Commentary

Tyrosine kinase signalling in breast cancer

Nancy E Hynes

Friedrich Miescher Institute, Basel, Switzerland

Received: 20 March 2000
Accepted: 27 March 2000
Published: 17 April 2000

Breast Cancer Res 2000, **2**:154–157

Abstract

Cells are continuously exposed to diverse stimuli ranging from soluble endocrine and paracrine factors to signalling molecules on neighbouring cells. Receptors of the tyrosine kinase family play an important role in the integration and interpretation of these external stimuli, allowing a cell to respond appropriately to its environment. The activation of receptor tyrosine kinases (RTKs) is tightly controlled, allowing a normal cell to correctly integrate its external environment with internal signal transduction pathways. In contrast, due to numerous molecular alterations arising during the course of malignancy, a tumour is characterized by an abnormal response to its environment, which allows cancer cells to evade the normal mechanisms controlling cellular proliferation. Alterations in the expression of various RTKs, in their activation, and in the signalling molecules lying downstream of the receptors play important roles in the development of cancer. This topic is the major focus of the thematic review section of this issue of *Breast Cancer Research*.

Keywords: cortactin, ErbB receptor tyrosine kinases, fibroblast growth factor receptor, G-protein coupled receptors, insulin-like growth factor-1, Src

Receptors of the tyrosine kinase family play an important role in the integration and interpretation of diverse extracellular stimuli, allowing a cell to respond appropriately to its environment. All members of this superfamily have in common an extracellular ligand-binding domain, a single membrane-spanning region and a cytoplasmic protein tyrosine kinase domain. Ligand binding promotes receptor dimerization, consequently stimulating kinase activity and triggering autophosphorylation of specific tyrosine residues within the cytoplasmic domain (for review [1]). These phosphorylated residues serve as docking sites for proteins that are involved in regulation of intracellular signalling cascades. The activation of RTKs is generally tightly controlled, allowing a normal cell to integrate external stimuli with internal signal transduction pathways correctly. In contrast, due to numerous molecular alterations that arise during the course of malignancy, a tumour is characterized by an abnormal response to its environment, which allows cancer cells to evade the normal mechanisms that control cellular proliferation.

Alterations in RTK expression and activation, and in the signalling molecules that lie downstream of the receptors play important roles in the development of cancer. This topic is the major focus of the thematic review section of the present issue of *Breast Cancer Research*. In particu-

EGF = epidermal growth factor; ER = oestrogen receptor; FGF = fibroblast growth factor; GPCR = G-protein-coupled receptor; IGF = insulin-like growth factor; MMTV = mouse mammary tumour virus; PLC = phospholipase C; RTK = receptor tyrosine kinase.

lar, Stern [2] writes on the interactions among the ErbB family members [epidermal growth factor (EGF) receptor, ErbB2, ErbB3 and ErbB4]; Andrechek and Muller [3] present information gleaned from transgenic models of mammary cancer developed with Neu, the rat ErbB2 equivalent; and Prenzel *et al* [4] describe the emerging role of the EGF receptor as an integrator for other classes of membrane receptors. The non-RTK Src is hyperactive in breast cancer and, as discussed in the review by Biscardi *et al* [5], there is a cooperative interaction between Src and the EGF receptor, which very likely contributes to malignancy. The insulin-like growth factor (IGF)-I signalling cascade and its interaction with the oestrogen receptor (ER) in breast tumours is discussed by Zhang and Yee [6], and the role of fibroblast growth factors (FGFs) and the cooperating Wnt signalling pathway in mammary mouse tumour virus (MMTV)-induced mouse mammary cancer is discussed by Dickson *et al* [7]. Finally, the signal transducers that lie downstream of the tyrosine kinases that have been implicated in breast cancer are reviewed by Kairouz and Daly [8].

It has been known for almost 15 years that deregulated expression of the EGF receptor and ErbB2 contribute to the development and malignancy of breast cancer. In fact, one of the first consistent genetic alterations found in breast tumours was c-*erbB2* gene amplification [9]. The ErbB family has evolved from a single ligand–receptor combination in *C elegans*, through *Drosophila*, which have one receptor and four ligands, to vertebrates, in which four ErbB receptors bind multiple EGF-related ligands. Consequently, in vertebrates numerous ErbB homodimer and heterodimer combinations are possible, reflecting the greater complexity of receptors and ligands, and suggesting that they have evolved to provide the high degree of signalling diversity that is necessary for their development. This complex ErbB receptor–ligand network and its role in breast cancer is described in the article by Stern [2].

Src is overexpressed or highly activated in numerous types of human cancers, including breast cancer. Src physically interacts with both EGF receptor and ErbB2, and has been implicated in the transformation process induced by both RTKs. Evidence arising from various types of experiments indicates the significance of Src in normal EGF receptor signalling. Src plays an important role in EGF receptor activation, because it phosphorylates the receptor at Tyr 845 in the activation loop, stimulating its kinase activity [10]. Furthermore, Src and EGF receptor reciprocally interact and appear to cooperate in the process of malignancy [5]. The mechanism that underlies the Src–ErbB2 interaction is less clear than that described for Src–EGF receptor. However, mammary tumours from Neu transgenic mice display elevated Src kinase activity compared with the adjacent normal epithelium [11], suggesting that there is cooperativity in transformation.

As discussed in the article by Prenzel *et al* [4], RTKs do not act in isolation but are integral components in the complex signalling network that is necessary for the correct response of a cell to its environment. There is a wealth of data that show that EGF receptor in particular becomes activated, serving as a convergence point for other classes of membrane receptors, including G-protein coupled receptors (GPCRs), cytokine receptors and integrins. GPCR-induced EGF receptor activation has been considered to be ligand-independent because of the rapidity of the response, among other reasons. Intriguingly, it has recently been shown [12] that GPCR-mediated EGF receptor activation involves the stimulation of a metalloproteinase activity, which cleaves membrane-bound pro-HB-EGF, one of the ligands for EGF receptor, enabling it to bind and activate the kinase. Considering the abundance of EGF receptor ligands expressed in breast tumours, it is possible that autocrine EGF receptor activation may in some instances arise from GPCR-mediated ligand processing.

The signalling intermediates that act downstream of the tyrosine kinases involved in breast cancer have also come under scrutiny, as discussed in the article by Kairouz and Daly [8]. Most of these proteins, including phospholipase C (PLC)γ, Shc, Grb2 and Grb7, have SH2 or phosphotyrosine binding domains, allowing them to bind to specific phosphotyrosine residues in the activated RTKs. Many of these signalling intermediates lie downstream of the ErbB family and, not unexpectedly, some (eg PLCγ) show increased activity in tumours that overexpress ErbB RTKs. Interestingly, EMS1, the human homologue of the Src substrate cortactin, is amplified and overexpressed in approximately 15% of breast tumours [13]. Overexpression of cortactin increases cell motility, and this is dependent on Src-mediated tyrosine phosphorylation. These results imply that during the process of malignancy there is not only cooperativity between Src and ErbB RTKs, but also selection for overexpression of a Src substrate that very likely has a role in tumour progression, probably by increasing the invasive or metastatic potential of breast tumour cells.

IGF-I and its receptor have recently generated much interest because of the ability of the receptor to inhibit apoptosis and the central role that it plays in oncogenic transformation [14]. In fact it has been speculated that IGF-I receptor activation is necessary to repress apoptosis that would be induced by the uncontrolled activity of certain oncoproteins. The fact that many oncoproteins, including v-Src and EGF receptor, require functional IGF-I receptor to transform cells gives support to this hypothesis (for review [15]). The IGF-I receptor is expressed in virtually all breast tumours. In addition to its role in antiapoptic signalling moiety, there is a reciprocal interaction between the IGF-I system and ER, which is described

in the article by Zhang and Yee [6]. This interaction leads to enhancement of the biological effects of oestrogens and IGFs. Specifically, oestrogens induce the expression of many of the players in the IGF-I network, including the IGF-I receptor and the downstream signalling protein insulin receptor substrate-1 [16], leading to an enhanced cellular response to IGF-I. In primary human breast cancers, insulin receptor substrate-1 levels correlate positively with ER levels. The negative effects of the antiestrogen tamoxifen on ER-positive tumour cells may in part be due to downregulation of these important IGF-I signalling molecules. Furthermore, high levels of IGF-I receptor signalling may impact on therapy, because the antiapoptotic effects of this pathway might protect tumour cells from radiation-induced death.

As discussed in the article by Dickson *et al* [7], there is conflicting data on the importance of the FGF receptor family in human breast cancer development. However, inappropriate expression of FGF receptor ligands has a clear role in murine mammary cancer. MMTV-induced murine models of cancer have been extremely useful, not only in discovering oncogenes that promote mammary cancer, but also for identifying oncogenes that cooperate in the induction of the tumours. MMTV induces cancer by insertional mutagenesis, leading to activation/mutation of genes at the genomic proviral insertion site. The first proto-oncogene identified by MMTV proviral insertion was Int-1/Wnt-1 [17]. An FGF family member, FGF-3, was found at another site of MMTV insertion, and it was soon recognized that many tumours contain proviruses at both Wnt-1 and FGF-3. These results suggest that the combination of inappropriate FGF receptor signalling and activation of Wnt-1 and its downstream transcription factor Tcf potently induces mammary cancer. In the future it will be important to determine the role of FGF receptor signalling in human breast cancer and to determine whether there is a collaboration between Wnt-1 signalling and any of the RTKs implicated in development of this malignancy.

A theme emerging from these articles is the concept of cooperativity during the transformation process. I would like to develop this idea using the ErbB RTKs as an example. It is worthwhile mentioning here that ErbB2 is the preferred heterodimeration partner for the other ErbB family members. ErbB2-containing heterodimers have more potent and prolonged signalling ability [18], providing an explanation for the propensity of this receptor to be overexpressed in human cancer. ErbB2 overexpression triggers ligand-independent activation of the kinase domain, apparently as a result of spontaneous dimer formation. Although ErbB2 homodimers alone very likely contribute to malignancy, a number of observations, many arising from the transgenic models of mammary cancer that are discussed in the article by Andrechek and Muller [3], suggest that ErbB2 cooperates with other ErbB receptors, including EGF receptor and ErbB3, during the malignant process. Many breast tumours that contain ErbB2 also exhibit autocrine stimulation of EGF receptor via expression of one of its numerous ligands (for review [19]). The ability of ErbB2 to potentiate EGF receptor signalling, due to the formation of EGF receptor–ErbB2 heterodimers, would provide tumour cells with a more potent growth stimulus and might lead to the activation of additional intracellular pathways. Furthermore, mammary tumours derived from Neu transgenic mice also exhibit cooverexpression of endogenous EGF receptor [20]. The ErbB2–ErbB3 heterodimer appears to be the most potent ErbB dimer with respect to proliferation and transformation [21]. Moreover, mammary tumours from Neu transgenic mice exhibit selective upregulation of ErbB3 expression and activity, suggesting that there might be selective pressure for the ErbB2–ErbB3 heterodimer in mammary cancer development [22]. Very recent results from our laboratory indicate that overexpressed ErbB2 and ErbB3 cooperate during transformation [23] and as therapeutic targets [24].

As these articles should make apparent, our increasing knowledge on the specific molecular alterations in breast tumours has paved the way for the development of therapeutic agents that are customized to the tumour, recognizing and inhibiting the proteins responsible for the malignant phenotype. One exciting example of a rational, targeted approach to breast cancer treatment is Herceptin™ (Genentech, San Francisco, California, USA), a recombinant humanized antibody targeted to ErbB2. Its development as a therapeutic agent followed from experimental observations that certain antibodies that bind the extracellular domain of ErbB2 inhibit the growth of tumour cells that overexpress that receptor [25]. Herceptin has shown clinical efficacy in ErbB2-overexpressing breast cancer patients, and is now being used for the treatment of advanced breast cancer [26]. Finally, considering the concept of cooperativity between proteins that induce breast cancer, it is likely that therapeutic combinations directed at multiple molecular targets may prove to be more efficacious than monospecific therapy in the treatment of breast cancer.

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Author's affiliation: Friedrich Miescher Institute, Basel, Switzerland

Correspondence: Nancy E Hynes, Friedrich Miescher Institute, PO Box 2543, CH-4002 Basel, Switzerland. Tel: +41 61 697 8107; fax: +41 61 697 8102; e-mail: hynes@fmi.ch