Effects of c-erbB2 Signaling on Morphogenesis and Cell Adhesion in Mammary Epithelial Cells

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Overexpression of the growth factor receptor subunit c-erbB2, leading to ligand independent homodimerization and activation, is frequently found in human breast cancer. In this study we have examined the mechanisms behind cellular regulation of cell adhesion and morphogenesis induced by c-erbB2 homodimer signaling. In order to follow the events leading to c-erbB2 induced changes in mammary epithelial cells, we have used an inducible hybrid receptor in which the extracellular domain of the trkA nerve factor (NGF) receptor is fused to the transmembrane and cytoplasmic domains of c-erbB2. In an immortalized mammary epithelial cell line (HB2) transfected with this construct, homodimerization of the c-erbB2 cytoplasmic domain can be induced by exposing the cells to NGF. We have investigated the cellular pathways whereby c-erbB2 influences integrin function in the initial steps of c-erbB2 homodimer signaling, by analyzing the capacity of cells to adhere and spread on collagen. Pharmacological inhibitors and transient transfections were used to identify pathways required for suppression of integrin function by c-erbB2. The mechanism of integrin inactivation was elucidated by using conformational sensitive antibodies and analysis of F-actin rearrangements. We showed that c-erbB2 signaling disrupts adhesion and spreading of mammary epithelial cells by inhibiting the adhesive function of the collagen receptor integrin $\alpha_2\beta_1$. This anti-adhesive effect was mediated by parallel activation of the MAP ERK kinase 1/2 (MEK 1/2), protein kinase B (PKB) and Rho pathways mediating suppression of adhesion by causing the extracellular domain of integrin β_1 to adopt an inactive conformation. Among these pathways, only the contribution by the PKB branch was mediated by cytoskeletal rearrangements. Also, integrin linked kinase (ILK) and a rapamycin sensitive pathway were shown to mediate c-erbB2 induced inhibition of integrins without changing the integrin conformation. Long term signaling from c-erbB2 leads to irreversible phenotypical changes like epithelial mesenchymal transition (EMT) and anchorage independent growth. We showed that cell-cell contact strongly inhibited c-erbB2-induced EMT, which appears to proceed through three stages: 1) A reversible "priming" phase, during which cell-cell contact and expression of epithelial markers is unaltered. 2) Cell-cell separation without E-cadherin downregulation. 3) Irreversible loss of epithelial phenotype and rapid switch to a mesenchymal expression pattern. The fibroblastic population of cells obtained after c-erbB2 induced EMT had acquired the ability to grow in an anchorage-independent manner. In order to study the anchorage independence, cells were grown in soft agar as well as in collagen and treated with integrin blocking antibodies. Intriguingly, the anchorage independent cells seemed to be dependent on integrins in collagen, which was did not seem to be due to ECM-dependence in agar. Instead, they may be subject to a growth inhibitory effect from the collagen in the absence of integrin signaling. In summary, this thesis reveals novel insights into the mechanisms of integrin inside-out signaling and c-erbB2 induced phenotypical conversion, phenomena of high potential significance for cancer progression.

Key words: c-erbB2, integrin $\alpha_2\beta_1$, integrin regulation, ILK, PKB, Rho, epithelial-mesenchymal transition (EMT), E-cadherin, Anchorage independent growth (AIG)

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