Review

Tyrosine kinase signalling in breast cancer
Tyrosine kinase-mediated signal transduction in transgenic mouse models of human breast cancer

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Abstract

The ability of growth factors and their cognate receptors to induce mammary epithelial proliferation and differentiation is dependent on their ability to activate a number of specific signal transduction pathways. Aberrant expression of specific receptor tyrosine kinases (RTKs) has been implicated in the genesis of a significant proportion of sporadic human breast cancers. Indeed, mammary epithelial expression of activated RTKs such as ErbB2/neu in transgenic mice has resulted in the efficient induction of metastatic mammary tumours. Although it is clear from these studies that activation these growth factor receptor signalling cascades are directly involved in mammary tumour progression, the precise interaction of each of these signalling pathways in mammary tumourigenesis and metastasis remains to be elucidated. The present review focuses on the role of several specific signalling pathways that have been implicated as important components in RTK-mediated signal transduction. In particular, it focuses on two well characterized transgenic breast cancer models that carry the polyomavirus middle T (PyV mT) and *neu* oncogenes.

Keywords: mammary gland, oncogene, signal transduction, transgenic mouse

Introduction

The ability of mammary epithelial cells to respond to growth factor is dependent on specific growth factor receptors that are coupled to a number of intracellular signalling pathways. Of relevance to this is that the development, maturation and differentiation of the mammary epithelial cell are dependent on the interplay of hormones and growth factors. The development of the mammary gland is thought to involve a series of defined steps that consist of cell proliferation, differentiation and programmed cell death (apoptosis). After of the formation of the primary mammary tree from its embryonic rudiment, there is a rapid expansion of ductal outgrowth through the mammary fat pad, which is accompanied by the formation of mammary terminal end-buds. By 10 weeks of age the mammary epithelium has reached the end of the fat pad and ceases further ductal outgrowth.

After pregnancy, a further rapid expansion of the lobuloalveolar epithelium occurs, which leads to induction of terminal differentiation and lactation at birth. After the pups have been weaned from the lactating mother, the mammary epithelium undergoes a rapid involution through the induction of programmed cell death (apoptosis). The balance of soluble growth factors, hormones and cell–substratum interactions controls the regulation of this cycle of proliferation, differentiation and apoptosis. Of particular relevance to these processes is the activity of the tyrosine kinase class of receptors that are thought play a key role in transducing these various extracellular signals. Elevated activity

MMTV = mouse mammary tumour virus; PI-3K = phosphatidyl inositol-3 kinase; PyV mT = polyomavirus middle T (antigen); RTK = receptor tyrosine kinase.

of certain tyrosine kinases can result in aberrant cell proliferation and ultimately cell transformation.

The present review examines the role of certain tyrosine kinases that have been implicated in mammary tumour progression.

Involvement of the Neu receptor tyrosine kinase in mammary tumourigenesis

The progression of the primary mammary epithelial cell to a malignant phenotype involves multiple genetic events, including the activation of dominant activating oncogenes and inactivation of specific tumour suppressor genes. Of relevance to the present review is the observation that the activation of certain RTKs is implicated in the genesis of human breast cancer. For example, amplification and overexpression of *neu*/*erbB2* proto-oncogene is observed in 20–30% human breast cancer, and is inversely correlated with the survival of the patient [1,2••,3]. Although amplification and elevated expression of *neu* has been established as an important event in sporadic breast cancer, comparatively little is known concerning the molecular mechanism by which activation of *neu* influences mammary tumourigenesis and metastasis.

Direct evidence in support of a role for *neu* in mammary tumourigenesis is derived from observations made in transgenic mice that express oncogenic forms of the *neu* oncogene under the transcriptional control of mouse mammary tumour virus (MMTV) enhancer. Mammary epithelial specific expression of activated *neu* results in the rapid induction of metastatic multifocal mammary tumours [4,5,6••,7•]. Although mammary epithelial expression of the activated *neu* oncogene is tumourigenic, no comparable activating mutations have been detected in the transmembrane domain of human breast cancer that overexpresses ErbB2 [8]. Thus, the primary mechanism by which ErbB2 induces mammary tumourigenesis in human breast cancer is through overexpression of the wild-type receptor.

The oncogenic potential of the wild-type *neu* proto-oncogene in the mammary epithelium was tested in transgenic mice through MMTV directed expression of the wild-type *neu* cDNA [9]. These animals develop focal mammary tumours in 50% of female mice by age 205 days, with frequent metastases in the lung. Further genetic and biochemical analyses of these strains revealed that, in addition to elevated expression of tyrosine phosphorylated Neu, elevated levels of tyrosine phosphorylated ErbB3 were consistently observed [7•]. It is interesting to note that ErbB3 is the epidermal growth factor receptor family member that is primarily responsible for recruiting the phosphatidyl inositol-3 kinase (PI-3K) signalling molecule to Neu [10•,11•]. Given the importance of this signalling pathway in providing cell survival signals [12–15], it is conceivable that elevated expression of ErbB3 in these mammary tumours is required to provide the necessary antiapoptotic signals.

Another potent tyrosine kinase that is implicated in murine mammary tumourigenesis and metastasis is that associated with PyV mT antigen [16]. Mammary epithelial expression of PyV mT results in the rapid induction of multifocal metastatic mammary tumours. Because these tumours occur early in mammary gland development and involve the entire mammary gland, expression of PyV mT is clearly sufficient for transformation of the primary mammary epithelium. The potent transforming activity of the PyV mT and *neu* oncogenes in the mammary epithelium of these transgenic strains is due to their capacity to associate with and activate a number of common signalling molecules. After activation of the associated tyrosine kinase activities of Neu and PyV mT, specific phosphotyrosine residues within these oncogenes provide specific binding sites for a variety of signalling molecules that harbour either SH2 or phosphotyrosine binding/interacting domains [17].

Activation of Src family kinases in mammary tumour progression

A class of signalling molecules that plays an important role in mammary tumourigenesis and metastasis is the Src family of tyrosine kinases. Both activated Neu and PyV mT form stable complexes with c-Src and c-Yes, resulting in an increase in the specific activity of these Src family kinases [17–21,22•,23–26,27•]. The importance of c-Src in PyV mT-mediated tumour progression has been demonstrated by crossing the MMTV/PyV mT strains to c-*src*- and c-*yes*-deficient mice [28••]. The results of that study demonstrated that c-Src was required for efficient mammary tumourigenesis and metastasis, whereas c-Yes function was dispensable for induction of mammary tumours. The difference in oncogenic potential between these crosses was not due to levels of tyrosine phosphorylated PyV mT, because the mammary tissue derived from each of the respective crosses had equal levels of tyrosine phosphorylated PyV mT. Although these observations argue that activation of c-Src function is a critical event in mammary tumour progression, mammary epithelial expression of an activated c-*src* oncogene in transgenic mice resulted in the induction of mammary epithelial hyperplasias rather than the multifocal mammary tumours observed in the PyV mT strains [29]. Taken together, these observations argue that, although c-Src function is necessary for mammary tumour progression, its activation is not sufficient to induce the rapid tumour progression that is observed in the PyV mT transgenic strains.

Although it is clear that c-Src function is required for PyV mT-mediated tumourigenesis, its requirement for tumourigenesis in the Neu-induced model remains to be firmly established. Like PyV mT transformed tumour cells,

however, c-Src derived from the Neu-induced mammary tumour cells is complexed with a 89-kDa phosphotyrosine protein that appears to be specific to the mammary epithelium [24]. These observations suggest that activation of c-Src by either PyV mT or activated Neu may result in recruitment of similar sets of mammary specific substrates. Future crosses of the activated Neu strains with *src*-deficient strains should allow these issues to be addressed.

Activation of the phosphatidyl inositol-3 kinase in mammary tumour progression

Another class of SH2 signalling molecules that are known to be associated and activated by both PyV mT and activated Neu oncogenes is PI-3K. Association of PI-3K with PyV mT occurs through its binding to phosphotyrosine residues (Tyr 315/322) within the PyV mT coding sequences [30]. In contrast, recruitment of the PI-3K by Neu occurs through the recruitment to ErbB3 [10•,11•]. Activation of PI-3K and consequent production of phosphoinotide-3 lipids stimulates a number of plekstrin homology-containing serine kinases, including PDK1 and integrin-linked kinase [31–33]. These activated serine kinases in turn activate the Akt/PKB class of serine kinases, which can stimulate a number of antiapoptotic signalling molecules such as nuclear factor-κB [34–36]. In addition, activation of Akt can inhibit proapoptotic proteins such as Bad, Forkhead transcription factors and caspase 9 [12,37,38].

The importance of the PI-3K signalling pathway has been highlighted by several recent studies. Mammary epithelial expression of mutant PyV mT decoupled from the PI-3K pathway results in the induction of extensive mammary epithelial hyperplasias [15]. Consistent with the importance of the PI-3K signalling pathway in promoting cell survival, these mammary epithelial hyperplasias were highly apoptotic. Conversely, inducible expression of a dominant-negative inhibitor of PI-3K in mammary tumour cells expressing wild-type PyV mT was capable of efficiently inducing apoptotic cell death. Despite the initial induction of global mammary epithelial hyperplasias, focal mammary tumours eventually developed in these mammary strains. Mammary tumour progression in these mutant PyV mT strains was further correlated with a dramatic upregulation of the ErbB2 and ErbB3 RTKs. It is conceivable that elevated levels of ErbB2/ErbB3 can indirectly recruit the PI-3K, and thus compensate for the inability of the mutant PyV mT to associate and activate the PI-3K.

Another interesting phenotype of tumours that are induced by this mutant PyV mT is that they are poorly metastatic by comparison with tumours that express the wild-type PyV mT oncogene [39]. The observed defect in the metastatic potential of mammary tumours induced by this mutant form of PyV mT was further correlated with a defect in neovascularization [39]. Taken together, these observations argue that activation of the PI-3K PyV mT may play a critical role in promoting metastatic invasion.

Although activation of Neu is not directly associated with activation of the PI-3K signalling pathway, it can heterodimerize with ErbB3, which possesses six PI-3K binding sites. Indeed, it is thought that recruitment of the PI-3K signalling pathway by members of the epidermal growth factor receptor family is through heterodimerization with the ErbB3 RTK [10•,11•]. Given the importance of ErbB3 in recruiting the PI-3K signalling molecule, elevated expression of ErbB3 may be an important step in Neu-induced mammary tumourigenesis. Consistent with this view, elevated expression of ErbB3 is observed during mammary tumour progression in transgenic mice that express Neu in the mammary epithelium [7•]. Interestingly, the observed upregulation of ErbB3 protein in the Neu-induced mammary tumours does not occur at the level of *erbB3* transcript, because both tumour and adjacent normal mammary tissue express comparable levels of *erbB3* transcript [7•]. The precise molecular mechanism by which elevated levels of ErbB3 protein is achieved during mammary tumour progression remains to be elucidated, however. Consistent with these transgenic mouse studies, a large proportion of ErbB2-expressing human breast cancers exhibit elevated levels *of erbB3* transcripts [7•]. Thus, coexpression of ErbB2 and ErbB3 RTKs appears to be common event in tumour progression in both humans and these transgenic mouse models.

Activation of the Ras signalling pathway in mammary tumour progression

Other cytoplasmic proteins such as Shc and Grb2 have been demonstrated to form specific complexes with both activated forms of Neu and PyV mT [40–42,43•,44–47, 48•,49,50]. The association of Grb2 and Shc with either of these activated oncoproteins is known to play a central role in stimulation of Ras signalling. For example, tyrosine phosphorylation of Shc either by the PyV mT complex or by Neu results in an association with Grb2. In turn, Grb2 stimulates a guanine nucleotide exchange protein, SOS, to convert Ras from the inactive GDP-bound state to the active GTP-bound form [45,51–56]. In contrast to PyV mT, which signals to Ras through its association with Shc, Neu can activate Ras through Grb2, Shc and several other unidentified adapter proteins [46,57]. Upon Ras activation, it can associate with a number of downstream effector molecules including PI-3K, Raf serine kinase, GAP and Ral [58–65].

Direct evidence in support of a role for Ras in mammary tumour progression stems from observations made with transgenic mice that express an oncogenic version of Ras under transcriptional control of the MMTV promoter. Mammary epithelial-specific expression of v-Ha-ras resulted in the induction of focal mammary tumours in

female transgene carriers [66••]. Because these tumours were focal in origin and arose after a long latency period, expression of activated ras is not sufficient to induce mammary tumours, but rather requires additional genetic events. Although insufficient for tumour induction alone, a growing body of evidence suggests that activation of the Ras signalling pathway is critical for the progression to the tumourigneic phenotype. For example, mammary-specific expression of a mutant PyV mT oncogene decoupled from the Shc/Grb2 signalling molecules results in the induction of widespread mammary epithelial hyperplasias [15]. In contrast to the rapid tumour progression observed in the wild-type PyV mT transgenic mice, focal mammary tumours arise in the mutant PyV mT strains after a long latency period. Interestingly, a certain proportion of tumours that arise in these mutant PyV mT strains exhibit reversion of Shc-binding site mutation [15]. The strong biological selection for retention of Shc-binding site suggests that retention of this signalling pathway is critical for tumour progression.

Further evidence in support of the importance of the Shc–Grb2–Ras signalling axis in mammary tumour progression stems from observations made by interbreeding the PyV mT transgenic strains with the Grb2 knockout mice. Because homozygous deletion of Grb2 is not compatible with embryonic viability [67••], it was not feasible to ascertain whether Grb2 function was absolutely required for PyV mT tumour progression. The results of those experiments, however, revealed that a reduction to one copy of Grb2 was sufficient to interfere with tumour progression [67••]. Conversely, ectopic expression of Grb2 or Shc in the mammary epithelium of transgenic mice cooperates with mutant PyV mT decoupled from the Shc adapter protein to accelerate mammary tumour progression [68]. Taken together, these observations argue that dosage of these key adapter proteins that couple to Ras can have profound effects on mammary tumour progression.

Although studies with PyV mT transgenic mice have clearly demonstrated the importance of PI-3K, c-Src and Shc/Grb2/Ras signalling pathways in mammary tumourigenesis and metastasis [15,28••], the role of these various signalling molecules in Neu-induced tumourigenesis is less well understood. In contrast to the well-defined signalling molecules emanating from the PyV mT oncogene, the binding sites for only a subset of signalling molecules that couple to Neu have been identified. These include Grb2 and Shc molecules, which bind tyrosine residues 1144 and 1227 in Neu [43•]. In addition to these signalling molecules that positively activate the Ras signalling pathway, an autophosphorylation site that negatively regulates Neu-mediated signal transduction has also been described [43•]. The identity of this signalling molecule remains to be elucidated, however. Thus, unlike PyV mT, the signalling molecules that modulate the Ras signalling pathway are probably more complex in Neu-mediated tumourigenesis. Future studies to investigate the role of Neu-coupled signalling molecules in mammary tumour progression should provide important insight into the molecular basis of breast cancer.

Conclusion

The studies outlined above strongly support the notion that tyrosine kinase-mediated signalling in the mammary epithelium involves the concerted activation of a number of signalling pathways that can cooperate to lead to malignant transformation of the mammary epithelial cell. Future strategies to interfere with the ability of tyrosine kinases to transform cells will independently target these coupled signalling pathways. The development of novel inhibitors of these signalling molecules will hopefully provide effective treatment for this prevalent, but poorly understood disease.

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