E-Cadherin Gene Mutations Frequently Occur in Synovial Sarcoma as a Determinant of Histological Features

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Synovial sarcoma is a mesenchymal tumor that has an epithelial character and two major histological subtypes, the biphasic type and the monophasic fibrous type. However, the mechanisms involved in its epithelial differentiation are unknown, and furthermore, the determinants for histological subtype in synovial sarcoma remain unclear. In this study, we immunohistochemically examined E-cadherin expression and screened for genetic alterations in the E-cadherin gene from exon 4 to exon 9 in 49 cases of synovial sarcoma. In addition, we also examined the mRNA expressions of E-cadherin and Snail, a direct repressor of E-cadherin gene expression, by reverse transcriptase-polymerase chain reaction in 20 samples of frozen material. Immunohistochemical E-cadherin membranous expression was observed in 12 cases (24.5%), and was predominant in biphasic tumors. Single-strand conformation polymorphism analysis followed by DNA direct sequencing revealed 15 missense E-cadherin mutations in 12 cases (24.5%: monophasic, 11 of 42; biphasic, 1 of 6; poorly, 0 of 1) and 7 silent mutations (14.3%) in 7 cases. Ten of the 12 cases with E-cadherin missense mutations did not show E-cadherin membranous expression. Reverse transcriptase-polymerase chain reaction demonstrated E-cadherin and Snail mRNA expressions in 14 cases (70%) and in all cases, respectively. E-cadherin gene expression was inactivated by missense mutations in three of the eight cases (37.5%) of monophasic fibrous tumors that showed E-cadherin mRNA expressions. The E-cadherin gene was potentially inactivated in a significant number of synovial sarcomas. E-cadherin dysfunction because of its mutation in the central region of the molecule was associated with its decreased immunohistochemical expression and histological fibroblastic and spindle-shaped features of monophasic tumors. Thus, E-cadherin gene

mutation may be one of the determinants of histological subtype in synovial sarcoma. (Am J Pathol 2001, 159:2117-2124)

Initial events in the metastatic spread of tumors involve loss of cell-cell adhesion within the primary tumor mass. The integrity and morphology of epithelial tumor cell colonies is maintained primarily by cell-cell adhesions mediated by E-cadherin and its associated intracellular catenin molecules.^{1,2} It has been reported that detachment of cell-cell adhesions is related to the reduced expression of E-cadherin in carcinoma.^{2,3} We have previously demonstrated that the dysfunction of E-cadherin and catenins contributes to an adverse clinical outcome in synovial sarcoma, and furthermore, that E-cadherin expression tends to be more common in biphasic tumors.⁴ However, the mechanism behind E-cadherin dysfunction in synovial sarcoma, especially in monophasic fibrous tumors, is still unknown.

Synovial sarcomas are known to have two major forms, the monophasic type, in which the tumors are composed of spindle cells, and the biphasic type, in which the tumors contain both epithelial cells arranged in glandular structures and spindle cells. In an effort to find possible determinants of the histological subtype of synovial sarcoma, studies have focused on the chimeric fusion transcripts, SYT-SSX1 and SSX2, that are caused by a characteristic chromosomal translocation, t(X;18)(p11;q11).⁵ It has been demonstrated that there is a significant relation between histological subtype and the type of fusion transcript, although the mechanisms involved in epithelial differentiation in synovial sarcoma remain unclear.

It has been suggested that reduced expression of E-cadherin is associated with the characteristics of invasiveness and loss of differentiation.⁶ Moreover, putative cause-effect relationships between E-cadherin inactiva-

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tion and the histological type have also been suggested in some carcinomas.^{7,8} On the other hand, the Snail family of transcription factors has previously been shown to be expressed in fibroblasts and implicated in the differentiation of epithelial-mesenchymal transitions during embryonic development.^{9,10} In addition, the transcription factor Snail has been shown to be a direct repressor of E-cadherin gene expression by binding to its proximal promoter in epithelial tumor cells.^{9,10} Furthermore, it has also been demonstrated that endogenous Snail protein is present in invasive human carcinoma cell lines and tumors in which E-cadherin expression has been lost, leading to the acquisition of a fibroblastic phenotype.¹⁰ These findings prompted us to investigate the possible involvement of E-cadherin and Snail expressions in the morphogenesis of synovial sarcoma that histologically shows epithelial-mesenchymal transitions.

In the present study, we screened for E-cadherin gene mutations in synovial sarcoma, and examined their possible association with histological subtype. In addition, we also examined E-cadherin and transcription factor Snail mRNA expressions in synovial sarcoma, and compared their expressions with E-cadherin immunohistochemical expression and histological features to evaluate the influence of E-cadherin and the transcription factor Snail on the development of synovial sarcoma, with special emphasis on its histological subtype.

Materials and Methods

Materials and DNA Preparation

Forty-nine cases of synovial sarcoma, most of which had been investigated in previous studies, combined with some recent additional cases, were used in this study.^{4,11} Materials were fixed in a 10% formaldehyde solution and embedded in paraffin. Histological subtypes comprised 42 cases of monophasic type, 6 cases of biphasic type, and 1 case of poorly differentiated type. Biphasic type of synovial sarcoma was defined as those cases in which apparent glandular structures could be recognized. Clinical data for these cases were collected from the medical records. Survival data were available for 44 cases. Follow-up ranged from 1 to 278 months (mean, 65.9 months). Genomic DNA was purified from materials fixed in a 10% formaldehyde solution and embedded in paraffin, using standard proteinase K digestion and phenol/chloroform extraction. In addition, genomic DNA from corresponding nontumoral tissue was also extracted in each case of synovial sarcoma showing aberrant single-strand conformation polymorphism (SSCP) bands in the tumor tissue to rule out the possibility of single nucleotide polymorphisms, and then analyzed subsequently.

Polymerase Chain Reaction (PCR)-SSCP and Mutational Analysis for E-Cadherin Gene

Six sets of intronic primers were used for genomic DNA screening of the E-cadherin gene from exon 4 to exon 9. The primer sequences were the same as those previously

described.^{7,12} PCR was performed in a Gene Amp PCR System 9600 (Perkin Elmer, Foster City, CA) for 40 cycles after initial denaturing at 96°C for 5 minutes in a total volume of 20 μ L of reaction mixture containing 50 ng of genomic DNA of each sample, 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 2.0 mmol/L MgCl₂, 25 mmol/L of dNTP, 1.0 U Taq DNA polymerase (Takara Biomedicals, Japan) and 1.0 mmol/L of each of the primers. Each cycle consisted of denaturation at 96°C for 1 minute, at 58°C for 1 minute, and at 72°C for 1 minute. After the final cycle of amplification, the extension was continued for an additional 7 minutes at 72°C. Annealing temperatures were the same for each pair of primers. Human genomic DNA (Clontech, Palo Alto, CA, USA), which was confirmed to have the same base sequences as those of GenBank (Accession No. L34791-34796), was used as a positive control for each PCR and the subsequent reactions. SSCP was performed using a gel containing 12.5% acrylamide (GenePhor; Amersham Pharmacia Biotech, Uppsala, Sweden) and a DNA fragment analyzer (GenePhor, Amersham Pharmacia Biotech) at 600 V, 25 mA, 15 W, and 5°C, for 120 minutes, and then the DNA bands were visualized by a DNA Silver Staining kit (GenePhor, Amersham Pharmacia Biotech). To increase the quantity of mutant DNA before sequencing, the extra bands that seemed to be aberrantly migrating were excised from the SSCP gel and re-amplified and then the sequences were performed, using the same primers. The sequence data were collected by ABI Prism 310 Collection Software and were analyzed by Sequencing Analysis and Sequence Navigator Software (Perkin Elmer). These procedures were performed two times for those cases that showed E-cadherin mutations to exclude the possibility of PCR artifacts.

RNA Extraction and Reverse Transcriptase (RT)-PCR to Detect Endogenous E-Cadherin and Snail Expression

Frozen materials were also available for 20 cases (15 cases of monophasic fibrous, 4 cases of biphasic, and 1 case of poorly differentiated type) of synovial sarcoma from among 49 cases that were analyzed for E-cadherin mutations. Total RNA was extracted, using Trizol reagent (Gibco BRL, Tokyo, Japan) according to the manufacturer's protocol. Total RNA was also extracted from two cases of sporadic desmoid tumors. Five μ g of RNA of each sample were used for the subsequent reverse transcription. After the reaction, RNase treatment was performed to eliminate RNA. Sequences of specific pairs of primers were as follows: E-cadherin (upper primer: 5'-GAC GCG GAC GAT GAT GTG AAC-3'; lower primer: 5'-TTG TAC GTG GTG GGA TTG AAG A-3'), Snail (upper primer: 5'-TCC TCT ACT TCA GCC TCT TCC TT-3'; lower primer: 5'-GGC ACT GGT ACT TCT TGA CAT CT-3'), and β -actin (upper primer: 5'-AGG CCA ACC GCG AGA AGA TGA CC-3'; lower primer: 5'-GAA GTC CAG GGC GAC GTA GCAC-3'). Each PCR product was obtained after 35 cycles of amplification with an annealing temperature of 56.3°C for E-cadherin and β -actin, and 58.8°C for Snail.

Table 1. E-Cadherin Mutations in Synovial Sarcoma

Case no.*	Age/sex	Subtype	Exon	Codon	Mutation		E-cadherin membranous expression	Prognosis
					Nucleotide change	Amino acid change		
Missense mutations								
49	62/M	Biphasic	4	159	CCT to TCT	Pro to Ser	(+)	DOD [†] (99 mos)
			4	170	CCA to CTA	Pro to Leu		
47	64/F	Monophasic	6	255	CAG to CAT	Gln to His	(−)	Unknown
28	28/F	Monophasic	7	309	CCT to TCT	Pro to Ser	(−)	Alive (118 mos)
38	38/F	Monophasic	7	311	CTC to TTC	Leu to Phe	(−)	DOD (31 mos)
25	41/F	Monophasic	8	339	CCT to CTT	Pro to Leu	(+)	DOD (113 mos)
10	43/F	Monophasic	8	339	CCT to CTT	Pro to Leu	(−)	DOD (10 mos)
36	20/M	Monophasic	8	344	GTG to ATG	Val to Met	(−)	DOD (28 mos)
41	21/F	Monophasic	8	353	GAG to AAG	Glu to Lys	(−)	Alive (181 mos)
2	56/M	Monophasic	9	386	GAG to AAG	Glu to Lys	(−)	DOD (69 mos)
16	45/M	Monophasic	9	401	GCT to ACT	Ala to Thr	(−)	DOD (27 mos)
21	11/M	Monophasic	9	414	ACC to ATC	Thr to Ile	(−)	Alive (77 mos)
4	50/F	Monophasic	9	385	CCT to CTT	Pro to Leu	(−)	DOD (11 mos)
			9	399	ACT to ATT	Thr to Ile		
			9	429	CCA to TCA	Pro to Ser		
Silent mutations								
5	65/F	Monophasic	6	266	GCT to GTT	Val to Val	(−)	DOD (12 mos)
32	30/F	Monophasic	8	344	GTG to GGG	Val to Val	(−)	Unknown
37	55/M	Monophasic	8	344	GTG to GGG	Val to Val	(−)	Alive (58 mos)
29	26/F	Monophasic	8	353	GAG to GAA	Glu to Glu	(−)	Alive (278 mos)
30	25/M	Monophasic	8	374	ATC to ATT	Ile to Ile	(−)	Alive (40 mos)
17	33/F	Monophasic	8	374	ATC to ATT	Ile to Ile	(+)	Alive (146 mos)
19	19/F	Biphasic	8	374	ATC to ATT	Ile to Ile	(+)	Alive (25 mos)

*Case numbers are identical throughout the manuscript including Figures and Tables.

[†]DOD, died of disease.

The PCR products were electrophoresed in 2.0% agarose gel and visualized with ethidium bromide. RNA from the HepG2 human hepatoblastoma cell line and two cases of extra-abdominal desmoid tumor was used as a positive control for E-cadherin and Snail expression.

Statistical Analysis

The significance of E-cadherin missense mutations on the overall survival rate was estimated using the log-rank test.

Results

E-Cadherin Gene Mutation

We performed mutational analysis of the E-cadherin gene from exon 4 to exon 9 in 49 cases, however, we could not obtain PCR amplicons for exon 7 in enough number of cases, this probably being because of the anticipated length of the PCR amplicon (329 bp) obtained in our study based on the formalin-fixed materials. Therefore, when the frozen materials were sufficiently prepared for DNA analysis, some of those could be used for the amplification of exon 7. As a result, we could obtain PCR amplicons for exon 7 in 23 cases. SSCP analysis followed by DNA direct sequencing revealed 15 missense E-cadherin mutations in 12 cases (24.5%), and 7 silent mutations in 7 cases (14.3%) of synovial sarcoma (Table 1 and Figure 1; A to C). Two cases contained more than one missense mutation at different sites: one case had three missense mutations, and the other had two missense

mutations. All cases but one with E-cadherin missense mutations were monophasic fibrous tumors, the remaining case being a biphasic tumor. In addition, the presence of E-cadherin missense mutations did not affect the patients' prognoses in this study (log-rank test: $P = 0.52$). Furthermore, none of the cases showing aberrant SSCP bands in the tumor tissue harbored aberrant SSCP bands in the nontumoral tissue, suggesting that the base substitutions detected in this study are not single nucleotide polymorphisms but somatic mutations restricted to the tumor tissue (Figure 1, D and E).

E-Cadherin Immunohistochemical Expression

Immunohistochemical findings were obtained from the previously published data.⁴ E-cadherin membranous expression was detected by immunohistochemistry in 12 of the 49 cases (monophasic fibrous, 6 of 42; biphasic, 6 of 6; poorly differentiated, 0 of 1) of synovial sarcoma (24.5%). All of the monophasic fibrous tumors that showed E-cadherin membranous expression contained epithelioid areas composed of a proliferation of relatively plump cells. Among the 12 cases with E-cadherin missense mutations, only 2 cases (monophasic fibrous tumor and biphasic tumor) showed E-cadherin membranous expression, with the monophasic fibrous case also showing cytoplasmic staining (Figure 2; A to C). Furthermore, both cases showed membranous expression of at least one of the catenins (α , β , γ).⁴ The remaining 10 cases with E-cadherin missense mutations did not show E-cadherin expression either at the cellular membrane or at the

Figure 1. A: Results of PCR-SSCP of E-cadherin gene exon 8. Aberrantly migrating bands can be observed in lanes 3, 4, 6, 11, 12, and 16 (arrows). Lane 1: Control; lanes 2–18: synovial sarcoma samples. B: Sequencing results for E-cadherin gene exon 8 in case 36. Tumor sequencing shows the substitution of ATG for GTG at codon 344, causing an amino acid change from Val to Met (below). Corresponding normal sequences are also shown (above). C: Sequencing results for E-cadherin gene exon 4 in case 49. Tumor sequencing shows the substitution of TCT for CCT at codon 159, causing an amino acid change from Pro to Ser (bottom left), and the substitution of CTA for CCA at codon 170, causing an amino acid change from Pro to Leu (bottom right). Corresponding normal sequences are also shown (top). D and E: Results of PCR-SSCP of E-cadherin gene exons 8 (D) and 9 (E) in both tumoral (T) and nontumoral (N) tissues. Aberrantly migrating bands can be observed only in the tumoral tissue of the both cases. C, control

nucleus/cytoplasm, while also showing no catenin membranous expression (Figure 2; D to F).

E-Cadherin and Snail mRNA Expressions by RT-PCR

The results are summarized in Table 2. Control HepG2 cells expressed both E-cadherin and Snail mRNA (Figure 3A). Snail mRNA expression was also found in the two cases of desmoid tumor, however, E-cadherin expres-

sion could not be detected in either of these desmoid tumors (Figure 3A). E-cadherin mRNA expression was observed in 14 of the 20 cases (monophasic fibrous, 10 of 15; biphasic, 4 of 4; poorly differentiated, 0 of 1) of synovial sarcoma where frozen materials were available (Figure 3B). E-cadherin expression was confirmed by both RT-PCR and immunohistochemistry in all four cases of biphasic tumor, whereas the one case of poorly differentiated tumor failed to show E-cadherin expression either with RT-PCR or with immunohistochemical examina-

Figure 2. A–C: Histological features (A) and immunohistochemical staining for E-cadherin (B) and β -catenin (C) in case 25 that contained a point mutation at exon 8 of the E-cadherin gene. Monoclonal antibodies to E-cadherin and β -catenin were supplied by Transduction Laboratories (diluted at 1:1000 and 1:200, respectively). This E-cadherin monoclonal antibody reacts with the cytoplasmic portion of the molecule. This case was entirely composed of a proliferation of short spindle-shaped or relatively plump cells (A). E-cadherin immunostaining showed distinct membranous staining, while also showing cytoplasmic staining (B). Tumor cells were positive for β -catenin at the cellular membrane, without any apparent nuclear staining (C). D–F: Histological features (D) and immunohistochemical staining for E-cadherin (E) and β -catenin (F) in case 4, which contained three point mutations at exon 9 of the E-cadherin gene. This case was composed of a proliferation of spindle-shaped cells with scanty cytoplasm in a fascicular formation with foci of tumor cell proliferation showing an incohesive appearance in the myxoid matrix (D). E-cadherin membranous staining could not be observed (E). This case showed no distinct membranous staining of β -catenin, but showed aberrant nuclear staining (F).

tions. None of the five cases of monophasic fibrous tumor without E-cadherin mRNA expression showed E-cadherin expression immunohistochemically. Furthermore, two of these contained E-cadherin missense mutation. Loss of

E-cadherin expression was confirmed by immunohistochemistry in 8 of the 10 cases of monophasic fibrous tumors demonstrating E-cadherin mRNA expression. The remaining two cases showed E-cadherin membranous

Table 2. E-Cadherin and Snail mRNA Expressions in Synovial Sarcoma

Case no.	Age/sex	Subtype	Epithelial nests	mRNA expression		E-cadherin mutation	E-cadherin membranous expression	Prognosis
				E-cadherin	Snail			
22	25/F	Biphasic		(+)	(+)	(-)	(+)	Alive (28 mos)
14	36/F	Biphasic		(+)	(+)	(-)	(+)	Alive (165 mos)
45	18/F	Biphasic		(+)	(+)	(-)	(+)	Alive (8 mos)
19	19/F	Biphasic		(+)	(+)	Silent	(+)	Alive (25 mos)
17	33/F	Monophasic	(+)	(+)	(+)	Silent	(+)	Alive (146 mos)
9	44/F	Monophasic	(+)	(+)	(+)	(-)	(+)	Alive (139 mos)
36	20/M	Monophasic	(+)	(+)	(+)	Missense	(-)	DOD* (28 mos)
2	56/M	Monophasic	(+)	(+)	(+)	Missense	(-)	DOD (69 mos)
16	45/M	Monophasic	(-)	(+)	(+)	Missense	(-)	DOD (27 mos)
37	55/M	Monophasic	(-)	(+)	(+)	Silent	(-)	Alive (58 mos)
8	53/F	Monophasic	(+)	(+)	(+)	(-)	(-)	DOD (46 mos)
13	22/F	Monophasic	(-)	(+)	(+)	(-)	(-)	DOD (45 mos)
1	32/M	Monophasic	(-)	(+)	(+)	(-)	(-)	DOD (8 mos)
46	61/F	Monophasic	(-)	(+)	(+)	(-)	(-)	Alive (2 mos)
38	38/F	Monophasic	(+)	(-)	(+)	Missense	(-)	DOD (31 mos)
47	64/F	Monophasic	(-)	(-)	(+)	Missense	(-)	Unknown
35	38/M	Monophasic	(-)	(-)	(+)	(-)	(-)	DOD (4 mos)
7	58/M	Monophasic	(-)	(-)	(+)	(-)	(-)	DOD (15 mos)
48	52/F	Monophasic	(-)	(-)	(+)	(-)	(-)	Alive (6 mos)
43	11/F	Poorly diff.	(-)	(-)	(+)	(-)	(-)	DOD (16 mos)

*DOD, died of disease.

expression immunohistochemically and were histologically composed of a proliferation of relatively plump epithelioid cells, occasionally demonstrating nest-like formations. Among the eight cases with E-cadherin mRNA expression but no immunohistochemical E-cadherin expression, three cases contained E-cadherin missense mutations and one case had a silent mutation. Five of these eight cases of monophasic fibrous tumor were entirely composed of a proliferation of spindle-shaped cells with scanty cytoplasm, whereas the remaining three cases demonstrated focal nest-like formations. On the other hand, Snail mRNA expression was confirmed almost equally by RT-PCR in all of the cases of synovial sarcoma (Figure 3B).

Discussion

E-cadherin inactivation caused by mutations has been reported in various epithelial malignancies and cell lines,^{7,12-16} however, it has not been reported in the field of sarcomas. We detected for the first time E-cadherin gene missense mutations in synovial sarcoma, and they were frequently seen, being noted in 24.5% of tumors. In addition, silent mutations were also detected in seven cases (14.3%). E-cadherin is often cited as a prime example of a tumor suppressor gene, based on the classic two-hit hypothesis of tumor suppressor gene inactivation: mutations in one allele are accompanied by deletion of the remaining normal allele. Although the allele status of the tumors with E-cadherin missense mutations remains unclear, in this study, dense mobility shift bands but loss of or only faint normal bands were observed in 10 of 12 cases with E-cadherin missense mutation (except cases 28 and 47), as shown in Figure 1A. These findings suggest that, in synovial sarcoma, the E-cadherin gene is potentially inactivated by the two-hit hypothesis in the

Figure 3. A: Results of RT-PCR to detect endogenous E-cadherin and Snail mRNA expressions in control samples. The expression of Snail was demonstrated in HepG2 cells and in both cases of desmoid tumor, whereas E-cadherin expression was detected only in HepG2 cells and not in the desmoid tumor cases. RT(-): negative control. B: Results of RT-PCR to detect endogenous E-cadherin and Snail mRNA expressions in synovial sarcoma samples. The expression of Snail was demonstrated in all cases of synovial sarcoma. E-cadherin expression was detected in **lanes 1, 2, 3, 5, and 6**, but not in **lane 4**. **Lanes 1-3:** Biphasic tumors; **lanes 4-6:** monophasic tumors.

majority of the cases with E-cadherin missense mutation. Furthermore, these findings seem to suggest that the E-cadherin gene is one of the loci of genetic susceptibility in synovial sarcoma.

Interestingly, RT-PCR demonstrated E-cadherin mRNA expression in 66.7% (10 of 15) of the monophasic tumors as well as in the biphasic tumors. This value was extremely high compared with the previously reported ratio of immunohistochemically detectable E-cadherin expression.⁴ Although frameshift mutations resulting in truncation of the E-cadherin protein could not be detected in this study, and it should be noted that we examined and evaluated the function of the E-cadherin molecule only by immunohistochemistry, it has been previously shown that conservative point mutations within the N-terminal calcium-binding pocket are enough to abolish cell-cell adhesion.¹⁷ Therefore, three cases of monophasic fibrous tumors containing E-cadherin missense mutations at this region, which actually showed E-cadherin mRNA expression but which did not show immunohistochemical E-cadherin expression, were expected to impair cell-cell adhesion by interference with the calcium-dependent cell adhesion. Thus, the E-cadherin gene in the monophasic fibrous type of synovial sarcoma was inactivated by E-cadherin mutations to some degree. In addition, silencing of E-cadherin by CpG hypermethylation within its promoter region has also been reported in other carcinomas such as breast, gastric, bladder, and thyroid carcinomas and several carcinoma cell lines.^{18–22} Therefore, CpG methylation within the promoter region may also occur in some cases of synovial sarcoma that are negative for E-cadherin mRNA expression.

A reduced E-cadherin expression has been shown to cause cellular morphological changes in epithelial cells, from epithelial features to a more fibroblastic and flattened phenotype.^{10,23,24} Synovial sarcoma is a mesenchymal tumor that has an epithelial character and that has tumor cells that frequently show a variety of cell shapes, varying from fibroblastic or flattened to epithelial morphology. Tumor cells in synovial sarcoma whose cell-cell adhesive function has been abolished by E-cadherin mutations, would also be expected to undergo morphological changes, acquiring a more fibroblastic and flattened shape. We previously demonstrated that E-cadherin was predominantly expressed in biphasic tumors of synovial sarcoma, especially in their glandular structure.⁴ In addition, the correlation between the down-regulation of E-cadherin and cellular differentiation, and the correlation between the down-regulation of E-cadherin and glandular disintegration have been reported in primary and metastatic gastric cancer.⁶ Taking all these findings into consideration, it seems likely that there is an association between the presence of E-cadherin gene mutations and histological features in synovial sarcoma.

Ten of the 12 cases demonstrating E-cadherin missense mutations were monophasic fibrous tumors that showed no E-cadherin membranous expression. One of the remaining two cases was a histologically biphasic tumor showing E-cadherin membranous expression. The presence of this case (case 49) may challenge the putative cause-effect relationship between E-cadherin dys-

function and histological subtype in synovial sarcoma. This case demonstrated two E-cadherin missense mutations at codons 159 and 170 (exon 4), these being present within the beginning region of the extracellular domain of the E-cadherin molecules.^{25–27} However, this region is not expected to have a key role to play in the adhesion process, compared to the central region of the E-cadherin gene that codes for the five extracellular cadherin domains of the protein.^{25–27} Thus, this case (case 49) could be considered to show E-cadherin membranous expression and could be expected to have epithelial morphology and furthermore to differentiate into a biphasic tumor. Another case (case 25) of the two remaining cases was a histologically monophasic fibrous tumor showing E-cadherin membranous expression, although it contained E-cadherin mutation at the central region. Some explanations can be offered for this discrepancy. The first is that this case also showed membranous expression of β -catenin, which has important roles to play in cell adhesion and in localizing E-cadherin at the cellular membrane.⁴ The second is that the E-cadherin immunohistochemical antibody used in this study recognizes the cytoplasmic domain of the molecule. The third is that both wild-type and mutant E-cadherin proteins may be expressed in this case: the normal molecule being located in the membrane, the mutated one in the cytoplasm, although SSCP revealed only a faint normal band in the tumor tissue of this case. Concerning this point, it was shown previously that E-cadherin mutated in exon 8 is localized in the cytoplasm of transfected cells whereas the normal molecule is seen at the cell membrane.²⁸ However, these explanations seem inadequate, because the latter case (case 25) was composed of rather plump cells, although a distinct biphasic pattern was not demonstrated. These findings suggest that not only E-cadherin, but also catenins, could contribute toward the acquisition of the epithelial morphology in synovial sarcoma.

Transcription factor Snail has been shown to be expressed by fibroblasts and some epithelial tumor cells, and to repress E-cadherin gene expression by binding directly to the E-boxes present in the proximal E-cadherin promoter.^{9,10} Furthermore, it has also been demonstrated that endogenous E-cadherin expression was inversely correlated with endogenous Snail expression.⁹ Therefore, we first expected that the transcription factor Snail could be a key factor in the epithelial morphology of synovial sarcoma and that it would be expressed in only monophasic fibrous tumors and not in biphasic tumors. However, to the contrary, Snail mRNA was expressed in all of the cases of synovial sarcoma as well as in the control HepG2 cells and samples of desmoid tumor, while furthermore, E-cadherin mRNA was expressed in the majority of synovial sarcoma samples. Most synovial sarcomas may have some mechanisms by which they can escape from the function of Snail to repress E-cadherin expression, although we cannot completely refute the possibility that Snail expression was derived from fibroblasts present in the tumor stroma. However, the existence of monophasic tumors that show E-cadherin expression but that do not demonstrate a distinct biphase-

sic pattern, in addition to the presence of spindle-cell components in biphasic tumors, suggests that other genes involved in epithelial morphogenesis such as extracellular matrix proteins are also important determinants of histological subtype in synovial sarcoma.¹⁰

In conclusion, E-cadherin gene mutations frequently occur in synovial sarcoma, particularly in those of the monophasic fibrous histological subtype. E-cadherin dysfunction because of its mutation is associated with its decreased protein expression and with histological features in synovial sarcoma. Mutations of the E-cadherin gene could therefore be thought of as one of the determinants of histological subtype in synovial sarcoma.

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