

# Talk to the Hand 'Cause the Nucleus Ain't Listening: Adhesion, Communication, and Tumor Cell Invasion in the Skin<sup>1</sup>

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Being a multicellular organism confers many advantages; however, size has its costs. Higher orders of regulation are required in order to coordinate the biology of so many disparate cells. The coordination of cell–cell interactions is most evident during development, where defects result in readily observable phenotypes. One of the characteristics that typifies a cancerous cell is the loss of these positional clues that often depend upon information relayed through cell adhesion complexes, i.e., the loss of contact inhibition. A variety of cell–cell and cell–substrate adhesion complexes have been described in mammalian tissues that are often aberrantly regulated in a malignant cell.

Along with desmosomes, adherens junctions are one of the major keratinocyte cell–cell adhesion complexes. They are composed of transmembrane homophilic binding proteins belonging to the cadherin family of calcium dependent cell adhesion proteins. The extracellular portion of the cadherin polypeptide binds to a second cadherin protein on the adjacent cell. The cytoplasmic cadherin tail binds to either  $\beta$ -catenin or plakoglobin, which in turn bind to  $\alpha$ -catenin.  $\alpha$ -catenin interacts with actin filaments, thereby linking the actin cytoskeleton to the transmembrane adherens junction complex. As such, a system is formed by this network of protein–protein interactions that by creating a continuous linkage between the cytoskeletons of adjacent cells provides adhesion and mechanical stability to the skin. Other adherens junction associated proteins have been described including the catenin p120 and IQGAP1.

When does an atypical cell become a cancer, that is, an invasive tumor? What changes are necessary for a dysplastic cell to invade? Although that cell may have expanded proliferative potential, is it a cancer if it is not invasive? Down-regulated or aberrant cadherin function has been associated with malignant cells. Initial investigations in the 1990s demonstrated that E-cadherin was exhibiting the biological properties of a tumor suppressor, namely, decreased or absent E-cadherin expression was associated with tumorigenesis (Schipper *et al*, 1991; Shiozaki *et al*, 1991; Oka *et al*, 1993); whereas, transfection of WT E-cadherin into carcinoma cell lines was associated with a reversion of their phenotype (Chen and Obrink, 1991; Frixen *et al*, 1991; Vleminckx and Vakaet, 1991).

In this issue of the Journal, Margulis *et al* describe the use of skin organotypic culture system (Andriani *et al*, 2003) to demonstrate that the loss of E-cadherin function is associated with a disruption of cell–cell adhesion and invasion of epithelial cells into the connective tissue stroma of the dermis. Because E-cadherin interacts with a number of different proteins, one mechanism for disrupting its function would be to saturate its binding part-

ners with a nonfunctional decoy protein. Margulis and colleagues have used this strategy to abolish E-cadherin function by transfecting keratinocytes with a gene encoding nonfunctional E-cadherin, a construct referred to as a dominant negative gene. Despite having normal levels of adherens junction proteins, cells transfected with the dominant negative E-cadherin construct were less adherent and more invasive. They used an immortalized cell line that does not display invasive behavior *in vivo* and generated an *in vitro* model of human skin including a stratified epidermis, basement membrane, and dermal matrix. When E-cadherin function was abolished by turning on expression of the dominant negative E-cadherin construct, the epidermal cells acquired an invasive phenotype; the basement membrane underwent proteolytic degradation and the transformed cells migrated from the epidermis to the dermis. This invasive behavior may be a consequence of loss of cell–cell adhesion itself, or likely, as a consequence of the subsequent aberrations in cadherin-mediated signaling resulting from the disruption of E-cadherin function.

Adherens junction signaling occurs by several mechanisms, including (i) the positioning of traditional membrane receptors resulting from the formation of stable cell–cell contacts, (ii) the clustering of cell surface and transmembrane proteins, and (iii) changes in the association of cadherins with intracellular partners as a consequence of alterations in cadherin–cadherin homotypic interactions. Primary signaling events occur as a consequence of ligand binding to E-cadherin, for example, the activation of Rac1 (Noren *et al*, 2001; Kovacs *et al*, 2002; Noren *et al*, 2003). Adherens junction formation, via binding of E-cadherin on apposing cells, results in binding and activation of PI3 kinase, which in turn activates the GTPase Rac. The recruitment of PI3 Kinase to Rho family GTPases has been implicated as a downstream component of cadherin mediated signaling. Additional secondary events, e.g., activation of CDC42, occur as a consequence of the formation of cell–cell adhesion contacts. An additional example of secondary signaling is the activation of receptor tyrosine kinases that results from E-cadherin mediated cell–cell adhesion. Formation of adherens junctions results in the association and ligand independent activation of the epidermal growth factor receptor, with subsequent activation of MAPK signaling cascades (Pece and Gutkind, 2000).

In tissue culture, the switch from nonconfluence to confluence is accompanied by reorganization of the actin cytoskeleton, formation of adherens junctions and engagement of E-cadherin by its homolog on apposing cells as cell–cell contacts are formed within the confluent epithelial sheet/monolayer. Reorganization of the actin cytoskeleton is believed to be one consequence of cadherin-mediated signaling that results from cadherin engagement and adherens junction formation. These morphologic and biologic changes are typical of the phenomenon of contact inhibition that characterizes nontransformed cells and can be thought

<sup>1</sup>“Talk to the Hand”: A saying used to ignore and disregard a comment or an insult when you can't think of a way to counter it. When this phrase is used, it is customary to raise hand, palm facing out. <http://www.urbandictionary.com>, accessed October 13, 2003.

of as the biologic opposite of a cell that is metastasizing, e.g. escaping the confines of its neighbors to travel to distant and different tissues.

Recent work from our lab has identified another signaling pathway in normal human keratinocytes that occurs when adherens junction mediated cell-cell adhesion is disrupted (Hu *et al*, 2001; Hu *et al*, 2003). One consequence of this event is the translocation of plakoglobin from the membrane to the nucleus where it competes with  $\beta$ -catenin for binding to TCF/LEF family member transcription factors. This functional antagonism of  $\beta$ -catenin mediated signaling is thought to down-regulate Wnt mediated proliferative signals and thus act as a control to prevent unchecked cell proliferation in the absence of cell-cell adhesion. Consistent with the idea that signaling is important for E-cadherin to function as a tumor suppressor, Wong and Gumbiner have used mammary and prostate cancer cell lines to demonstrate that E-cadherin's tumor suppressor activity is not dependent upon its cell adhesion function. They demonstrated that the  $\beta$ -catenin binding domain of E-cadherin is essential for its tumor suppressor activity suggesting that signaling, rather than the mechanical property of cell-cell adhesion, is important for the tumor suppressor activity (Wong and Gumbiner, 2003).

The organotypic model described by Margulis provides an experimental system that reproduces the tissue properties of skin, namely a three dimensional structure of a differentiated epidermis, basement membrane, and dermis, that can readily and reproducibly be manipulated. This system provides a unique opportunity to study in detail the mechanism of epithelial tumor invasion.

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