[CANCER RESEARCH 56, 49–52, January 1, 1996]

Advances in Brief

No Evidence for Mutations in the α- and β-Catenin Genes in Human Gastric and Breast Carcinomas

Sonja Candidus,[1] Petra Bischoff, Karl-Friedrich Becker, and Heinz Höfler

GSF-Forschungszentrum für Umwelt und Gesundheit, Institut für Pathologie, Neuherberg, Postfach 11 29, D-85758 Oberschleissheim [S. C., P. B., K-F. B., H. H.], and Institut für Pathologie der Technischen Universität, Klinikum rechts der Isar, Ismaningerstrasse 22, D-81675 München [H. H.], Germany

Abstract

Disturbed function of E-cadherin and/or of one of its anchoring proteins, the catenins, is thought to destabilize E-cadherin-mediated cell-cell adhesion, which may enhance the invasiveness of epithelial cells and thus favor carcinoma progression. Reduced expression of E-cadherin and α-catenin, as well as mutations in the E-cadherin gene, have been found in various carcinomas, whereas mutations in the α- and β-catenin genes have been described only in carcinoma cell lines. Using reverse transcription-PCR, followed by agarose gel electrophoresis and single-strand conformational polymorphism, we examined 16 diffuse- and 5 intestinal-type gastric carcinomas, as well as 9 lobular and 2 ductal breast carcinomas, for mutations of α- and β-catenin cDNA. All of the investigated tumors were analyzed previously for E-cadherin mutations. Comparing tumorous and nontumorous samples, we detected neither deletions nor aberrant single-strand conformational polymorphism patterns. At nucleotide 2220 of the α-catenin gene, we identified one frequent polymorphism. Our findings suggest that, in contrast to E-cadherin, mutations of α- and β-catenin do not contribute to the pathogenesis or the diffuse growth patterns of gastric or breast carcinomas.

Introduction

Metastasis starts with a reduction of cell adhesion, resulting in cells that are able to invade neighboring tissue and then are released into the blood or lymphatic stream. Although cell-cell adhesion is a mechanism that involves different proteins, several lines of evidence indicate that the E-cadherin cell adhesion complex is indispensable for tight cell-cell adhesion (1, 2). Reduction or loss of E-cadherin expression in several carcinomas is described (for a review, see Ref. 3), as well as mutation of E-cadherin in 50% of diffuse type gastric carcinomas (4) and in about 40% of breast carcinomas.[2] The cytoplasmic domain of E-cadherin is associated with three anchoring proteins, α-, β-, and γ-catenin (for a review, see Ref. 5). The interaction between cadherins and catenins seems to be crucial for the adhesive function of E-cadherin (6, 7). Therefore, disturbed cell adhesion in tumors with normal E-cadherin may be also explained by impaired function of one of the catenins. α-Catenin is essential for the linking of cadherins to the cytoskeleton (8, 9). Reduced or total loss of α-catenin expression was observed in 55% (10) and 70% (11, 12) of gastric tumors, in 75% (10) and 81% (13) of breast carcinomas, in 80% of esophageal cancers (11, 14), and in 80% of colon carcinomas (11). These findings suggest that, in addition to E-cadherin, α-catenin may be an useful indicator of a functional cell adhesion complex apparatus. In predicting invasive capability, reduced α-catenin expression seems to be an even more sensitive marker than E-cadherin (13). Moreover, deletion of parts of the α-catenin gene, resulting in insufficient E-cadherin function, was found in PC9, a human lung cancer cell line (15), and in PC3, a human prostate cancer cell line (16). Transfection of PC9 cells, which normally show very weak cell-cell adhesiveness, with neural α-catenin cDNA resulted in tight cell-cell adhesions mediated by E-cadherin (17). β-Catenin plays a role in the linkage between E-cadherin and α-catenin (18). It is thought that this linkage is regulated by tyrosine phosphorylation of β-catenin mediated by EGF[3] (19, 20). The reported interaction of β-catenin with APC, a tumor-suppressor gene product, may also interfere with the function of the E-cadherin/catenin adhesion complex (21–25). Recently, two groups have independently described a 321-bp in-frame deletion in the NH_2-terminal part of β-catenin in two cell lines (HSC-39 and HSC-40A) established from one patient with a signet ring cell carcinoma of the stomach (26, 27). This truncated β-catenin molecule failed to link E-cadherin and α-catenin and resulted in a nonfunctional E-cadherin complex.

Thus far, mutations of the α- and β-catenin genes have been described only for cell lines, not in tumors *in vivo*. Tumors with a diffuse growth pattern, which might be expected to have disturbed E-cadherin function but which show normal E-cadherin expression and/or no E-cadherin mutations, could be anticipated to be good candidates for mutations of the catenins. Therefore, we investigated gastric and breast carcinomas, which were analyzed previously for E-cadherin mutations (4), for deletions and point mutations in both α- and β-catenin. The tumors included 21 gastric carcinomas [16 of the diffuse type and 5 of the intestinal type according to the classification of Laurén (28)] and 11 breast carcinomas (9 lobular and 2 ductal type).

Materials and Methods

Tissues. Fresh tumor tissue (n = 32) and nontumorous mucosa (n = 20; nontumorous tissue was not available for one diffuse type gastric carcinoma and all breast carcinomas) were obtained at surgery, snap-frozen, and stored in liquid nitrogen. The tumors included 16 diffuse-type and 5 intestinal-type gastric carcinomas, according to the Laurén classification, as well as 9 lobular and 2 ductal type breast carcinomas. The examined tumors were analyzed previously for E-cadherin mutations in our laboratory (Ref. 4 and Table 1).

Isolation of RNA. Total cellular RNA was isolated by guanidinium thiocyanate extraction and the cesium chloride centrifugation method (29). These RNA samples were used for mutation analysis of E-cadherin as well as α- and β-catenin. Sequences of primers are available from the authors upon request.

RT-PCR. After RT of mRNA, each PCR amplification was performed in a final volume of 25 μl containing 2 μl of cDNA (1:10 diluted), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1–2 mM $MgCl_2$, 0.01% (w/v) gelatin, 200 μM dNTP, 0.8 μM of each primer, and 1.25 units Taq polymerase (Perkin Elmer Cetus); for some of the fragments, 10% glycerol, 5% DMSO, or 10% polyethylene glycol was added. The PCR conditions consisted of 1 cycle at 95°C for 5 min, followed by 35 or 40 cycles at 95°C for 1 min, annealing temperature (between 50 and 62°C depending on the primer pairs) for 30 s, and 72°C for 30 s (small fragments) or 72°C for 1 min 30 s (large fragments). Detailed

Received 9/18/95; accepted 11/14/95.

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[1] To whom requests for reprints should be addressed.

[2] K-F. Becker and H. Höfler, unpublished data.

[3] The abbreviations used are: EGF, epidermal growth factor; RT-PCR, reverse transcription-PCR; SSCP, single-strand conformational polymorphism; LOH, loss of heterozygosity; APC, adenomatous polyposis coli.

Table 1 *Summary of mutation analysis of E-cadherin, α-catenin, and β-catenin in gastric and breast carcinomas*

	Number of tumors						
		E-cadherin mutation		α-Catenin mutation		β-Catenin mutation	
Type of tumor	Tested	+[a]	−	+	−	+	−
Stomach (n = 21):							
Diffuse type	16	4[b]	12	0	16	0	16
Intestinal type	5	0	5	0	5	0	5
Breast (n = 11):							
Lobular type	9	4[c]	5	0	9	0	9
Ductal type	2	1[d]	1	0	2	0	2

[a] +, mutation detectable; −, no mutation detectable.
[b] E-cadherin mutations in diffuse type gastric carcinomas were published previously (4).
[c] All 4 E-cadherin mutations detected in lobular breast carcinomas result in translational stop (manuscript in preparation).
[d] A point mutation in exon 12 resulting in the replacement of alanine by threonine was detected (manuscript in preparation).

PCR conditions used for amplification of each fragment are available from the authors upon request.

Screening for Deletions in the α- and β-Catenin cDNAs. All patients were examined for α- and β-catenin cDNA deletions. Three large fragments per gene (α-catenin: 1267, 943, and 842 bp; β-Catenin: 959, 983, and 650 bp) were amplified by PCR. These overlapping fragments included the whole length of the α- and β-catenin genes. Reaction products were separated on a 1% agarose gel and stained in ethidium bromide for visualization.

SSCP Analysis. To cover the whole length of the α- and β-catenin genes, we amplified overlapping fragments in the range of 250–380 bp. The amplified fragments of the α- and β-catenin genes were analyzed for mutations by SSCP. When available, comparative analyses of normal *versus* tumor tissue pairs from the same individual were performed to identify possible polymorphisms. PCR products were diluted 1:2 in denaturing buffer (10 mM NaOH-95% formamide-0.05% xylencyanol-0.05% bromophenolblue), heat denatured, and loaded onto a 8% nondenaturing polyacrylamide gel (acrylamide/bisacrylamide, 29.5:0.5) containing 2% of glycerol. Each fragment was analyzed at two temperatures (4 and 15°C) and using two kinds of electrophoresis. We performed a homogenous gel and buffer system with 1× MOPS buffer (0.02 M 3-[*N*-morpholin o]propanesulfonic acid-0.001 M EDTA, pH 8.0) and an isotachophoresis with 1× Tris-phosphate buffer (0.09 M Tris-phosphate-0.002 EDTA, pH 8.0) in the gel and 1× Tris-borate buffer (0.09 M Tris-borate-0.002 EDTA, pH 8.0) as electrophoresis buffer. The gels were silver stained for visualization of the DNA.

Sequence Analysis. The polymorphism in α-catenin, detected as an aberrant SSCP pattern, was sequenced. PCR amplified DNA fragments were purified using the QIAquick gel extraction kit (QIAGEN GmbH, Hilden, Germany) and sequenced by the dideoxy method (30) using [α-^{35}S]dATP. A 6% denaturing polyacrylamide gel at 50 W for 3 h was utilized for electrophoresis.

Results and Discussion

We examined tumors that had been characterized previously for E-cadherin mutations (4) for mutations in the α- and β-catenin cDNA. Only 4 of 16 diffuse-type gastric and 5 of 11 breast carcinomas showed an E-cadherin mutation. Nevertheless, we detected neither band shifts of PCR fragments by agarose gel electrophoresis nor aberrant SSCP patterns, suggesting that there are no deletions or point mutations of α- and β-catenin in either gastric or breast carcinomas. These results are listed in Table 1. E-cadherin mutations were identified in 4 of 16 diffuse-type gastric carcinomas (4) and in 4 of 9 lobular carcinomas, as well as in 1 of 2 ductal breast carcinomas. The E-cadherin mutations detected in the 4 lobular breast carcinomas all result in translational stop, whereas the one found in ductal breast carcinoma consists of a point mutation in exon 12, resulting in the replacement of alanine by threonine.[4] These E-cadherin mutations were detected in the same samples used for mutation analysis of the catenins; therefore, the fraction of neoplastic cells in the samples analyzed should also have been sufficient to find all existing catenin mutations.

The identified frequent polymorphism in α-catenin illustrates the sensitivity of SSCP analysis in identifying even small changes in the DNA sequence (Fig. 1, Table 2). Sequencing revealed an exchange of guanine by adenine at nucleotide position 2220, changing the triplet TCG to TCA, both coding for serine. The frequency of heterozygosity (43.8% on average) of this intragenic polymorphism makes it a feasible marker for LOH studies of α-catenin.

The absence of detectable genetic abnormalities in α- and β-catenin in the tumors examined argues that the catenins are not a frequent target of mutations involved in the pathogenesis of these tumors. Discrepancies of reported deletions in α- and β-catenin in cancer cell lines may be explained by changes due to cell culture of the tumor cells before examination. However, deletion of the NH_2-terminal part of β-catenin was described for two cell lines (26, 27) established from a single patient with signet ring cell carcinoma of the stomach using different methods. This indicates that the genetic abnormality of β-catenin in these cells had occurred before cultivation and was already present in the tumor *in vivo*. On the other hand, our results are in line with those of Furukawa *et al.* (31). They also described the absence of mutations in the α-catenin gene in a variety of tumors (49 hepatocellular carcinomas, 53 lung cancers, 48 gastric cancers, and 50 esophageal cancers) analyzed by RNase protection analysis of the conserved exons 2, 10, 13, and 16 of α-catenin. Therefore, other mechanisms for inactivation or regulation of α- and β-catenin should be considered.

The high percentage (between 55 and 81%) of tumors showing reduction or loss of α-catenin expression (10–14) points to the importance of α-catenin for a functional E-cadherin-mediated cell adhesion. If this frequently observed reduced α-catenin immunoreactivity cannot be explained by a gene mutation, then impaired transcription or translation of this gene, or destabilization of the α-catenin transcript or protein should be considered. *In vitro*, a post-transcriptional regulatory mechanism for α-catenin has been described (9). If this is also relevant *in vivo*, tumor cells with down-regulated cadherin expression will not preserve α-catenin expression, resulting in tumor cells showing neither E-cadherin nor α-catenin expression. However, tumors with a reduction or loss of α-catenin expression were much more frequent than those negative for E-cadherin, indicating another inactivation mechanism as perhaps post-translational modification of the protein, resulting in destabilization or degradation of α-catenin.

For β-catenin, tyrosine phosphorylation by the v-*src* oncogene is described, which results in a suppressed cadherin function correlated with a cellular transformation *in vitro* (32–34). Elevated levels of β-catenin phosphorylation were also reported in two human breast

[4] K-F. Becker, I. Becker, C. Schott, and H. Höfler. Mutational inactivation of the E-cadherin gene and loss of the wild-type protein is a feature of lobular-type breast carcinoma, manuscript in preparation.

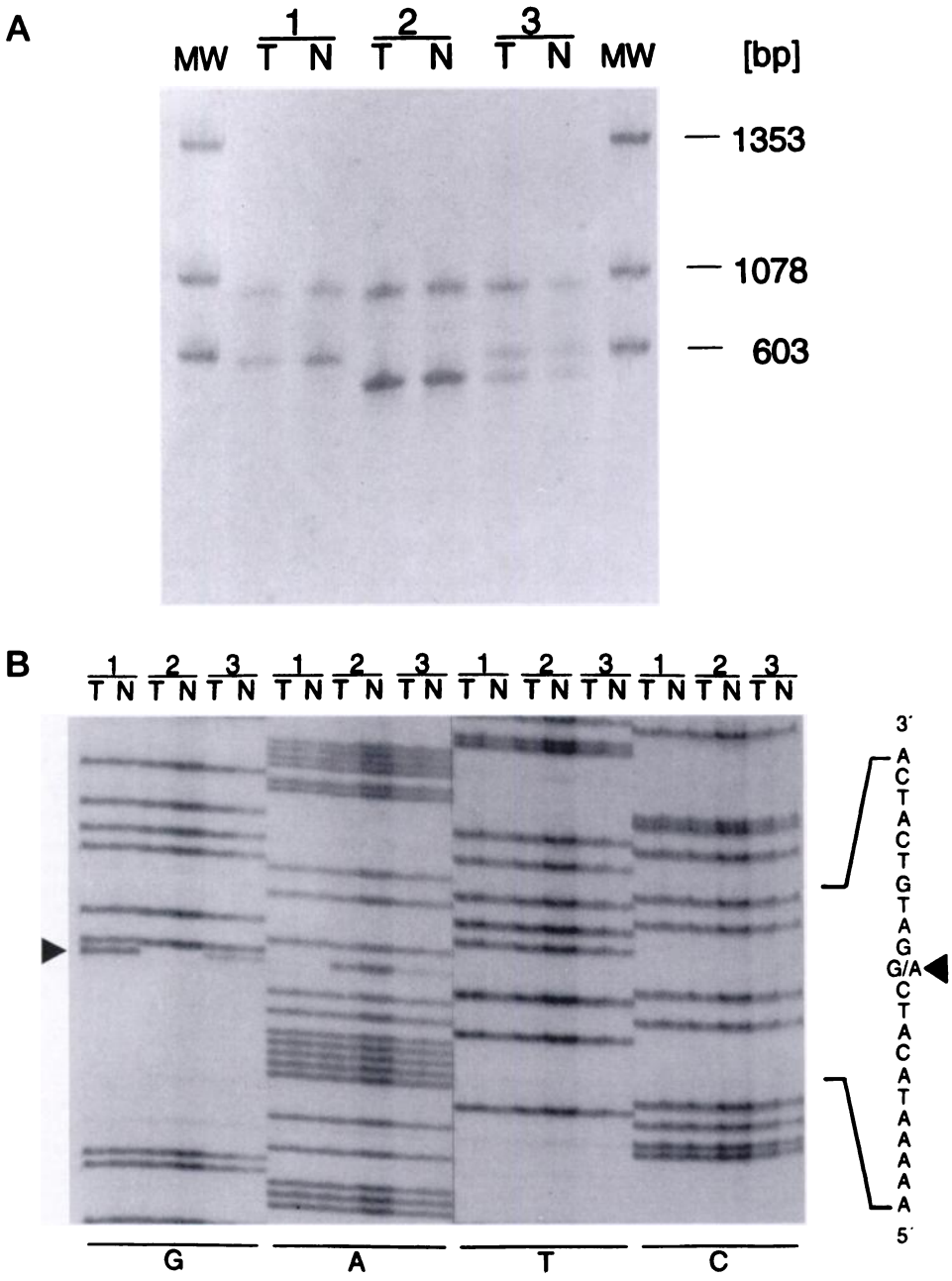

Fig. 1. Polymorphism found in α-catenin. *A*, SSCP gel (continuous gel and buffer system, 15°C), α-catenin, fragment k. *B*, sequencing gel. Guanine at nucleotide position 2220 is exchanged by adenine (transition). Both triplets, TCG, and TCA, encode the same amino acid (serine). *Arrowhead*, position 2220. *1*, homozygous, α-catenin sequence corresponding to that in the GenBank database library under the accession number D13866 (16); *2*, homozygous, α-catenin sequence with adenine instead of guanine at position 2220; *3*, heterozygous; *T*, tumor; *N*, normal; *MW*, molecular weight marker (ϕX174/*Hae*III).

cancer cell lines (BT549 and HS578t; Ref. 35). The examination of two gastric cancer cell lines (MKN-74 and Okajima) for phosphorylated proteins coimmunoprecipitated with E-cadherin revealed, besides E-cadherin and β-catenin, a M_r 185,000 molecule identified as c-*erb*B2 oncogene product (36). This tyrosine kinase was shown to bind to β-catenin and plakoglobin. Furthermore, treatment with TGF-α, which stimulates tyrosine kinase activity of c-*erb*B2 gene product, activated the tyrosine phosphorylation of β-catenin and E-cadherin in gastric cancer cells. In this context, it is of interest that EGF also induces an immediate tyrosine phosphorylation of β- and γ-catenin, and that the EGF receptor directly binds to the core region of β-catenin (19, 20). These results together suggest that β-catenin might be an important regulatory protein for the link between receptor-mediated signaling and cadherin function. β-Catenin was also shown to interact in a competitive fashion with APC, as well as with E-cadherin (21–24), indicating that APC might modulate the interaction between cadherins and catenins. Recent results suggest that the tumor suppressor activity of mutant APC may be compromised due to a defect in its ability to regulate β-catenin (25).

In summary, loss of cell-cell adhesion during malignant progression is likely to be a complex process. Mutations of E-cadherin itself, as detected in diffuse-type gastric carcinomas (4) and also in breast carcinomas,[4] may contribute to impaired cell adhesion within the tumor. Those tumors for which E-cadherin mutations could be excluded might then be expected to have mutations in the α- or β-catenin gene. Our results indicate that mutation of the α- or β-catenin gene does not seem to take place, at least in gastric and breast carcinomas. However, the growth pattern of diffuse-type gastric and lobular breast carcinomas points to a problem with cell adhesion. Therefore, other defects in the E-cadherin/catenin system have to be considered. Altered expression patterns have already been described for E-cadherin and α-catenin. Modification of α- and particularly β-catenin on the protein level also seems to play a role in carcinogenesis and tumor progression. Furthermore, the possibility remains that alterations in

Table 2 *Frequency of the polymorphism found in α-catenin*

Type of tumor	No. (%)	Polymorphism		
		Homozygous 1[a] (%)	Homozygous 2 (%)	Heterozygous (%)
Stomach	21 (100)	11 (52.4)	2 (9.5)	8 (38.1)
Diffuse type	16	10	1	5
Intestinal type	5	1	1	3
Breast	11 (100)	5 (45.5)	0	6 (54.5)
Lobular type	9	4	0	5
Ductal type	2	1	0	1
Total	32 (100)	16 (50.0)	2 (6.3)	14 (43.8)

[a] Homozygous 1, α-catenin sequence corresponding to that in the GenBank under accession number D13866 (16); homozygous 2, α-catenin sequence with adenine instead of guanine at position 2220.

the genes that regulate the expression of E-cadherin and the catenins may be the crucial factor.

References

1. Frixen, U. H., Behrens, J., Sachs, M., Eberle, G., Voss, B., Warda, A., Löchner, D., and Birchmeier, W. E-cadherin-mediated cell-cell adhesion prevents invasiveness of human carcinoma cells. J. Cell. Biol., *113:* 173–185, 1991.
2. Vleminckx, K., Vakaet, Jr., L., Mareel, M., Fiers, W., and Van Roy, F. Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. Cell, *66:* 107–119, 1991.
3. Birchmeier, W., and Behrens, J. Cadherin expression in carcinomas: role in the formation of cell junctions and the prevention of invasiveness. Biochim. Biophys. Acta, *1198:* 11–26, 1994.
4. Becker, K.-F., Atkinson, M. J., Reich, U., Becker, I., Nekarda, H., Siewert, J. R., and Höfler, H. E-cadherin gene mutations provide clues to diffuse type gastric carcinomas. Cancer Res., *54:* 3845–3852, 1994.
5. Kemler, R. From cadherins to catenins: cytoplasmic protein interactions and regulation of cell adhesion. Trends Genet., *9:* 317–321, 1993.
6. Nagafuchi, A., and Takeichi, M. Cell binding function of E-cadherin is regulated by the cytoplasmic domain. EMBO J., *7:* 3679–3684, 1988.
7. Ozawa, M., Ringwald, M., and Kemler, R. Uvomorulin-catenin complex formation is regulated by a specific domain in the cytoplasmic region of the cell adhesion molecule. Proc. Natl. Acad. Sci. USA, *87:* 4246–4250, 1990.
8. Herrenknecht, K., Ozawa, M., Eckerskorn, C., Lottspeich, F., Lentner, M., and Kemler, R. The uvomorulin-anchorage protein α catenin is a vinculin homologue. Proc. Natl. Acad. Sci. USA, *88:* 9156–9160, 1991.
9. Nagafuchi, A., Takeichi, M., and Tsukita, S. The 102 kd cadherin-associated protein: similarity to vinculin and posttranscriptional regulation of expression. Cell, *65:* 849–857, 1991.
10. Ochiai, A., Akimoto, S., Shimoyama, Y., Nagafuchi, A., Tsukita, S., and Hirohashi, S. Frequent loss of α catenin expression in scirrhous carcinomas with scattered cell growth. Jpn. J. Cancer Res., *85:* 266–273, 1994.
11. Shiozaki, H., Iihara, K., Oka, H., Kadowaki, T., Matsui, S., Gofuku, J., Inoue, M., Nagafuchi, A., Tsukita, S., and Mori, T. Immunohistochemical detection of α-catenin expression in human cancers. Am. J. Pathol., *144:* 667–674, 1994.
12. Matsui, S., Shiozaki, H., Inoue, M., Tamura, S., Doki, Y., Kadowaki, T., Iwazawa, T., Shimaya, K., Nagafuchi, A., Tsukita, S., and Mori, T. Immunohistochemical evaluation of α-catenin expression in human gastric cancer. Virchows Arch., *424:* 375–381, 1994.
13. Rimm, D. L., Sinard, J. H., and Morrow, J. S. Reduced α-catenin and E-cadherin expression in breast cancer. Lab. Invest., *72:* 506–512, 1995.
14. Kadowaki, T., Shiozaki, H., Inoue, M., Tamura, S., Oka, H., Doki, Y., Iihara, K., Matsui, S., Iwazawa, T., Nagafuchi, A., Tsukita, S., and Mori, T. E-cadherin and α-catenin expression in human esophageal cancer. Cancer Res., *54:* 291–296, 1994.
15. Shimoyama, Y., Nagafuchi, A., Fujita, S., Gotoh, M., Takeichi, M., Tsukita, S., and Hirohashi, S. Cadherin dysfunction in a human cancer cell line: possible involvement of loss of α-catenin expression in reduced cell-cell adhesiveness. Cancer Res., *52:* 5770–5774, 1992.
16. Oda, T., Kanai, Y., Shimoyama, Y., Nagafuchi, A., Tsukita, S., and Hirohashi, S. Cloning of the human α-catenin cDNA and its aberrant mRNA in a human cancer cell line. Biochem. Biophys. Res. Commun., *193:* 897–904, 1993.
17. Hirano, S., Kimoto, N., Shimoyama, Y., Hirohashi, S., and Takeichi, M. Identification of a neural α-catenin as a key regulator of cadherin function and multicellular organization. Cell, *70:* 293–301, 1992.
18. Nagafuchi, A., and Takeichi, M. Transmembrane control of cadherin-mediated cell adhesion: a 94 kD protein functionally associated with a specific region of the cytoplasmic domain of E-cadherin. Cell Regul., *1:* 37–44, 1989.
19. Hoschuetzky, H., Aberle, H., and Kemler, R. β-Catenin mediates the interaction of the cadherin-catenin complex with epidermal growth factor receptor. J. Cell Biol., *127:* 1375–1380, 1994.
20. Shiozaki, H., Kadowaki, T., Doki, Y., Inoue, M., Tamura, S., Oka, H., Iwazawa, T., Matsui, S., Shimaya, K., Takeichi, M., and Mori, T. Effect of epidermal growth factor on cadherin-mediated adhesion in a human oesophageal cancer cell line. Br. J. Cancer, *71:* 250–258, 1995.
21. Rubinfeld, B., Souza, B., Albert, I., Müller, O., Chamberlain, S. H., Masiarz, F. R., Munemitsu, S., and Polakis, P. Association of the *APC* gene product with β-catenin. Science (Washington DC), *262:* 1731–1733, 1993.
22. Su, L-K., Vogelstein, B., and Kinzler, K. W. Association of the APC tumor suppressor protein with catenins. Science (Washington DC), *262:* 1734–1737, 1993.
23. Hülsken, J., Birchmeier, W., and Behrens, J. E-cadherin and APC compete for the interaction with β-catenin and the cytoskeleton. J. Cell Biol., *127:* 2061–2069, 1994.
24. Rubinfeld, B., Souza, B., Albert, I., Munemitsu, S., and Polakis, P. The APC protein and E-cadherin form similar but independent complexes with α-catenin, β-catenin, and Plakoglobin. J. Biol. Chem., *270:* 5549–5555, 1995.
25. Munemitsu, S., Albert, I., Souza, B., Rubinfeld, B., and Polakis, P. Regulation of intracellular β-catenin levels by the adenomatous polyposis coli (APC) tumor-suppressor protein. Proc. Natl. Acad. Sci. USA, *92:* 3046–3050, 1995.
26. Oyama, T., Kanai, Y., Ochiai, A., Akimoto, S., Oda, T., Yanagihara, K., Nagafuchi, A., Tsukita, S., Shibamoto, S., Ito, F., Takeichi, M., Matsuda, H., and Hirohashi, S. A truncated β-catenin disrupts the interaction between E-cadherin and α-catenin: a cause of loss of intercellular adhesiveness in human cancer cell lines. Cancer Res., *54:* 6282–6287, 1994.
27. Kawanishi, J., Kato, J., Sasaki, K., Fujii, S., Watanabe, N., and Niitsu, Y. Loss of E-cadherin-dependent cell-cell adhesion due to mutation of the β-catenin gene in a human cancer cell line, HSC-39. Mol. Cell. Biol., *15:* 1175–1181, 1995.
28. Laurén, P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type of carcinoma. Acta Pathol. Microbiol. Scand., *64:* 31–49, 1965.
29. Sambrook, J., Fritsch, E. F., and Maniatis, T. Molecular Cloning: A Laboratory Manual, Ed. 2, pp. 7.19–7.22. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1989.
30. Sanger, F., Nicklen, S., and Coulson, A. R. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA, *74:* 5463–5467, 1977.
31. Furukawa, Y., Nakatsuru, S., Nagafuchi, A., Tsukita, S., Muto, T., Nakamura, Y., and Horii, A. Structure, expression and chromosome assignment of the human catenin (cadherin-associated protein) α 1 gene (*CTNNA1*). Cytogenet. Cell Genet., *65:* 74–78, 1994.
32. Matsuyoshi, N., Hamaguchi, M., Taniguchi, S., Nagafuchi, A., Tsukita, S., and Takeichi, M. Cadherin-mediated cell-cell adhesion is perturbed by v-*src* tyrosine phosphorylation in metastatic fibroblasts. J. Cell Biol., *118:* 703–714, 1992.
33. Hamaguchi, M., Matsuyoshi, N., Ohnishi, Y., Gotoh, B., Takeichi, M., and Nagai, Y. $p60^{v\text{-}src}$ causes tyrosine phosphorylation and inactivation of the N-cadherin-catenin cell adhesion system. EMBO J., *12:* 307–314, 1993.
34. Behrens, J., Vakaet, L., Friis, R., Winterhager, E., Van Roy, F., Mareel, M. M., and Birchmeier, W. Loss of epithelial differentiation and gain of invasiveness correlates with tyrosine phosphorylation of the E-cadherin/β-catenin complex in cells transformed with a temperature-sensitive v-*SRC* gene. J. Cell Biol., *120:* 757–766, 1993.
35. Sommers, C. L., Gelmann, E. P., Kemler, R., Cowin, P., and Byers, S. W. Alterations in β-catenin phosphorylation and plakoglobin expression in human breast cancer cells. Cancer Res., *54:* 3544–3552, 1994.
36. Ochiai, A., Akimoto, S., Kanai, Y., Shibata, T., Oyama, T., and Hirohashi, S. c-*erbB*-2 gene product associates with catenins in human cancer cells. Biochem. Biophys. Res. Commun., *205:* 73–78, 1994.