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The effect of BRK on EGF signalling via *erb*B3 in mammary epithelial cells

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Keywords

Apoptosis, BRK, EGF, epithelial cells, *erb*B3, mammary, PI 3-kinase

Introduction

BRK is an intracellular protein-tyrosine kinase that is expressed in 60% of human breast cancers but is undetectable in normal and benign breast tissues. The introduction of the BRK gene into the normal breast epithelial cell lines HB4a and MCF10-A leads to a potentiation of their growth response to the mitogen EGF and their ability to proliferate in an anchorage-independent manner. BRK and the EGF receptor (EGFR) have also been shown to form a constitutive association in mammary epithelial cells.

Aims

To investigate how BRK influences EGF downstream signalling in human mammary epithelial cells.

Comments

The ability of breast receptor kinase (BRK) to deregulate cellular proliferation together with its expression in 60% of breast cancers and the documented importance of protein-tyrosine kinases in tumorigenesis makes this an important study. BRK is shown to provide a link between epidermal growth factor (EGF) binding to its receptor and the phosphorylation of *erb*B3 which leads to an increase in cell number, either by increased proliferation or decreased apoptosis. As *erb*B2 is thought to mediate in the activation of *erb*B3 and, as both of these receptors have been shown to be overexpressed in a proportion of breast cancers, further studies will be needed to determine the role of *erb*B2 in this BRK/*erb*B3 interaction. Also, does neu differentiation factor/heregulin, which is known to bind directly to erbB3 leading to both the recruitment of phosphoinositide (PI) 3-kinase's p85 subunit and increased mitogenesis, also interact with BRK?

Methods

HB4a cells were transiently transfected with empty or *brk* expression vectors. Following overnight quiescence in 0.5% FCS, cells were treated with EGF (100 ng/ml) for 5 minutes. BRK or *erb*B3 was then immunoprecipitated from lysates of stimulated or mock-stimulated cells, resolved by SDS-PAGE, Western blotted and probed with antibodies to BRK, phosphotyrosine, *erb*B3, the p85 subunit of PI 3-kinase (PI 3-kinase p85) or Akt/protein kinase B (Akt). PI 3-kinase activity was assayed in *erb*B3 precipitates. BRK and *erb*B3 were also co-expressed in human embryonic kidney (HEK293) cells.

Results

Mock stimulated BRK transfected cells showed an increase in phosphorylation of *erb*B3 above control cells together with the recruitment of the PI 3-kinase p85. Treatment of BRK-transfected HB4a cells with EGF led to enhanced tyrosine phosphorylation of both BRK and *erb*B3 and an increased association of PI 3-kinase p85. In HEK293 kidney cells overexpressing both *erb*B3 and BRK, immunoprecipitation of *erb*B3 demonstrated not only enhanced phosphorylation but also the formation of *erb*B3/BRK complexes. Mutant BRK, lacking kinase activity, failed to induce *erb*B3 phosphorylation but still co-precipitated with it. PI 3-kinase assays showed that there was an increase in enzyme activity associated with *erb*B3 in response to EGF in HB4a control cells. This increased twofold in BRK-expressing cells. A potential downstream target of PI 3-kinase, Akt, was also found to exhibit a low level of phosphorylation at serine 473 following EGF treatment of BRK-expressing cells. This is one of two sites whose phosphorylation is required for full activation of Akt.

Discussion

This study shows that the expression of BRK in human mammary epithelial cells significantly enhanced the degree to which *erb*B3 became phosphorylated in response to EGF. Results using the expression of a kinase inactive BRK mutant suggests that BRK may directly phosphorylate *erb*B3, possibly by physical interaction. The recruitment of the p85 subunit of PI 3-kinase, its increased activity and the phosphorylation of Akt are further evidence of a role for BRK in EGF downstream signalling. Thus the expression of BRK in human breast cancer cells could lead to a greater efficiency of mitogens such as EGF.

Additional information

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