Small GTPases and regulation of cadherin dependent cell–cell adhesion

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Abstract
Cadherins belong to a superfamily of cell–cell adhesion receptors that bind to the same type of molecules (homotypic interaction) in a calcium dependent manner. Different members of the family are found in a wide variety of cell types and cadherin adhesive function plays a role in cell fate, segregation, and differentiation, which ensures the higher order of organisation found in many tissues. This review will focus on the role that cadherin adhesiveness plays in the differentiation of epithelial cells, and how cadherin function can be regulated by proteins of the small GTPase family. In the text, readers are referred to recent reviews and other chapters covering important topics that are not discussed here because of space limitation.

Keywords: cadherins; catenins; small GTPases; cell–cell adhesion

E-cadherin and P-cadherin are found in epithelia and their function is essential to establish and maintain the differentiated epithelial phenotype (reviewed by Gumbiner). It is possible that the role of cadherin receptors during epithelial differentiation is purely mechanical: the close apposition of membranes may facilitate the formation of other junctional components and cytoskeletal rearrangement. Alternatively (or in conjunction with their mechnano-adhesive function), adhesion mediated by cadherin receptors may effectively trigger signalling events. Evidence is now accumulating for the involvement of cadherin in the induction of gene expression, cellular differentiation, growth control, and the distribution of cytoplasmic proteins.

During tumorigenesis in epithelial cells, E-cadherin adhesiveness is frequently reduced or abolished in a variety of different ways (reviewed by Christofori and Semb). Loss of E-cadherin mediated adhesion results in increased dedifferentiation of tumour cells (transformation from adenoma to carcinoma). Interestingly, in some cases, the transformed cells switch on the expression of other types of cadherin receptors normally found in mesenchymal and fibroblast cells. However, in contrast to E-cadherin, expression of these receptors can neither restore the epithelial morphology nor prevent invasiveness. Thus, the reduction of metastatic potential by the expression of functional E-cadherin may be the sum of two factors: sticking cells together and influencing the differentiation status of tumour cells.

Regulation of cadherin function
Formation of a cadherin mediated adhesive contact can be achieved by three steps that have different requirements and use distinct receptor domains (for a recent review see Yap et al). (1) Cadherins dimerize at the cell surface, and the extracellular domain alone is sufficient to induce dimerisation in the absence of calcium ions (fig 1A). (2) Homophilic binding occurs as: the receptors interact with dimers on opposing cells in an antiparallel fashion. Formation of this cadherin adhesive unit requires the extracellular domain and calcium ions. (3) Adhesive receptors cluster laterally at sites of cell–cell adhesion (fig 1A), in a process in which interaction of the cadherin tail with intracellular proteins and the actin cytoskeleton are determinant factors. These three steps yield an increase in the number of binding sites and in the adhesive strength of the receptors for each unit area of the membrane. In addition, the interaction with the cytoskeleton keeps the clustered receptors together and provides a framework for the localisation of many different cytoskeletal and signalling proteins at intercellular junctions (see below; reviewed by Yamada and Geiger). Because of the important cellular functions in which cadherins participate, there is much interest in understanding how their function is regulated. The association of cadherins with actin filaments is mediated by proteins called catenins. Cadherin interacts directly with β-catenin, and α-catenin links this complex to actin filaments. The direct interaction of cadherin complexes with tyrosine kinases, receptor tyrosine phosphatases, and kinase substrates suggests that the phosphorylation of the complex may be modulated. However, the functional importance of phosphorylation for cadherin adhesion is not clear. Activation of many signalling pathways can perturb intercellular contacts, but neither their specificity, with respect to cadherin receptors, nor the mechanism involved have been established (reviewed by Yap et al). Recently, key regulators of cadherin mediated adhesiveness were identified as proteins of the small GTPase family, and their role is discussed below.

Small GTPases
The Ras superfamily of small GTPases contains proteins whose function is dependent on the type of guanine nucleotide bound. The Ras
proteins with the actin cytoskeleton, which is indirectly mediated by the actin binding adhesion requires calcium ions (to stabilise the extracellular domain) and association of the lateral clustering or zipping up of cadherin complexes at sites of cell–cell contact. Functional (homophilic binding). This interaction occurs in a antiparallel fashion and results in the catenins. The receptors interact with the same type of molecules in neighbouring cells exist as dimers at the cell surface, constitutively associated with cytoplasmic proteins called catenins. The phosphorylation of the cadherin tail and/or -catenin, vinculin, and -actinin. The phosphorylation of the cadherin tail and/or the catenins might be involved in the clustering process, interaction with the cytoskeleton, or the shutting of catenins from cytosolic pools to adhesive sites. The turnover of -catenin cytosplastic pools also involves phosphorylation events. (B) The GTPase cycle: most small GTPases are found associated with GDP in an inactivated state. Replacing GDP with GTP activates the small GTPase, and this is the form competent for intracellular signalling. The hydrolysis of GTP to GDP occurs very rapidly and switches the molecule off. These two steps are tightly controlled by regulatory proteins: activation is mediated by guanine nucleotide exchange factors (GEFs), whereas GTP hydrolysis is facilitated by GTPase activating proteins (GAPs).

subfamily members are involved in growth control and differentiation. The Rho subfamily (Rho, Rac, and Cdc42) participates in cellular events involved primarily in cytoskeletal reorganisation, but these proteins can also activate kinase cascades, induce gene transcription, and induce DNA synthesis (reviewed by Van Aelst and D’Souza-Schorey and Mackay and Hall). Inside the cell, members of the Ras family are normally found associated with GDP in an inactivated state (fig 1B). Activation is brought about by binding to GTP, a process that is tightly modulated by the GAP (GTPase activating protein) and GEF (guanine nucleotide exchange factor) regulatory proteins (fig 1B). The importance of appropriate control of the GTPase cycle is reflected by mutations that lock the molecule in an activated state. For example, activating mutations are frequently present in the Ras protein found in tumour cells (oncogenic form, H-Ras). Similar mutations in the Rho genes have not been found in tumours, even though activation of Rho proteins in tissue culture can induce transformation. However, deletions have been identified in exchange factors specific for the Rho subfamily members that result in the activation of small GTPases (such as the oncogenes Lbc, Vav, and Dbl) (reviewed by Cerione and Zheng).

The Rho subfamily
One of the first clues that the small GTPases might be involved in cell–cell adhesion came from work on drosophila. In mammalian epithelial cells, the activity of endogenous Rho and Rac is required for the formation of cadherin dependent contacts, as well as for the cytoskeletal reorganisation that stabilises cadherin receptors in the plasma membrane (fig 2). Inhibition of the small GTPases specifically removes cadherins from stable contacts, and this temporally precedes the release of other molecules involved in cell–cell adhesion. Moreover, the regulation of cadherin function by Rho or Rac depends on the maturation status of the junctions and the cellular context (table 1).

In Madin–Darby bovine kidney (MDCK) cells, exogenously expressed Rac is found at cell–cell contacts, but both the activated and inactivated forms show the same staining pattern.

The functional importance of this is not clear but, in MDCK cells, proteins that can either activate (Tiam-1) or inactivate (IQGAP) the small GTPase Rac also localise to cell–cell contact sites. Interestingly, IQGAP can bind directly to E-cadherin– -catenin complexes, in an apparent competition with -catenin. The physiological importance of this association is not clear, but IQGAP can also bind and crosslink actin filaments and so could potentially replace -catenin in the interaction of cadherin complexes with the cytoskeleton. Although expression of the IQGAP gene does not remove E-cadherin from cell–cell contacts when cotransfected into fibroblasts, it is thought that the IQGAP–cadherin interaction renders the receptors less adhesive. The latter is possibly the result of a weaker association of the complex with the actin cytoskeleton and/or an inactivation of the small GTPases at junctional structures.
It appears that Rho and Rac are required in distinct pathways in the regulation of cell–cell adhesiveness, as opposed to spreading on the substratum, in which a hierarchy among the small GTPases has been demonstrated. In epithelial cells, actin recruitment to clustered cadherin receptors is dependent on the activity of Rac, but not of Rho. It is conceivable that Rac can modulate cadherin function by regulating the association of the complexes with actin filaments, and this is in line with the reported role of Rac in actin polymerisation. However, Rac function is necessary, but not sufficient, to promote accumulation of actin at the cell periphery because the presence of functional cadherin mediated adhesion is also required.

On the other hand, transfection of activated Rac into MDCK cells results in an enhanced immunostaining of cadherin receptors and actin at sites of intercellular contacts, but its importance has not been established. Although a strengthening of cadherin mediated adhesion by activated Rac is suggested by these results, it is not clear whether the augmented cadherin staining signal reflects an increase in the density of receptors at cell–cell contact sites.

The Ras subfamily
Another member of the superfamily, H-Ras, also interferes with cadherin adhesiveness (fig 2). Activating mutations in H-Ras are found frequently in human tumours, and are accompanied by loss of epithelial characteristics and increased migration. Oncogenic H-Ras can activate different intracellular pathways that contribute to Ras transformation: phosphatidylinositol 3 (PI3) kinase, mitogen activated protein kinase (MAPK), and the small GTPases Rac and Ral (reviewed by Van Aelst and D’Souza-Schorey). In addition, activation of each of these pathways individually is sufficient to promote morphological transformation in fibroblasts.

Microinjection of activated H-Ras into MDCK cells promotes the disassembly of cadherin receptors from junctions (fig 2). In epithelial cells, oncogenic Ras transfection leads to changes in both catenin phosphorylation and the association of the cadherin complex with the actin cytoskeleton. In some cases, these changes do not necessarily result in the abrogation of cell–cell adhesion and epithelial morphology, but rather a weakening of intercellular contacts. It is possible that different levels of expression of ras and the balance between the different activated pathways in distinct cell types can account for these discrepancies (reviewed by Marshall).

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<tr>
<th>Receptor</th>
<th>Cell type</th>
<th>Regulation by</th>
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<tr>
<td>E-cadherin</td>
<td>Keratinocytes</td>
<td>++</td>
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<tr>
<td></td>
<td>MCDK (kidney epith.)</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>MCF10 (breast epith.)</td>
<td>++</td>
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<tr>
<td></td>
<td>L-cells (fibroblasts)</td>
<td>−</td>
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<tr>
<td></td>
<td>Small lung cells</td>
<td>ND</td>
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<tr>
<td>β-cadherin</td>
<td>Keratinocytes</td>
<td>++</td>
</tr>
<tr>
<td>VE-cadherin</td>
<td>Endothelial cells</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>CHO cells</td>
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Cdc42 activity has no effect on E-cadherin adhesiveness. In small lung carcinoma cells, Rho inactivation leads to an enhanced aggregation of cells in suspension, as opposed to in the other adherent cell types, where Rho inactivation inhibits E-cadherin mediated adhesion.

CHO, Chinese hamster ovary cells; ND, not determined.
Small GTPases are ideal candidates to participate in complex biological processes involving cell–cell and cell–matrix adhesion during epithelial morphogenesis, wound healing, and metastasis. Identification of the putative targets of the small GTPases that can modulate cadherin function will greatly enhance our understanding of the molecular mechanisms that operate in these important cellular processes.

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