Interaction of the DF3/MUC1 Breast Carcinoma-associated Antigen and β-Catenin in Cell Adhesion*

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The DF3/MUC1 mucin-like glycoprotein is aberrantly overexpressed in human breast carcinomas. The functional role of DF3 is unknown. The present studies demonstrate that DF3 associates with β-catenin. Similar findings have been obtained for γ-catenin but not α-catenin. DF3, like E-cadherin and the adenomatous polyposis coli gene product, contains an SXXXXXXXSSL site that is responsible for direct binding to β-catenin. The results further demonstrate that interaction of DF3 and β-catenin is dependent on cell adhesion. These findings and the role of β-catenin in cell signaling support a role for DF3 in the adhesion of epithelial cells.

The human DF3 (MUC1, episialin, PEM) gene encodes a high molecular mass membrane-associated glycoprotein with a mucin-like external domain. The DF3 glycoprotein is expressed on the apical borders of secretory mammary epithelial cells and aberrantly expressed over the entire surface of carcinoma cells (1). The ectodomain consists of varying numbers of 20-amino acid tandem repeats that are subject to O-glycosylation and that contribute to the expression of a polymorphic protein (2–4). The N-terminal region contains hydrophobic signal sequences that vary as a consequence of alternate splicing (5–7). The C-terminal region includes a transmembrane domain and a 72-amino acid cytoplasmic tail that contains tyrosine phosphorylation sites (8, 9). The function of DF3 is unclear. However, high levels found on carcinoma cells reduce cell-cell and cell-extracellular matrix adhesion in a nonspecific manner (10–12). These studies have suggested that DF3 interferes with cellular adhesion by steric hindrance from the rigid ectodomain (11).

Cadherin cell adhesion molecules form complexes with the cytoplasmic α, β-, and γ-catenin proteins (13). α-Catenin is required for cadherin-mediated cell adhesion and links cadherins to the actin cytoskeleton (14, 15). β-Catenin links α-catenin to the cadherins and is highly related to plakoglobin (γ-catenin) (16–18). β-Catenin is homologous to the Drosophila segment polarity gene product Armadillo (19) that acts downstream of Wingless (20). Armadillo forms complexes with Drosophia E-cadherin and α-catenin (21, 22). These findings have supported a role for β-catenin in morphogenetic signals. Other studies have demonstrated that β-catenin binds directly to the adenomatous polyposis coli (APC) gene product (23–25). The APC protein and E-cadherin form independent complexes with β-catenin (25). γ-Catenin mediates similar interactions among APC, α-catenin, and the cytoskeleton (16).

The present results demonstrate that DF3 interacts directly with β-catenin. An SXXXXXXSSL motif in the DF3 cytoplasmic domain is responsible for binding to β-catenin. We also demonstrate that cell adhesion induces the interaction between DF3 and β-catenin.

MATERIALS AND METHODS

Cell Culture—Human ZR-75-1 breast carcinoma cells were grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum, 100 μg/ml streptomycin, 100 units/ml penicillin, and 2 mM l-glutamine. Cells were grown in suspension (0.3 × 10⁷/ml) with gentle rocking or as a monolayer on polystyrene culture dishes.

Cell Lysate—Cells (~70% confluent) were lysed in ice-cold lysis buffer (150 mM NaCl, 50 mM Tris, pH 7.6, 0.5% Brij 97, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 1 mM sodium vanadate, 1 mM phenylmethylsulfonyl fluoride, and 1 mM dithiothreitol) for 30 min on ice. Lysates were cleared by centrifugation at 14,000 × g for 15 min.

Immunoprecipitation and Immunoblotting—Lysates were incubated with monoclonal antibody (mAb) DF3 (1), anti-α-catenin (Zymed Laboratories, Inc., San Francisco, CA), anti-β-catenin (Zymed), anti-γ-catenin (Zymed), or anti-E-cadherin (Transduction Laboratories, Lexington, KY) for 2 h at 4 °C. Immunoprecipitates were prepared by incubation with rabbit anti-mouse IgG (Upstate Biotechnology, Inc., Lake Placid, NY) and protein A-Sepharose (Pharmacia Biotech Inc.) for 1 h at 4 °C. The precipitates were subjected to electrophoresis in 7.5% or 6% SDS-polyacrylamide gels. Proteins were transferred to nitrocellulose membranes by dry transfer. The membranes were blocked in 5% nonfat dry milk in phosphate-buffered saline containing 0.05% Tween 20 and then incubated with an appropriate antibody for immunoblot analysis. Reactivity was detected by horseradish peroxidase-conjugated second antibodies and chemiluminescence (ECL, Amersham Corp.).

Direct Binding Studies—The GST fusion construct expressing the DF3 cytoplasmic domain (CD) was prepared by polymerase chain reaction cloning and ligation into the pGEX2T vector. GST or GST-DF3/CD was affinity-purified with glutathione-Sepharose 4B beads and suspended in elution buffer (50 mM Tris-HCl, pH 8.0, 5 mM glutathione). Nitrocellulose filters were incubated with GST or GST-DF3/CD for 1.5 h at room temperature. Reactivity was detected with an anti-GST antibody (Santa Cruz Biotechnology).

RESULTS AND DISCUSSION

To identify proteins that associate with DF3, we analyzed mAb DF3 immunoprecipitates by SDS-PAGE and silver staining. The detection of a coprecipitated protein of 92 kDa was confirmed by reactivity with an antibody against β-catenin (Fig. 1A). Since E-cadherin forms complexes with α-, β-, and γ-catenins (26), we analyzed anti-DF3 immunoprecipitates for an association with α- and γ-catenins. While there was no detectable α-catenin in the precipitates, the results indicate that DF3 forms complexes with γ-catenin (Fig. 1, B and C). In the reciprocal experiments, anti-catenin immunoprecipitates were analyzed by immunoblotting with anti-DF3. The findings confirm binding of DF3 to β- and γ-catenins (Fig. 1D). As previously shown (26), E-cadherin formed complexes with all three of the catenins (Fig. 1D).

To determine if binding to DF3 is direct, we subjected anti-β-
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The functional role of the association between DF3 and β-catenin was studied in cells grown in suspension and then grown as a monolayer. There was no detectable β-catenin in the mAb DF3 immunoprecipitates prepared from the suspension cells. By contrast, binding of DF3 to β-catenin was detectable at 1 and 3 h of adherence (Fig. 4A). Cell adhesion was also associated with formation of a complex with DF3 and γ-catenin (Fig. 4B), but not α-catenin (data not shown). A similar analysis of E-cadherin immunoprecipitates demonstrated little if any difference in binding to β- or γ-catenin in suspension as compared with adherent cells (Fig. 4C).

β-Catenin is involved in the formation of adherens junctions of epithelial cells. The cell adhesion E-cadherin protein and the APC tumor suppressor gene product compete for binding to the arm repeats of β-catenin (16) that are also found in Armadillo, γ-catenin, and certain other junctional proteins (29). The present studies demonstrate that DF3 also binds directly to β-catenin and that the SXXXXSSL motif in DF3 is responsible for...
this interaction. Similar results were obtained with the highly related γ-catenin. Whereas the cytoplasmic domain of DF3/MUC1 is phosphorylated on tyrosine (8, 9), it is not known if tyrosine sites influence binding of catenins to the serine-rich motif. The formation of a complex between DF3 and β-catenin (or γ-catenin) may differ from those found in other β-catenin complexes. The interaction of E-cadherin or APC complexes to the cytoskeleton is mediated by binding of β-catenin to α-catenin (16). By contrast, there was little if any α-catenin in the complex of DF3 and β-catenin. Moreover, while E-cadherin forms a stable complex with β-catenin in suspension and adherent cells, the interaction of DF3 with β-catenin is detectable following cell adhesion. Similar findings were obtained for the interaction of DF3 and γ-catenin. These findings support a role for DF3 in the adhesion of cells and provide support for a novel interaction of DF3 with catenins.

REFERENCES
1. Kufe, D., Inghirami, G., Abe, M., Hayes, D., Justi-Wheeler, H., and Schlom, J. (1984) Hybridoma 3, 223–232
2. Siddiqui, J., Abe, M., Hayes, D., Shani, E., Yunis, E., and Kufe, D. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 2320–2323
3. Gendler, S. J., Burchell, J. M., Duhig, T., Lamport, D., White, R., Parker, M., and Taylor-Papadimitriou, J. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 6060–6064
4. Gendler, S., Taylor-Papadimitriou, J., Duhig, T., Rothbard, J., and Burchell, J. A. (1988) J. Biol. Chem. 263, 12820–12825
5. Abe, M., and Kufe, D. (1989) Biochem. Biophys. Res. Commun. 165, 644–649
6. Kufe, D., Inghirami, G., Abe, M., Hayes, D., Justi-Wheeler, H., and Schlom, J. (1984) Hybridoma 3, 223–232
7. Wreschner, D. H., Hareuveni, M., Tsarfaty, I., Smorodinsky, N., Horev, J., Zaretsky, J., Rotkes, P., Weiss, M., Lathe, R., Dion, A., and Keydar, I. (1990) Eur. J. Biochem. 189, 463–473
8. Pandey, P., Kharbanda, S., and Kufe, D. (1995) Cancer Res. 55, 4000–4003
9. Zriban-Licht, S., Baruch, A., Keydar, I., and Wreschner, D. H. (1994) FEBS Lett. 356, 130–137
10. Li, W., Buijs, F., Vos, H. L., and Hilkens, J. (1992) Cancer Res. 52, 2318–2324
11. Wesseling, J., van der Valk, S. W., and Hilkens, J. (1996) Mol. Biol. Cell 7, 565–577
12. van de Wiel-van Kempen, E., Li, W., Buijs, F., Vos, H. L., Melief, C. J. M., Hilkens, J., and Figdor, C. G. (1993) J. Immunol. 151, 767–776
13. Ozawa, M., Baribault, H., and Kemler, R. (1988) EMBO J. 8, 1711–1717
14. Ozawa, M., Ringwald, M., and Kemler, R. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 4246–4250
15. Rimm, D. L., Koolov, E. R., Kebrerias, P., Cianci, C. D., and Morrow, J. S. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 8813–8817
16. Hubmair, J., Birchmeier, W., and Behrens, J. (1994) J. Cell Biol. 127, 2061–2069
17. Knudsen, K. A., and Wheelock, M. (1992) J. Cell Biol. 118, 671–679
18. Peifer, M., McCrea, P. D., Green, K. J., Wieschaus, E., and Gumbiner, B. M. (1992) J. Cell Biol. 118, 681–689
19. McCrea, P. D., Turel, C. W., and Gumbiner, B. (1991) Science 254, 1359–1361
20. Noordermeer, J., Klingensmith, J., Perrimon, N., and Nusse, R. (1994) Nature 367, 80–83
21. Oda, H., Uemura, T., Shiomi, K., Nagafuchi, A., Tsukita, S., and Takeuchi, M. (1993) J. Cell Biol. 121, 1133–1140
22. Oda, H., Uemura, T., Harada, Y., Iwai, Y., and Takeuchi, M. (1994) Dev. Biol. 165, 716–726
23. Su, L., K. Vogelstein, B., and Kinzler, K. W. (1993) Science 262, 1734–1737
24. Rubinfeld, B., Souza, B., Albert, I., Muller, O., Chamberlain, S. H., Mastarz, F. R., Munemitsu, S., and Polakis, P. (1993) Science 262, 1731–1734
25. Rubinfeld, B., Souza, B., Albert, I., Munemitsu, S., and Polakis, P. (1995) J. Biol. Chem. 270, 5549–5555
26. Kintner, C. (1992) Cell 69, 225–236
27. Stappert, J., and Kemler, R. (1994) Cell Adhes. Commun. 2, 319–327
28. Haukzuzky, H., Aher, H., and Kemler, R. (1994) J. Cell Biol. 127, 1375–1380
29. Peifer, M., Berg, S., and Reynolds, A. B. (1994) Cell 76, 789–791
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