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PKB mediates c-erbB2-induced epithelial β_1 integrin conformational inactivation through Rho-independent F-actin rearrangements

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Abstract

Signalling from the growth factor receptor subunit and proto-oncogene c-erbB2 has been shown to inhibit the adhesive function of the collagen receptor integrin $\alpha_2\beta_1$ in human mammary epithelial cells. This anti-adhesive effect is mediated by the MAP ERK kinase 1/2 (MEK1/2) and protein kinase B (PKB) pathways. Here, we show that both pathways mediate suppression of matrix adhesion by causing the extracellular domain of the β_1 integrin subunit to adopt an inactive conformation. The conformational switch was also dependent on rapid and extensive actin depolymerisation. While neither activation nor inhibition of the Rho GTPase affected this rearrangement, Rho was found to be activated by c-erbB2 and to be necessary for conformation-dependent integrin inactivation and, apparently by a different mechanism, a delayed re-formation of stress fibers which did not restore integrin function. Interestingly, the initial actin depolymerisation as well as its effects on integrin function was shown to be mediated by PKB. These results demonstrate how oncogenic growth factor signalling inhibits matrix adhesion by multiple pathways converging on integrin conformation and how Rho signalling can profoundly influence integrin activation in a cytoskeleton-independent manner.

Keywords: Integrin; Epithelial cells; erbB2; Protein kinase; Cytoskeleton

Introduction

It has long been recognised that integrins, the cell's major tools for adhering to and sensing the extracellular matrix, are subjected to a complex and refined regulation of their matrix-binding capacity, so-called inside-out signalling [1,2]. This regulation is thought to occur at several levels, including changes in integrin conformation, cytoskeletal rearrangements and changes in the cell surface distribution of integrins. Recently, striking advances have been made in the elucidation of the structural basis of integrin affinity regulation, demonstrating that transitions between active and inactive states of the integrin heterodimer involve dramatic conformational rearrangements of its highly flexible extracellular domains [3,4].

Less well defined are the intracellular events that trigger the changes in integrin activity. Although the significance of certain integrin-binding proteins (especially talin, [5]) and of components of the intracellular signalling machinery [6] have been firmly established, a coherent picture of the pathways employed by a particular physiological or pathophysiological stimulus in regulating integrin function is in most cases lacking. This is especially true of integrin regulation in epithelial cells, since hematopoietic and, to

Abbreviations: ca, constitutively active; dn, dominant-negative; ERK, extracellular signal-regulated kinase; GFP, green fluorescent protein; GST, glutathione–*S*-transferase; ILK, integrin-linked kinase; LPA, lysophosphatidic acid; LTB, latrunculin B; mDia, mammalian homologue of Diaphanous; MEK, MAP ERK kinase; mTOR, mammalian target of rapamycin; NGF, nerve growth factor; PBS, phosphate-buffered saline; PI3K, phosphoinositide-3-kinase; PKB, protein kinase B; RBD, Rho-binding domain of rhotekin; ROK, Rho-dependent kinase; S6K, S6 kinase; wt, wild-type.

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some extent, fibroblastic cells have been the preferred systems in most studies. Furthermore, the mechanisms of regulation of the β_1 family, the largest group of integrins, are considerably less well known as those governing β_2 or β_3 integrins.

In this and previous reports, we have investigated how β_1 integrin-mediated events in human mammary epithelial cells are regulated by signalling from c-erbB2, a growth factor receptor subunit closely related to the epidermal growth factor (EGF) receptor. c-erbB2 has also been identified as a proto-oncogene, the overexpression of which is strongly associated with poor prognosis in breast carcinoma. Indeed, Herceptin (Trastuzumab), a humanised monoclonal antibody against the extracellular domain of c-erbB2, has become an accepted therapeutic agent in the clinical treatment of c-erbB2-positive mammary tumours [7], although far from all patients with such tumours who respond favourably to Herceptin treatment. The oncogenic effects of c-erbB2 overexpression are generally believed to be mediated by ligand-independent c-erbB2 homodimerisation. We have established a system for studying the effects of c-erbB2 homodimer signalling, consisting of an immortalised human mammary epithelial cell line (HB2, [8]) into which a hybrid "trk-neu" receptor has been introduced [9]. trk-neu was generated by fusion of the extracellular domain of the trkA nerve growth factor (NGF) receptor to the transmembrane and cytoplasmic domains of c-erbB2 [10]. Addition of NGF to cells expressing this construct causes homodimerisation and activation of the intracellular c-erbB2 tyrosine kinase domain, thus mimicking the signalling caused by a c-erbB2 homodimer. In our previous studies, [9,11], we have described how c-erbB2 signalling causes pronounced disruption of morphogenesis in collagen and reduced adhesion and spreading on collagen surfaces, phenomena that depend on the functional inactivation of the collagen-binding integrin $\alpha_2\beta_1$. We have also shown that this c-erbB2-induced integrin inactivation is mediated by several intracellular pathways, including the MAP ERK kinase-extracellular signal-regulated kinase (MEK-ERK) and phosphoinositide-3-kinase-protein kinase B (PI3K-PKB) pathways, acting in a parallel fashion [11]. Here, we show that both these pathways as well as a separate pathway dependent on the Rho GTPase and its effector, Rho-dependent kinase (ROK), all mediate integrin suppression downstream of c-erbB2 by causing the extracellular domains of β_1 integrins to adopt an inactive conformation. We also show that c-erbB2 induces a striking depolymerisation of the actin cytoskeleton and that this rearrangement is required for the c-erbB2-induced integrin conformational switch and suppression of adhesion. Surprisingly, the actin-destabilising effects of c-erbB2 signalling were not affected by interfering with the function of Rho or ROK; rather, PKB was shown to be the main mediator of the cytoskeleton-dependent integrin inactivation induced by c-erbB2.

Materials and methods

Reagents, antibodies and oligonucleotides

Solubilised bovine collagen I (Vitrogen 100) was purchased from Nutacon BV, Netherlands. 2.5 S nerve growth factor (NGF) from mouse submaxillary gland was obtained from Promega. Latrunculin B, dimethyl sulfoxide, the anti-actin monoclonal antibody (mAb) 20-33 and *o*-nitrophenyl-β-D-galactopyranoside (ONPG) were from Sigma. PD98059, wortmannin and Y27632 were purchased from Calbiochem. Polyethyleneimine (25 kDa) was from Aldrich. The three conformation state-sensitive integrin β_1 mAbs were obtained as follows: 9EG7 (rat IgG2a) was purchased from BD; B44 (mouse IgG1) and HUTS-4 (mouse IgG2b) were from Chemicon. The integrin β_1-recative mAbs TS2/16 (ATCC# HB-243) and P5D2 (Developmental Studies Hybridoma Bank, University of Iowa) were obtained from hybridoma culture supernatant. The mAb 26C4 against RhoA and antisera against total and Ser-3-phosphorylated cofilin were from Santa Cruz Biotechnology. Antibodies to MEK1/2 and total and Ser-473-phosphorylated PKB were from Cell Signaling Technology. The ILK mAb (clone 3) was from BD. Antibody for detection of the V5 tag was from Invitrogen. Glutathione–Sepharose and reagents for enhanced chemiluminescence (ECL) were purchased from Amersham Biosciences. Alexa Fluor 488-labeled goat anti-mouse antiserum, Alexa Fluor 546-labeled phalloidin and ZenonOne reagents for direct fluorescent labeling of mouse IgG1 were obtained from Molecular Probes. Jasplakinolide (Molecular Probes) was a kind gift from Dr. Margareta Wallin, Department of Zoology, University of Göteborg. Double-stranded oligoribonucleotides for RNAi of PKB and ILK were purchased from Ambion (siRNA id# 42811 and 1461, respectively). Control siRNAs and the Oligofectamine siRNA transfection reagent were purchased from QIAGEN.

cDNA constructs

cDNA coding for the constitutively activated V12Rac and V14Rho as well as dominant-negative N17Rac and N19Rho (all myc-tagged), originally from Dr. Marc Symons, Picower Institute for Medical Research, Manhasset, NY, were provided by Dr. Henrik Semb, University of Lund, Sweden. These cDNAs were excised from their original vectors with *Eco*RI and ligated into pcDNA3.1-based vectors. pCMV5.SNE/PKBα and pCMV5.SNE/PKBα(K179A) used for expression of HA-tagged wild-type and dominant-negative PKBα, respectively, were from Dr. Brian A Hemmings, Friedrich Miescher Institut, Zürich, Switzerland. Two plasmids containing dominant-negative alleles of the integrin-linked kinase (ILK) were used: pcDNA3.1V5-His/ILK-kd coding for the E359K mutant, provided by Dr. Shoukat Dedhar, University of British