

Commentary

Essential function for ErbB3 in breast cancer proliferation

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Abstract

The overexpression of the ErbB family of tyrosine kinase receptors is thought to be important in the development of many breast tumours. To date, most attention has focused on the ErbB2 receptor. Now, in a recent report, it has been shown that ErbB3 is a critical partner for the transforming activity of ErbB2 in breast cancer cells. Importantly, the proliferative signals from this transforming complex appear to act via the PI-3 kinase pathway.

Keywords: breast, ErbB2, ErbB3, PI-3K, PKB

A hallmark of cancer cells is that they are able to proliferate in the absence of normal proliferative signals. This self-sufficiency in growth signals is often achieved through genetic changes that result in the overexpression of growth factors or their receptors. In breast epithelial cells, normal proliferation is regulated by both steroid hormone receptors and the ErbB family of peptide growth factor receptors (also called Type I receptor tyrosine kinases or RTKs). Overexpression of these receptors is frequently found in breast cancer, and identification of the molecular pathways involved has permitted the development of new breast cancer therapies.

Most anti-breast cancer drugs under investigation or in current use act to inactivate the oestrogen receptor or the ErbB receptors, ErbB2 and ErbB1. In particular, much research has focused on the ErbB2 oncogene, because this gene is frequently overexpressed in breast tumours and is associated with a poorer prognosis. Now, however, in a recent report, Holbro and colleagues [1] highlight the oncogenic potential of another ErbB receptor, the ErbB3 receptor, by showing that ErbB3 functions as an essential partner of ErbB2 to drive the proliferation of ErbB2-overexpressing breast tumour cells.

The ErbB family of receptors form homodimers and heterodimers on ligand binding to the extracellular domain.

The receptor dimer is activated through intrinsic tyrosine kinase-mediated autophosphorylation of the receptors in the cytoplasmic domain. These phosphorylations result in the recruitment of downstream effector proteins that activate multiple signalling pathways including the mitogen-activated protein (MAP) kinase and phosphoinositide 3-kinase (PI-3K) pathways [2–4]. ErbB3 is distinct in that it has an impaired protein tyrosine kinase activity and activation of this receptor is achieved only through the formation of heterodimeric receptor complexes with other kinase-active family members [5–7]. In this way, the ErbB2–ErbB3 complex can act as a high-affinity heregulin co-receptor [8]. The ErbB3 receptor is also unique among the ErbB family in its ability to directly recruit and activate PI-3K [5,9].

Elevated levels of tyrosine-phosphorylated ErbB3 are frequently found in breast tumours that overexpress ErbB2 [10,11]. In addition, *in vitro* assays in NIH 3T3 cells and a transgenic mouse model of breast cancer development have shown that ErbB2 cooperates with ErbB3 to transform cells [12,13]. This has led to speculation that ErbB3 might be an important partner to ErbB2 in the development of breast tumours overexpressing ErbB2. To address this issue definitively, Holbro and colleagues used elegant techniques to disrupt ErbB3 and ErbB2 function selectively in breast cancer cells. They blocked ErbB2

activity by using either the ErbB2-selective kinase inhibitor PKI166 [14] or by functionally inactivating the receptor through expression of the single-chain antibody scFv-5R, which targets ErbB2 to the endoplasmic reticulum [15]. To inhibit ErbB3 expression, they infected the cells retrovirally with an artificial transcription factor, E3, composed of a polydactyl zinc-finger domain, which specifically recognizes the 5' untranslated region of c-erbB3 and downregulates its expression [16].

With these techniques they demonstrated that ErbB2 inactivation blocked proliferation in ErbB2-overexpressing cells and that this was associated with a decrease in the phosphotyrosine content of ErbB3, confirming that ErbB2 activity was required to activate ErbB3. Inactivation of ErbB2 was also associated with a decrease in the level of ErbB3-PI-3K complex and the activity of the PI-3K target, protein kinase B (PKB), demonstrating that activation of the PI-3K pathway was downstream of this receptor. Importantly, they found that specific downregulation of the ErbB3 receptor, using the E3 transcription factor, had similar antiproliferative effects on the cell lines overexpressing ErbB2. The specificity of this effect was confirmed by re-expressing ectopic ErbB3 under the control of a promoter that was insensitive to the E3 transcription factor. Interestingly, some of the cell lines tested also express ErbB1 and ErbB4, suggesting that neither of these receptors is able to substitute for ErbB3. This idea was confirmed by the observation that re-expression of ErbB4 was unable to rescue loss of ErbB3. Together, these results clearly show that ErbB3 is required for ErbB2 to be transforming in these cells.

Looking at downstream signalling pathways, the authors observed that, although loss of ErbB3 had no effect on the levels of signalling through the MAP kinase pathway, signalling through the PI-3K pathway was blocked in one cell line. This result suggested that the proliferative signal from this receptor complex was transmitted via this pathway. This finding was corroborated by the observation that constitutive activation of this pathway by the expression of an active form of PKB was sufficient to overcome the proliferative arrest seen in response to the loss of either receptor. Thus, the ErbB2-ErbB3 complex stimulates an oncogenic, proliferative signal via the PI-3K pathway.

The clear demonstration of the requirement of ErbB3 as a partner for ErbB2 in breast cell transformation and the observation that the proliferative drive requires signalling through the PI-3K pathway are in line with previous findings. Using a dominant-negative version of ErbB3, Ram and colleagues reported similar findings that ErbB3 function seemed to be required for the growth factor and anchorage independence of an ErbB2-overexpressing breast cancer cell line [17]. In addition, mutagenesis of the ErbB3 receptor to assess its role in the transformation

ability of the ErbB2-ErbB3 complex in NIH 3T3 cells points to the importance of the PI-3K pathway in the induction of cellular transformation [18].

The importance of the PI-3K pathway in breast cancer is also suggested by the finding of PTEN deletions and overexpression of PKB in primary breast tumours [19,20]. How does this pathway lead to deregulation of the cell cycle? Holbro and colleagues show that inactivation of this pathway is correlated with a loss of cyclin D3 expression and with a decrease in the phosphorylation of retinoblastoma protein. PI-3K signalling is known to regulate cyclin D levels at both the transcriptional and translational levels [21] and is also known to regulate the concentrations and localization of the cyclin-dependent kinase inhibitor p27^{Kip1} [22]. It will be of interest to see whether these are the signalling pathways responsible for driving the proliferation of these cells in this context.

These studies clearly show an important role for the PI-3K pathway in the ability of the ErbB2-ErbB3 complex to drive proliferation. However, other signalling pathways originating from the ErbB2-ErbB3 oncogenic unit undoubtedly contribute to transformation *in vivo*. The ErbB2-ErbB3 heterodimer has been reported to increase cell motility and to induce morphological changes that seem to require both PI-3K and MAP kinase signalling [23]. In addition, the ErbB2-ErbB3 heterodimer has been reported to upregulate matrix metalloproteinase-9 expression, which is thought to be important in promoting tumour invasiveness and metastasis [24]. MAP kinase, p38, protein kinase C and PI-3K signalling pathways all seem to have a role in this process. In addition, Src family signalling is thought to be important in the transformation process downstream of ErbB2, through regulation of the cytoskeletal changes that accompany malignant transformation [25].

These findings are relevant to a large proportion of breast malignancies but might also be useful for our understanding of other malignancies. In colorectal cancer, an increase in ErbB2 expression is often associated with an increase in ErbB3 expression [26]. Moreover, co-expression of ErbB2 and ErbB3 is also found in oral squamous cell carcinoma, ovarian tumours and lung adenocarcinomas and in many cases seems to be important for the transformed phenotype of the tumour [27,28].

Advances in our understanding of the molecular pathways responsible for ErbB-mediated transformation should result in the development of novel strategies for treating breast cancer. In particular, the demonstration of an important role of ErbB3 and the PI-3K pathway in driving the proliferation of ErbB2-overexpressing breast cancer cells makes them attractive targets for anti-breast cancer drugs for this subset of patients. One such drug currently

undergoing trials is the monoclonal antibody 2C4, which can disrupt the process of heterodimerisation of the ErbB2–ErbB3 receptor by hindering the recruitment of ErbB2 into ErbB3 complexes [29]. It is to be hoped that the identification of ErbB3 as a requisite component of ErbB2 signalling will result in the development of further promising new drugs.

Competing interests

None declared.

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