Minimal Mutation of the Cytoplasmic Tail Inhibits the Ability of E-cadherin to Activate Rac but Not Phosphatidylinositol 3-Kinase

DIRECT EVIDENCE OF A ROLE FOR CADHERIN-ACTIVATED Rac SIGNALING IN ADHESION AND CONTACT FORMATION

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Classic cadherins are adhesion-activated cell signaling receptors. In particular, homophilic cadherin ligation can directly activate Rho family GTPases and phosphatidylinositol 3-kinase (PI3-kinase), signaling molecules with the capacity to support the morphogenetic effects of these adhesion molecules during development and disease. However, the molecular basis for cadherin signaling has not been elucidated, nor is its precise contribution to cadherin function yet understood. One attractive hypothesis is that cadherin-activated signaling participates in stabilizing adhesive contacts (Yap, A. S., and Kovacs, E. M. (2003) J. Cell Biol. 160, 11–16). We now report that minimal mutation of the cadherin cytoplasmic tail to uncouple binding of p120-ctn ablated the ability of E-cadherin to activate Rac. This was accompanied by profound defects in the capacity of cells to establish stable adhesive contacts, defects that were rescued by sustained Rac signaling. These data provide direct evidence for a role of cadherin-activated Rac signaling in contact formation and adhesive stabilization. In contrast, cadherin-activated PI3-kinase signaling was not affected by loss of p120-ctn binding. The molecular requirements for E-cadherin to activate Rac signaling thus appear distinct from those that stimulate PI3-kinase, and we postulate that p120-ctn may play a central role in the E-cadherin-Rac signaling pathway.

Classic cadherins are fundamental determinants of morphogenesis and tissue patterning (1, 2). Cadherins function as membrane-spanning macromolecular complexes: the cadherin ectodomains engage in adhesive binding whereas the cytoplasmic tails associate with a variety of proteins that potentially allow cadherins to interact with the actin cytoskeleton and cell signaling pathways (3). A deep understanding of how cadherins exert their morphogenetic effects must explain how adhesive engagement of the cadherin ectodomain is translated into changes in cellular behavior, particularly productive adhesion and cell-cell recognition.

An important advance comes from the recent discovery that classical cadherins function as adhesion-activated cell signaling receptors (4–7). Homophilic ligation of the cadherin ectodomain activates intracellular signaling through Rho-family GTPases and the lipid kinase, PI3-kinase. These signals have the potential to allow cadherin engagement to regulate the actin cytoskeleton and the cadherin/catenin apparatus, thereby translating adhesive recognition into changes in cell shape and surface adhesion.

In particular, Rac activity is consistently stimulated as an early-immediate response to cadherin ligation (4, 5) and Rac signaling appears to be important for cells to initiate productive adhesive contacts (5, 8). However, the molecular basis for cadherin-activated Rac signaling remains to be elucidated, and the biological significance of direct cadherin signaling is not thoroughly understood. Although recent data point to a role in adhesive recognition and strengthening (reviewed in Ref. 9), both Rac and PI3-kinase signals can have more diverse targets that include transcriptional regulation and control of cell population dynamics (10, 11). Indeed, although it is attractive to postulate that cadherin-activated signaling might support adhesive contact formation, this notion has not yet been directly tested.

In this study we sought to pursue the functional significance of E-cadherin-activated cell signaling. We focused on the potential role of p120-ctn binding in this process, based on data suggesting that p120-ctn might provide insights into the relationship between cadherin signaling and adhesion. p120-ctn is an armadillo family protein that associates directly with the membrane-proximal region of the cadherin cytoplasmic tail (12). Mutation of the cytoplasmic tail to eliminate p120-ctn binding revealed an important contribution of this catenin to cadherin adhesion, albeit with complex effects. In several instances, loss of the capacity to bind p120-ctn reduced cadherin adhesion (13, 14), but in other cases, actual potentiation of adhesion was observed (15). Some of these discrepancies may reflect differences in the cadherin studied, the cell types utilized, and the potential for other proteins to interact with the membrane-proximal region of the cytoplasmic tail. The molecular basis for these functional effects remains unknown.

When overexpressed in the cytoplasm p120-ctn can also

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1 The abbreviations used are: PI3-kinase, phosphatidylinositol 3-kinase; CHO, Chinese hamster ovary; GFP, green fluorescent protein; PAK, p21-activated kinase; ConA, concanavalin A; PMA, phorbol 12-myristate 13-acetate.

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A431D cells (14), in contrast to wild-type E-cadherin, the hE-764AAA mutant does not recruit p120-ctn. Protein complexes bound to E-cadherin were immunoprecipitated using monoclonal antibody HECD-1, which binds the ectodomain of E-cadherin (identical in wild-type E-cadherin and the hE-764AAA mutant). Complexes were separated by SDS-PAGE and immunoblotted for E-cadherin, β-catenin, or p120-ctn.

RESULTS

As a first step to analyzing the molecular basis of cadherin signaling, we utilized a minimal E-cadherin mutant (hE-764AAA) that contains three sequential point mutations (EED to AAA) that effectively ablated binding of p120-ctn to E-cadherin in A431D cells (14). We stably expressed hE-764AAA in Chinese hamster ovary cells (hE-CHO cells) and chose clones that displayed surface levels of mutant protein similar to CHO cell lines stably expressing wild-type E-cadherin (hE-CHO cells; see Fig. 1A). In both hE-CHO cells and in hE-764-CHO cells the majority of cellular cadherin was found at the cell surface (preserved by trypsinization in the presence of calcium but degraded in the absence of calcium). B, recruitment of catenins to cell-cell contacts. Near-confluent cultures of P-CHO cells, hE-CHO cells, and hE764-CHO cells were fixed and stained by indirect immunofluorescence for E-cadherin, β-catenin, and p120-ctn. The hE-764AAA mutant recruits β-catenin but not p120-ctn to cell-cell contacts, whereas both catenins are recruited by wild-type E-cadherin. C, the hE-764AAA mutation does not co-immunoprecipitate p120-ctn. Protein complexes bound to E-cadherin were immunoprecipitated using monoclonal antibody HECD-1, which binds the ectodomain of E-cadherin (identical in wild-type E-cadherin and the hE-764AAA mutant). Complexes were separated by SDS-PAGE and immunoblotted for E-cadherin, β-catenin, or p120-ctn.

FIG. 1. Minimal mutation of the cadherin cytoplasmic tail un-couples p120-ctn binding in Chinese hamster ovary cells. The hE-764AAA cadherin mutant was stably expressed in CHO cells (hE764-CHO cells) and compared with cells stably expressing wild-type E-cadherin (hE-CHO cells). A, surface expression of cadherin proteins. E-cadherin (HECD-1 monoclonal antibody) Western blots were performed on samples from parental, untransfected CHO cells (P-CHO), hE-CHO cells (hE-CHO), or hE-764-CHO cells (hE-764). Samples were prepared from cells lysed directly into SDS sample buffer (total; T), from cells initially exposed to surface trypsinization (0.05% (w/v) crystalline trypsin) in the presence of extracellular calcium (2 mM) to preserve the cadherin ectodomain (+ Ca), or from cells initially exposed to surface trypsin in the absence of extracellular calcium (5 mM EDTA, −Ca), which renders surface E-cadherin (but not cytoplasmic E-cadherin) sensitive to degradation by trypsin. Similar amounts of total E-cadherin (E-cad) are found in hE764-CHO cells and hE-CHO cells. In both lines, the majority of E-cadherin is found at the cell surface (preserved by trypsinization in the presence of calcium but degraded in the absence of calcium). B, recruitment of catenins to cell-cell contacts. Near-confluent cultures of P-CHO cells, hE-CHO cells, and hE764-CHO cells were fixed and stained by indirect immunofluorescence for E-cadherin, β-catenin, and p120-ctn. The hE-764AAA mutant recruits β-catenin but not p120-ctn to cell-cell contacts, whereas both catenins are recruited by wild-type E-cadherin. C, the hE-764AAA mutation does not co-immunoprecipitate p120-ctn. Protein complexes bound to E-cadherin were immunoprecipitated using monoclonal antibody HECD-1, which binds the ectodomain of E-cadherin (identical in wild-type E-cadherin and the hE-764AAA mutant). Complexes were separated by SDS-PAGE and immunoblotted for E-cadherin, β-catenin, or p120-ctn.

As a first step in assessing this issue, we now report that a minimal mutation in the cytoplasmic tail that abolishes the association of p120-ctn inhibits the ability of E-cadherin to signal to Rac but does not affect its ability to activate P13-kinase. Analysis of this mutant provides direct evidence for the central role of cadherin-activated signaling in adhesion and cell recognition.
contacts (Fig. 1B) nor did it co-immunoprecipitate with p120-ctn (Fig. 1C). However, the hE-764AAA mutant efficiently co-immunoprecipitated β-catenin and recruited this protein to cell-cell contacts as predicted, because the minimal mutation does not affect the β-catenin binding site.

The hE-764AAA Mutation Is Associated with Defects in Adhesion and Contact Zone Extension—To assess the specific functional consequences of this cadherin mutation, we utilized a reductionist assay that exploits the ability of purified recombinant proteins incorporating the entire cadherin ectodomain to engage the ectodomains of cellular cadherins (4, 5, 13, 18–21). We used a fusion protein consisting of the ectodomain of human E-cadherin expressed as a constitutive dimer fused to the Fc portion of IgG (hE/Fc). Immobilized hE/Fc induces cadherin clustering, supports adhesion, and directly activates signaling by the Rac GTPase (5, 18, 22) and PI3-kinase (5), thereby allowing cadherin-specific cellular sequelae to be isolated.

An important sign of productive cadherin-based cell recognition is the rapid conversion of limited, nascent contacts into broad zones of adhesion (8, 23). This process of contact zone extension is also represented by the characteristically large lamellipodial extensions that cadherin-expressing cells make when they adhere to hE/Fc-coated substrata (5, 18). We found that, although weakly adhesive (Fig. 2C) (14), hE764-CHO cells attached to hE/Fc-coated substrata. However, whereas hE-CHO cells rapidly extended numerous broad cadherin-based lamellipodia (Fig. 2, A and B), hE764-CHO cells characteristically formed spindle-shaped cells, with very limited lamellipodial protrusions (Fig. 2, A and B). Phase contrast videomicroscopy revealed that as hE-CHO cells attached to hE/Fc they initially extended numerous small protrusions, which reinforced into a few dominant lamellipodia after ~45 min (Fig. 2A). In contrast, hE764-CHO cells continuously formed labile extensions that did not reinforce into any dominant lamellipodia (Fig. 2A).

This was further reflected by changes in cell adhesion, as measured by the resistance of cells to detachment from hE/Fc-coated substrata. As shown in Fig. 2C, cells expressing wild-type E-cadherin increased adhesion rapidly upon attachment to hE/Fc, whereas parental CHO cells bound poorly. hE764-CHO cells adhered to hE/Fc but always much more weakly than hE-CHO cells. This is consistent with earlier studies that reported a positive role for p120-ctn in cadherin adhesion (13, 14, 24).

The Functional Defects Associated with the hE-764AAA Mutation Are Rescued by Rac Signaling—We observed recently (5) that Rac signaling was essential for cells to effectively extend cadherin-based lamellipodia in these planar adhesion assays. The inability of hE764-CHO cells to form lamellipodia thus suggested that the functional defects associated with the hE-764AAA mutation might be due to changes in Rac signaling. As a first test of this notion, we asked whether restoration of Rac signaling could affect cadherin-based lamellipodial formation in hE764-CHO cells. As shown in Fig. 2B, we found that expression of constitutively active V12-Rac induced hE764-CHO cells to form extensive, nearly circumferential, lamellipodia upon hE/Fc-coated substrata, to a similar extent as occurred when V12-Rac was expressed in wild-type hE-CHO cells (Fig. 2B). Therefore provision of Rac signaling could restore the ability of hE764-CHO cells to support robust adhesion contact formation. In contrast, cadherin-based lamellipodial formation in hE764-CHO cells was not rescued by expression of constitutively active or dominant-negative mutants of RhoA or Cdc42 or by dominant-negative Rac (not shown).

Importantly, restoration of Rac signaling by expression of GFP-L61Rac also stimulated adhesion pronouncedly in hE764-CHO cells (Fig. 2C), whereas expression of GFP alone had no significant effect. Taken together, these data suggested that alterations in Rac signaling might be implicated in the functional consequences of the hE-764AAA mutation.
E-cadherin characteristically accumulated at the regions respectively adhered to the dorsal surfaces of hE-CHO cells. Cellular (Fig. 3). We found that latex beads coated with hE/Fc effecto to recruit Rac to adhesion sites was affected by this mutation mutation, we first examined whether the ability of E-cadherin cadherin-activated signaling was altered by the hE-764AAA contact (reviewed in Ref. 9). Therefore, to test whether direct Productive cadherin ligation recruits Rac to sites of adhesive binding site from PAK (Fig. 4), adhesion of hE-CHO cells to planar substrata homophilic ligation to activate endogenous Rac1 signaling. As This disparity in recruitment of GFP-Rac1 to sites of adhesion was accompanied by a marked difference in the ability of E-cadherin to recruit Rac1 to sites of cell adhesion. The hE-764AAA Mutation Does Not Activate Rac Signaling—Productive cadherin ligation recruits Rac to sites of adhesive contact (reviewed in Ref. 9). Therefore, to test whether direct cadherin-activated signaling was altered by the hE-764AAA mutation, we first examined whether the ability of E-cadherin to recruit Rac to adhesion sites was affected by this mutation (Fig. 3). We found that latex beads coated with hE/Fc effectively adhered to the dorsal surfaces of hE-CHO cells. Cellular E-cadherin characteristically accumulated at the regions where beads bound (Fig. 3A), and transiently expressed GFP-Rac1 was efficiently recruited to these sites of homophilic E-cadherin adhesion, appearing as distinct bands of fluorescence immediately around the beads (Fig. 3B). In contrast, GFP-Rac1 was not recruited to sites of adhesion with beads coated with either concanavalin A (ConA; see Fig. 3E) or poly-L-lysine (not shown), excluding the possibility that Rac recruitment was a nonspecific response to bead adhesion. GFP alone did not accumulate around beads coated with hE/Fc (Fig. 3F), indicating that Rac1 accumulation was not because of an edge artifact.

In contrast to hE-CHO cells, hE764-CHO cells failed to recruit Rac to sites of adhesion. As shown in Fig. 3C, hE/Fc-coated beads accumulated hE-764AAA at sites of adhesion, but this was not accompanied by any localized recruitment of GFP-Rac1 above the levels expressed on the free cell surface away from the adhesive contacts (Fig. 3D). Indeed, when quantitated by digital image analysis, the recruitment of GFP-Rac1 to hE/Fc beads in hE764-CHO cells was identical to that of GFP-Rac1 to ConA beads in hE-CHO cells (Fig. 3G). This suggested that the 764AAA mutation effectively abrogated the ability of E-cadherin to recruit Rac1 to sites of cell adhesion.
tide dissociation inhibitor-like activity for Rho in vitro (16), we found that PMA stimulated Rac activation to a comparable extent in both hE764-CHO cells and wild-type hE-CHO cells (Fig. 4B). Therefore failure of the hE764AAA mutant to activate Rac in response to homophilic ligation cannot be because of a generalized inhibition of Rac competence by cytoplasmic p120-ctn.

To further assess the ability of cadherin ligation to activate potential signals downstream of Rac, we examined the recruitment of PAK1 to sites of cadherin adhesion (Fig. 5). PAK1 is a well characterized effector of Rac signaling that is recruited to the membrane upon activation of Rac (25). Consistent with this, we found that endogenous PAK1 (Fig. 5B) accumulated with wild-type E-cadherin (Fig. 5A) at sites of adhesion to hE/Fc-coated beads. In contrast, PAK1 was not recruited to sites of contact when the hE764AAA mutant bound to hE/Fc-coated beads (Fig. 5, C and D). Taking these data together, we conclude that mutation of the p120-ctn binding site rendered E-cadherin incapable of signaling to Rac, blocking recruitment of Rac to sites of adhesion, stimulation of the GTPase itself, and the activation of a potential down-stream signaling pathway.

Uncoupling of p120-ctn Binding Does Not Affect Cadherin-activated PI3-kinase Signaling—We then turned to assess whether the ability of E-cadherin to activate PI3-kinase signaling was affected by the hE764AAA mutation. As with growth factors (26), stimulation of PI3-kinase by E-cadherin entails recruitment of PI3-kinase to sites of activated receptors and stimulation of down-stream targets (18, 27).

We found that hE/Fc beads recruited GFP-p85 to sites of cadherin adhesion in hE-CHO cells (Fig. 6, A and B), corroborating earlier demonstrations that homophilic ligation induces PI3-kinase to interact biochemically with the cadherin-catenin complex (18, 27). Recruitment of GFP-p85 to cadherin-coated beads was not, however, materially altered in hE764-CHO cells (Fig. 6, C and D), suggesting that this mutation did not affect the ability of E-cadherin to recruit PI3-kinase.

To pursue this notion, we then assessed the ability of Akt (protein kinase B) to be recruited to sites of cadherin adhesion (Fig. 7). Akt is a downstream effector of PI3-kinase signaling that translocates to the plasma membrane upon activation of PI3-kinase by growth factors (11), as well as E-cadherin ligation (5). Consistent with the pattern for GFP-p85, endogenous Akt was recruited to hE/Fc beads both in cells expressing wild-type E-cadherin (Fig. 7, A and B) and in cells expressing the hE764AAA mutation (Fig. 7, C and D). At the membrane
Akt is activated by phosphorylation in a PI3-kinase-dependent fashion (11). A further test of whether signals downstream of PI3-kinase were activated, we then used activation-specific phospho-Akt antibodies to assess the functional status of Akt in cells adherent to hE/Fe-coated substrata (Fig. 7G). Akt was concentrated by immunoprecipitation from membrane fractions, then probed for phospho-Akt. We found that membrane-associated phospho-Akt levels rose to a similar extent in hE-764 CHO cells as in hE-CHO cells when cells adhered to hE/Fe compared with poly(t-lysine). Taken together, these findings indicate that ablation of p120-ctn binding did not affect the ability of E-cadherin to either recruit PI3-kinase to adhesive contacts or activate its downstream signaling pathways.

**DISCUSSION**

We draw two conclusions from these data. First, limited mutation of the cadherin cytoplasmic tail effectively ablates cadherin-activated Rac signaling but does not affect the ability of E-cadherin to activate PI3-kinase signaling. Second, the functional defects associated with this mutation arise, in significant degree, from loss of the cadherin-activated Rac signaling pathway. Taken together these findings provide direct evidence of a key role for ligation-activated Rac signaling in cadherin adhesion and contact formation.

A number of recent studies have established that classical cadherins function as adhesion-activated cell signaling receptors (reviewed in Ref. 9). To date, the best characterized direct consequences of cadherin signaling include activation of the Rac GTPase and PI3-kinase (5, 6, 17, 27). We found that the minimal 764AAA mutation of the E-cadherin cytoplasmic tail abolished detectable evidence of signaling through the Rac pathway. Thus, Rac protein failed to recruit to sites of cadherin-specific contact in hE764-CHO cells compared with contacts mediated by wild-type E-cadherin, localization that is predicted to be essential for Rac to control cell morphology and the actin cytoskeleton in response to cadherin ligation (5, 8). Also, GTP loading of Rac in response to cadherin binding was abolished by this mutation, but neither baseline GTP-Rac levels nor PMA-stimulated GTP-Rac responses were significantly affected. This cadherin mutant thus appeared able to discriminate cadherin-activated Rac signaling from other pathways that can also stimulate Rac. In addition, PAK1, a well characterized (though not exclusive) downstream target of Rac signaling (25), failed to recruit to adhesions mediated by the hE-764AAA mutant, in contrast to wild-type E-cadherin contacts. These parameters assay different upstream and downstream steps already proven, or predicted, to be involved in cadherin-activated Rac signaling. Taken together they demonstrate that minimal mutation of the cytoplasmic tail effectively ablated the ability of E-cadherin to activate this signaling pathway.

In contrast, cadherin-activated PI3-kinase signaling appeared unaffected. Signaling from E-cadherin to PI3-kinase is distinguished by recruitment of the enzyme to the plasma membrane at sites of cadherin adhesion and the local activation of down-stream targets, especially Akt (protein kinase B). In line with earlier biochemical data that we and others have reported (5, 27), in the current study we found that the p85 subunit of PI3-kinase consistently accumulated in response to spatially restricted E-cadherin signals, accompanied by the accumulation of Akt at those contacts and its activation at the plasma membrane. Neither of these processes was materially altered by the 764AAA mutation. This is consistent with evidence that PI3-kinase may recruit to the cadherin-catenin complex by an association with β-catenin (28), which binds effectively to the hE-764AAA mutant (14). Thus, although we cannot exclude subtle quantitative changes in cadherin-activated PI3-kinase signaling, our data showed a clear qualitative difference in the ability of the hE-764AAA mutant to activate Rac signaling compared with its ability to stimulate PI3-kinase. This mutant thus provides a useful tool to both dissect out and discriminate between cadherin-activated signaling pathways.

Importantly, our findings indicate that this discrepancy in cadherin-activated signaling has direct consequences for adhesive function. Recent studies demonstrated that Rac signaling plays an important role in junctional integrity (reviewed in Refs. 9 and 29) and conditions the ability of cells to form productive cadherin-based contacts (5, 8). These studies did not, however, distinguish whether the requirement for Rac reflected cadherin-activated signaling or signaling from pathways separate from those directly activated by the cadherin. It was thus noteworthy that not only was cadherin-activated Rac signaling abrogated by the hE-764AAA mutant, but the adhesive defects characteristic of cells expressing this mutant were rescued by sustained Rac signaling.

Thus, whereas hE-764 CHO cells adhered weakly in detachment-based adhesion assays and were unable to productively extend adhesive contacts, expression of constitutively active Rac V12 or Rac 61L stimulated both these parameters of cadherin activity. This implies that the poor adhesive activity that results from ablating the p120-ctn binding site is unlikely to be because of an intrinsic structural defect affecting the ability of this cadherin mutant to support adhesion. Moreover, although we found previously (5) that inhibitors of PI3-kinase signaling profoundly reduced cadherin adhesion and lamellipodial formation, in our current studies the apparent preservation of cadherin-activated PI3-kinase signaling was not sufficient to support adhesive activity in the absence of Rac signaling. Instead, taken together, these observations suggest that alterations in cadherin-activated Rac signaling are, to a significant degree, responsible for the adhesive defects associated with the hE-764AAA mutation. By implication, a role for cadherin-activated Rac signaling may account for the positive adhesive contribution of p120-ctn identified in earlier reports (13, 14, 24).

Interestingly, however, a contribution of Rac signaling to cell-cell adhesion was not apparent when Madin-Darby canine kidney cells were allowed to aggregate over a longer time frame (1–24 h; see Ref. 8), when other adhesion systems may also contribute (30). This contrasts with our experience using more rapid assays of cadherin adhesion and contact formation (occurring over 15–90 min). This discrepancy suggests that the early immediate activation of Rac by E-cadherin may be most important for the phase of cadherin adhesive strengthening that occurs immediately after homophilic ligation, a critical time for productive cell-cell recognition to be initiated. Regulation of the actin cytoskeleton is one likely target for Rac during this period (18).

What mechanism might account for the ability of this cadherin mutant to ablate Rac signaling? Given the documented inability of the hE-764AAA mutant to bind p120-ctn, one intriguing possibility is that the cadherin-catenin complex must incorporate p120-ctn to activate Rac. This inference is consistent with reports that, when overexpressed in fibroblasts, p120-ctn can interact functionally with Rho family GTPases (though the spectrum of interactions may vary with cell type) (16, 17, 31). Furthermore, in Drosophila embryos p120-ctn appears to be necessary for Rho to be recruited to, and function in, epithelial adherens junctions (32). Indeed, purified p120-ctn can interact functionally with recombinant Rho (16).

We cannot, of course, exclude the possibility that other proteins that interact with the membrane-proximal region of the
cadherin tail might play a role in signaling to Rac. Both presenlin (33) and Hakai (34) bind directly to this region of the cadherin tail, although only binding of p120-catenin has as yet been reported to be affected by the hE-764AAA mutation (14). Presenlin may influence binding of β-catenin to E-cadherin (33), whereas Hakai can induce cadherin internalization (34). However, we observed no differences in the amount of β-catenin that co-immunoprecipitated with hE-764AAA or in the steady-state levels of surface versus cytoplasmic E-cadherin in our cell lines.

We conclude that analysis of the hE-764AAA mutant demonstrates a key role for directly activated Rac signaling in cadherin adhesion and contact zone formation. We suggest that p120-catenin may play a central role in the E-cadherin-Rac-signaling pathway. Further studies will be necessary to conclusively test this hypothesis and establish the mechanism responsible for this activity. Certainly, it was noteworthy that the hE-764AAA mutation appeared to so markedly inhibit Rac signaling, despite preserving PI3-kinase signaling. This was surprising, because PI3-kinase signaling is potentially capable of stabilizing Rac (26, 35), and indeed, cadherin-activated PI3-kinase signaling contributes to Rac activation by E-cadherin (5, 6). We had thus expected that cadherin-activated PI3-kinase signaling by the hE-764AAA mutant might have been able to support some degree of Rac activation. It is possible that, directly or indirectly, the membrane-proximal region of the cadherin tail recruits guanine nucleotide exchange factors that are absolutely essential for Rac activation by E-cadherin. Alternatively, p120-catenin (or another protein that interacts with this region of the cytoplasmic tail) may participate in recruiting Rac to the plasma membrane, a fundamental initial step in Rac activation, because PI3-kinase signaling is potentially capable of directly or indirectly, the membrane-proximal region of the cytoplasmic tail) may participate in recruiting Rac to the plasma membrane, a fundamental initial step in Rac activation, because PI3-kinase signaling is potentially capable of GTPase activation that is necessary for Rac to be able to interact with exchange factors and downstream effector molecules (36). Indeed, our observation that Rac recruitment to cadherin contacts was abolished by the hE-764AAA mutation is consistent with just such an idea. Whatever the precise molecular mechanism, our current findings directly substantiate the notion that cadherin-activated Rac signaling plays a key role in translating homophilic ligation of the cadherin ectodomain into productive adhesion.

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