

## **The Role of E-Cadherin and PIX in Contact Inhibition of Proliferation in Breast Cancer Cells**

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Contact inhibition is a poorly understood process that blocks both cell motility and proliferation upon formation of cell-cell contacts. Pak1 and betaPIX are two interacting effector molecules of the Rho-GTPase, Rac. In subconfluent cells Pak:PIX complexes localize to focal contacts, and regulate cell motility. We previously found that contact inhibition of normal differentiated epithelial cells depends on translocation of Pak:PIX-containing signaling complexes to cell-cell contacts.

In epithelial cells, E-cadherin mediates the establishments of cell-cell contacts, which is likely to be the first signal in the establishment of contact inhibition of the cells. Many tumors have lost E-cadherin, which is thought to stimulate both the proliferation and motility of tumor cells. We have shown that normal epithelial cells that lack functional E-cadherin do not recruit PIX to lateral membranes and, concomitantly, do not establish contact inhibition of proliferation. These findings led us to hypothesize that contact inhibition of proliferation in epithelial cells depends on an E-cadherin-dependent recruitment of PIX-containing protein complexes to the sites of cell-cell contacts.

The human breast cancer cell line, MDA-MB-435, is metastatic and has lost expression of E-cadherin. Here we have determined whether the re-introduction of E-cadherin into MDA-MB-435 cells is sufficient to recruit PIX complexes laterally. In addition, we have correlated these results to the establishment of contact inhibition of proliferation. In wild-type MDA-MB-435 cells, PIX was not observed at the lateral membranes. When E-cadherin was re-expressed at levels similar to those seen in normal mammary cells, only negligible PIX staining was observed laterally. Furthermore, E-cadherin re-expression did not lead to changes in the rate of proliferation of the MDA-MB-435 cells. In addition, we found that hScribble, a tumor suppressor molecule which interacts with PIX and has been implicated in lateral PIX recruitment, was found laterally in MDA-MB-435 cells with and without E-cadherin expression. This suggests that the presence of hScribble is not sufficient to recruit PIX laterally in these cells. Taken together our data show that E-cadherin expression is not sufficient to recruit PIX to lateral membranes of MDA-MB-435 or to confer contact inhibition of proliferation in these cells.