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c-*erb*B2 homodimerisation in mammary epithelial cells

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Keywords

Apoptosis, c-*erb*B2, epithelial-mesenchymal, integrin, mammary, scattering

Introduction

C-*erb*B2 is a tyrosine kinase receptor of the epidermal growth factor (EGF) receptor family. It has been found to be overexpressed in a proportion of breast cancers which have a poorer prognosis. Constitutive expression of c-*erb*B2 in mammary epithelial cells can confer a growth advantage or impairment depending on the level of its expression. At its highest levels it has also been associated with scattered colony formation, epithelial-mesenchymal conversion, anchorage-independent growth and a downregulation in the expression of α2 integrin and E-cadherin.

Aims

To examine the effects of the activation of the c-*erb*B2 receptor expressed at different levels in normal mammary epithelial cells transfected with the hybrid trk-neu receptor.

Comments

The use of a ligand-dependent system to activate the c-*erb*B2 receptor and analyse its effects on cell morphology, proliferation and behaviour is a good idea. As the authors say, when you constitutively overexpress an oncogene in transfected cells the signals generated may have widely different responses depending on the level of stimulation in a particular cell type. Consequently the experiments carried out in this paper go some way in explaining the variety of effects seen in the literature of c-*erb*B2 overexpression. However, it would have been appropriate for the authors to check the native level of nerve growth factor (NGF) receptor in the cell types that they used. Also, the appearance of fibroblastic cells in long-term culture could be indicative of a heterogeneous population with mesenchymal cells possessing their own NGF receptors.

Methods

The non-malignant mammary epithelial cell line MTSV1-7 and its subclone HB2 were transfected with trk-neu receptor consisting of the extracellular domain of the trkA nerve growth factor (NGF) receptor and the transmembrane and cytoplasmic domains of c-*erb*B2 (neu). In cells expressing this construct c-*erb*B2 homodimerisation could be achieved by the addition of NGF. Proliferation, soft agar and apoptosis assays were carried out on transfected cells. Immunofluorescent staining of vimentin and cytokertain 18, as well as western blotting for α2 integrin and E-cadherin, was also carried out.

Results

The trk-neu receptor was expressed at widely different levels in the MTSV1-7 and HB2 transfected cells. NGF treatment of transfected clones also led to a corresponding range of enhanced tyrosine phosphorylation of a band coinciding in size with the hybrid receptor. After 1 week of NGF treatment HB2 transfectants exhibiting a modest expression of the trk-neu receptor showed an increase in proliferation in collagen. However, cells expressing higher levels of the trk-neu receptor exhibited cell scattering, reduced viability and increased apoptosis following NGF treatment. At this stage, western blotting revealed no change in the levels of α2 integrin and E-cadherin although prior treatment with antibodies that activate the adhesive capacity of α2β1 integrin completely reversed the cell dissociation and apoptotic effects. Long-term NGF treatment (10 passages) of high-expressing transfectants led to the emergence of fibroblastic cells amongst the epithelial cells. When cloned, these cells were shown to have very high levels or the trk-neu receptor with increased tyrosine phosphorylation, together with significantly reduced levels of α2 integrin and E-cadherin. Only transfectants with the highest levels or trk-neu expression displayed anchorage-independent growth in soft agar.

Discussion

These results show that the outcome of c-*erb*B2 signalling is dependent on both the level of the receptor present and thus the intensity of the signal as well as the length of time of activation. In the short-term, low levels of expression led to increased cell proliferation and higher levels of expression to cell scattering and apoptosis. That this effect was not accompanied by reduced integrin expression but was reversed by antibody treatment suggests that it may be the conformation of α2β1 integrin and thus its interaction with the collagen matrix that is affected by signalling through the c-erbB2 receptor. Prolonged NGF treatment of high-expressing trk-neu transfectants was required before epithelia-mesenchymal conversion was seen, with anchorage-independent growth only being observed in the highest expressing clones suggesting that there are critical thresholds of signalling to be reached before these events can take place.

References

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