, in addition to the application of S100 protein and the melanoma-associated marker, HMB-45.

The expression of E-cadherin has been shown to correlate with tumor grade and behavior in a variety of human carcinomas. In the present study, although the varying intensity of E-cadherin staining was observed only in grade 2 and grade 3 tumors, it was not statistically significant (P = .07). Furthermore, no prognostic differences were noted between the tumors with and without E-cadherin expression.

The protein products of the wild-type p53 gene activate the expression of downstream genes that negatively control growth. Mutations, either nonsense or missense, create functional inactivation of p53 through reduction of wild-type p53 tetramers. A number of studies have been reported concerning p53 overexpression and p53 gene mutations in various types of human cancers. In sarcomas, the p53 gene is altered in 25% to 65% of cases. The results are similar to our findings of 100%. These studies suggest that immunostaining for E-cadherin would assist in confirming the diagnosis of clear cell sarcoma, in addition to the application of S100 protein and the melanoma-associated marker, HMB-45.

The expression of E-cadherin has been shown to correlate with tumor grade and behavior in a variety of human carcinomas. In the present study, although the varying intensity of E-cadherin staining was observed only in grade 2 and grade 3 tumors, it was not statistically significant (P = .07). Furthermore, no prognostic differences were noted between the tumors with and without E-cadherin expression.

The results possibly linking the important regulators of the cell cycle machinery to the expression of cell-cell adhesion molecules involved in tumor formation have been reported previously in colon cancers. Mueller et al demonstrated that E-cadherin was induced in colon carcinoma cell lines containing wild-type p53 and p21, but not in those lacking functional p21. Alteration of the gene may lead to a genomic instability, detected as mutations and/or amplifications at the gene level and as altered expression at the messenger RNA and protein level. The p53 protein acts as a transcription factor by both transactivating some genes and suppressing transcription of others. After DNA damage, normal cells stop in G1/S to allow time for repair. Cells with altered p53 do not stop in G1/S. Thus, DNA damage will not be repaired and genomic instability will appear, with accumulation of deletions and amplifications. Loss of heterozygosity of several genes, including the gene coding for E-cadherin, may be the result of such instability. Wang et al found in hepatocellular carcinomas a significant correlation between p53 gene mutation and loss of heterozygosity in the region of chromosome 16q21-q23, where E-cadherin is located. The loss of heterozygosity at the E-cadherin locus may be associated with mutations in the E-cadherin gene with subsequent abnormalities in its protein expression. The strong association between p53 alterations and down-regulation of the E-cadherin protein has been demonstrated in breast carcinomas. In our samples, however, did not correlate with E-cadherin expression, suggesting that p53 may not play a role in regulation of E-cadherin protein expression in soft tissue tumors. A large number of histologically homogeneous series of sarcomas should be investigated to verify this finding in future studies.

Our results indicate that E-cadherin is not an important factor in the cell-cell adhesion system in soft tissue sarcomas, with the exception of tumors with epithelial features, in which E-cadherin may play a role in maintaining their epithelial architecture. E-Cadherin protein expression in tumors without detectable p53 alteration and the absence of association between E-cadherin and p53 suggest that other mechanisms independent of p53 regulate E-cadherin protein expression in these tumors.

References