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c-*erbB*-2 Gene Product Associates with Catenins in Human Cancer Cells

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Phosphorylation of β-catenin, an intracytoplasmic cadherin-binding protein, causes disruption of the cadherin-mediated cell adhesion system in cancer cells. A 185-kDa phosphorylated protein, identified as the c-*erbB*-2 gene product, was co-immunoprecipitated with the E-cadherin-catenin complex. Association of the c-*erbB*-2 gene product with the cadherin-catenin complex was proven to be mediated through β-catenin and plakoglobin using an *in vitro* protein-protein precipitation system. These results indicate that the c-*erbB*-2 gene product associates with catenins and may regulate the cell adhesion and invasive growth of cancer. © 1994 Academic Press, Inc.

The cadherins are a family of cell-cell adhesion molecules essential for tight connection between cells, of which E-cadherin is the major cadherin molecule expressed by epithelial cells (1). Cadherins form a complex with cytoplasmic proteins called catenins, which comprise three molecules, α- and β-catenin and plakoglobin (2). The cadherin-mediated cell adhesion system has been shown *in vitro* to act as an "invasion suppressor system" in cancer cells (3). Disruption of the E-cadherin system is caused by mutation of the E-cadherin, α-catenin or β-catenin gene in some cancer cell lines, which show loose cell adhesiveness (5-7). However, these mutations seem to explain only part of the cell dissociation mechanism. Another process contributing to the disruption of cadherin function in cancer cells is the aberrant phosphorylation of a

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cadherin-catenin complex, especially that of β-catenin (8). However, the protein-tyrosine kinase which associates with and phosphorylates the cadherin-catenin complex in human cancer cells has not yet been elucidated.

Materials and Methods

The cell lines used in this study were: MKN-74, a well differentiated adenocarcinoma of the stomach; KATO-III, a signet-ring cell carcinoma of the stomach; Okajima and HSC-39, poorly differentiated adenocarcinomas of the stomach.

The methods of cell lysis and immunoprecipitation using anti-E-cadherin antibody (HECD-1) have been described previously (9). Immunoprecipitates were electrophoresed in 7.5% SDS-polyacrylamide gel followed by immunoblotting and analyzed using anti-phosphotyrosine antibody (PY-20, Seikagaku Co. 1:250 dilution), anti-c-*erbB*-2 antibody (Nichirei, rabbit polyclonal serum, 1:200 dilution), anti-EGF receptor antibody (DAKO, rabbit polyclonal serum, 1:50 dilution), anti-α-catenin (α-18, rat monoclonal antibody against α-catenin provided by Dr. A. Nagafuchi), anti-β-catenin (2D4, rat monoclonal antibody against β-catenin provided by Dr. A. Nagafuchi) and anti-plakoglobin antibody (CBL175, Cymbus Bioscience, 1:10 dilution).

GST fusion proteins of β-catenin and plakoglobin were synthesized as described previously (10). The fusion proteins were expressed in *E. coli* strain JM 109 and purified and coupled to CN4B-Sepharose using the methods recommended by the supplier (Pharmacia). *In vitro* protein-protein precipitation was performed as described previously (10).

Results

Phosphorylated proteins co-immunoprecipitated with the E-cadherin-catenin complex using anti-human E-cadherin antibody (HECD-1) were examined in lysates of two human cell lines. Phosphorylation of E-cadherin and catenins was detected in both cell lines. A single band representing a 185-kDa molecule was co-immunoprecipitated with HECD-1 in both cell lines as shown in Fig. 1A. Considering the molecular mass of this phosphorylated molecule, we attempted to identify it as epidermal growth factor (EGF) receptor or c-*erbB*-2 gene product in the immunoprecipitates obtained with HECD-1. The c-*erbB*-2 gene product was found to be co-immunoprecipitated with the E-cadherin-catenin complex (Fig. 1B). On the other hand, EGF-receptor protein, was not co-

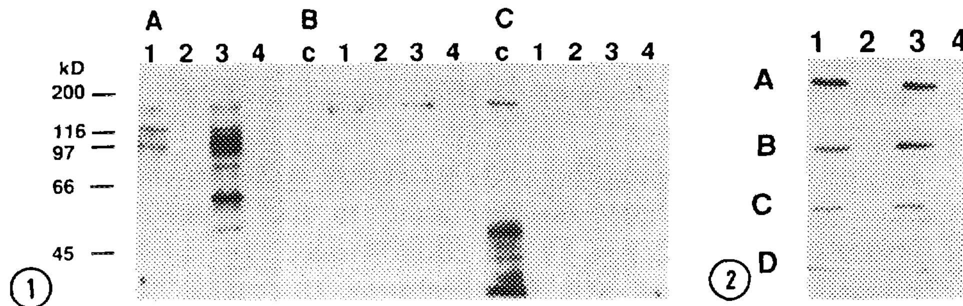

Fig. 1. Immunoblot detection of proteins co-precipitated with E-cadherin. Immunoprecipitates obtained with HECD-1 from cell lysates of two human cancer cell lines were subjected to immunoblot detection using each antibody. Anti-phosphotyrosine antibody (A), anti-c-*erbB-2* antibody (B) and anti-EGF receptor antibody (C). Lanes 1 and 2: MKN-74, Lanes 3 and 4: Okajima, Lanes 1 and 3: immunoprecipitation by HECD-1, Lanes 2 and 4: immunoprecipitation by normal mouse IgG as negative control, Lane c: whole cell lysates from MKN-7 (B) and from A431 (C) as a positive control for each antibody, respectively.

Fig. 2. Detection of E-cadherin-catenin complex after immuno-precipitation using anti-c-*erbB-2* antibody. Lanes 1 and 2: MKN-74, Lanes 3 and 4: Okajima, Lanes 1 and 3: immunoprecipitation using anti-c-*erbB*-2 antibody, Lanes 2 and 4: immunoprecipitation using normal rat serum as negative control. All molecules constituting the cadherin-catenin complex, E-cadherin (A), α-catenin (B), β-catenin (C) and plakoglobin (D), were co-immunoprecipitated with the c-erbB-2 gene product.

immuno-precipitated with molecules of the cadherin-catenin complex (Fig. 1C). As shown in Fig. 2, E-cadherin and all catenin molecules were also co-immunoprecipitated with the *c-erbB*-2 gene product. Neither E-cadherin nor either of the catenins and plakoglobin was detected in the immunoprecipitates with anti-EGF receptor antibody (data not shown).

The association of the c-*erbB*-2 gene product with cadherin-catenin cell adhesion molecules was analyzed in three cell lines, KATO-III, HSC-39 and MKN-74 by immunoprecipitation analysis using an antibody specific to c-*erbB*-2. The KATO-III cell line possesses a DNA mutation of the E-cadherin gene resulting in markedly reduced expression of E-cadherin protein while possessing normal α- and β-catenin molecules (5). The HSC-39 cell line has a 107-amino-acid deletion in the β-catenin protein, thus disrupting the interaction between E-cadherin and α-catenin even though the latter two molecules are expressed normally (7). The MKN-74 cell line possesses no mutation in the molecules of the cadherin-catenin cell adhesion system. Alpha-

and β-catenins and plakoglobin were co-immunoprecipitated with c-*erbB*-2 protein in KATO-III cells, and E-cadherin and the truncated form of β-catenin and plakoglobin were co-immunoprecipitated in HSC-39 cells (Fig. 3).

Beta-catenin and plakoglobin were expressed in *Escherichia coli* as GST-fusion proteins and tested for their ability to bind the c-*erbB*-2 gene product in KATO-III, HSC-39 and MKN-74 cells. The c-*erbB*-2 gene product was co-precipitated with GST-Sepharose 4B-coupled β-catenin and plakoglobin in all of the cell lines (Fig. 4A, B).

Discussion

The present study clearly demonstrated the association of the c-*erbB*-2 oncogene product with β-catenin and plakoglobin, forming a complex with E-cadherin and α-catenin. This is the first reported detection of an oncogenic protein-tyrosine kinase which directly associates with the cadherin-catenin complex in human cancer cells. Amplification of the c-*erbB*-2 gene copy number and over-expression of the c-*erbB*-2 protein are frequently detected in highly malignant cancers and are associated with poor prognosis in breast, gastric and other cancers (11). Treatment of TGF-α, which stimulates tyrosine-kinase activity of c-*erbB*-2 gene product,

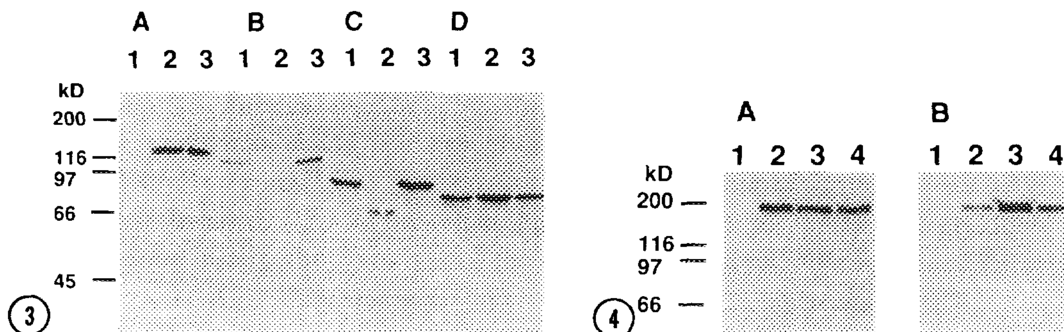

Fig. 3. Co-precipitation of the cadherin-catenin complex with the c-*erbB*-2 gene product. The immunoprecipitates obtained using anti-c-*erbB*-2 antibody were analyzed by immunoblotting to detect E-cadherin (A), α-catenin (B), β-catenin (C), and plakoglobin (D). Lane 1: KATO-III, Lane 2: HSC-39, Lane 3: MKN-74.

Fig. 4. Detection of association between the c-*erbB-2* gene product and GST-fused β-catenin (A) or plakoglobin (B). Sepharose-coupled-GST-fused β-catenin and plakoglobin were mixed with lysates of the three cell lines used in Fig. 3 and precipitated, followed by Western blot analysis using the anti-c-*erbB*-2 antibody. Lane 1 and 2: KATO-III, Lane 3: HSC-39, Lane 4: MKN-74. Lane 1: Negative control for GST-fused protein using GST-Sepharose.

activated the tyrosine phosphorylation of β-catenin and E-cadherin in gastric cancer cells, which showed marked scattered growth *in vitro* (data not shown). These results indicate that interactions involving the c-*erbB*-2 oncogene product and the cadherin-mediated cell adhesion apparatus acting as an "invasion suppressor system" may be critical for initiation of invasion and metastasis.

Beta-catenin and plakoglobin have recently been found to interact with the protein encoded by the tumor suppressor gene APC (10,12,13). In addition, Wnt-1, which was originally identified in vertebrates as a proto-oncogene and found to participate in the induction of mammary hyperplasias in mice, has been demonstrated to modulate cell adhesion molecules through stabilization of β-catenin (14). These findings combined with our present data suggest that the products of tumor suppressor genes, oncogenes and the molecules of the cadherin cell adhesion system are closely interrelated and exchange signals governing both cell proliferation and differentiation.

Acknowledgments

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References

1. Takeichi, M. (1991) Science 251, 1451-1455
2. Ozawa, M., Ringward, M. and Kemler, R. (1990) Proc. Natl. Acad. Sci. USA. 87, 4246-4250
3. Vleminckx, K., L. Vakaet, J., Mareel, M., Fiers, W. and Roy, F. V. (1991) Cell 66, 107-119
4. Shimoyama, Y. and Hirohashi, S. (1991) Cancer Res. 51, 2185-2192
5. Oda, T., Kanai, Y., Oyama, T., Yoshiura, K., Shimoyama, Y., Birchmeier, W., Sugimura, T. and Hirohashi, S. (1994) Proc. Natl. Acad. Sci. USA, 91, 1858-1861
6. Oda, T., Kanai, Y., Shimoyama, Y., Nagafuchi, A., Tsukita, S. and Hirohashi, S. (1993) Biochem. Biophys. Res. Commun. 193, 897-900
7. T. Oyama *et al.* submitted
8. Matsuyoshi, N., Hamaguchi, M., Taniguchi, S., Nagafuchi, A., Tsukita, S. and Takeichi, M. (1992) J. Cell Biol. 118: 703-714
9.Shimoyama, Y., Nagafuchi, A., Fujita, S., Gotoh, M., Takeichi, M., Tsukita, S. and Hirohashi, S. (1992) Cancer Res. 52: 5770-5774
10. Shibata, T., Gotoh, M., Ochiai, A., and Hirohashi, S.(1994) Biochem. Biophys. Res. Comm. 203, 519-522

11. Slamon, D.J., Clark, G.M., Wong, S.G., Levin, W.J., Ullrich, A., and BcGuire, W.L (1987) Science 235, 177-181
12. Rubinfeld, B., Souza, B., Albert, I., Muler, O., Chanberlain, S.C., Masiarz, F.R., Munemitsu, S., and Polakis, P. (1993) Science 262, 1731-1734
13. Su, L.K., Vogelstein, B., and Kinzler, K.W. (1993) Science 262, 1734-1737
14. Bradley, R.S., Cowin, P., and Brown, A.M.C. (1993) J. Cell Biol. 123, 1857-1865