Paradoxes of the EphB4 Receptor in Cancer

Nicole K. Noren and Elena B. Pasquale

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Abstract

Recent findings have started to uncover the intriguing roles of the Eph family of receptor tyrosine kinases in normal epithelial cells and during oncogenic transformation. This review focuses on EphB4, an Eph receptor that has both tumor-suppressing and tumor-promoting activities in breast cancer. Understanding the multifaceted role of EphB4 in tumorigenesis may allow the development of new anticancer therapies. [Cancer Res 2007;67(9):3994–7]

Introduction

The Eph receptors are the largest family of receptor tyrosine kinases. They are divided into an EphA and an EphB class, which respectively bind the glycosylphosphatidylinositol-linked ephrin-A ligands and the transmembrane ephrin-B ligands (1). Although Eph receptors are present in most cell types, thus far, their activities have been best characterized in the developing and adult nervous system and in the developing vascular system. Recent work is beginning to address the functions of the Eph family in the immune system, bone, stem cells, normal epithelial cells, and tumors. Well-known effects of Eph receptor activation by their ephrin ligands involve regulation of cell shape and movement, although modulation of cell survival and proliferation has also been described. Here, we review recent data that begin to shed light on the multiple roles in breast cancer of EphB4, one of the predominant Eph receptors expressed in epithelial cells.

EphB4/Ephrin-B2 Interaction Promotes an Epithelial Phenotype

The EphB4 receptor tyrosine kinase has been detected in the epithelial cells of human breast tissue (2, 3). Furthermore, regulated expression of EphB4 and its preferred ligand ephrin-B2 during development and during the estrous cycle has been proposed to play a role in mammary gland morphogenesis (4). Both EphB4 and ephrin-B2 are transmembrane proteins, and by immunofluorescence microscopy, they seem to be segregated in different membrane microdomains of the MCF-10A nontransformed human mammary epithelial cell line, where they are coexpressed (Fig. 1A). This segregated distribution is similar to that previously reported for Eph receptors and ephrins that are coexpressed in neurons (5). Patches of EphB4 immunoreactivity are juxtaposed to patches of ephrin-B2 immunoreactivity in MCF-10A cell-cell junctions (Fig. 1A and B), consistent with Eph activation occurring at sites of cell-to-cell contact (1). EphB4 is indeed substantially tyrosine phosphorylated in the MCF-10A cells, and treatment with a soluble form of the EphB4 extracellular domain (EphB4 Fc, which blocks the interaction of ephrin-B2 with endogenous EphB receptors) decreases EphB4 tyrosine phosphorylation (6).

Interestingly, EphB4 Fc treatment of MCF-10A cells also inhibits the activity of Abl, a cytoplasmic tyrosine kinase that phosphorlates and inactivates the adaptor protein Crk (6). The Abl/Crk pathway seems to be important for the maintenance of epithelial characteristics in the MCF-10A cells (Fig. 1B) because disruption of EphB4/ephrin-B2 complexes with EphB4 Fc or with an EphB4 antagonistic peptide disturbs the organization of cell-cell junctions, suggesting a shift to a more mesenchymal morphology (6). Consistent with these results, Crk adaptor activity has been shown to induce epithelial-to-mesenchymal transition (7). EphB4 Fc treatment also promotes other aspects of cell transformation, including increased cell growth in two-dimensional cultures and in soft agar (6).

Given that activation of receptors of the EphA class has also been reported to promote epithelial characteristics in cells of the mammary gland, kidney, and somites (1, 8), it will be important to determine whether other Eph receptors also regulate the Abl/Crk pathway. The finding that EphB4 Fc, which should not interfere with EphA signaling, greatly inhibits Crk phosphorylation suggests that EphA receptors may signal predominantly through other pathways in mammary epithelial cells. For example, ligand-stimulated EphA2 inhibits the Ras/mitogen-activated protein kinase pathway (9, 10). It should be noted that the ligand ephrin-B2 also has the ability to generate signals through its cytoplasmic domain, which are known as reverse signals (1). These signals can be stimulated by endogenous EphB4 or by EphB4 Fc (1, 11) and may contribute to maintain an epithelial phenotype (12), but their identity in epithelial cells is unknown (Fig. 1B).

EphB4 Signaling Inhibits Breast Cancer Cell Tumorigenicity

Up-regulation of EphB4 expression has been found in mouse mammary tumor models and in more than half of the human breast cancer specimens examined (2–4, 13). EphB4 is also widely expressed in human breast cancer cell lines (6, 13). Increased EphB4 expression may be a common occurrence during epithelial cell transformation because it has been reported in many types of cancer (refs. 6, 13–15 and references therein). Several signaling pathways involved in tumorigenesis can indeed promote EphB4 expression, including the Janus-activated kinase/signal transducer and activator of transcription and phosphatidylinositol 3-kinase/Akt pathways downstream of ErbB family receptors and the Wnt/β-catenin/Tcf4 pathway (13, 14). Estrogen has also been shown to drive EphB4 expression in the mouse mammary gland (4). In addition, EphB4 is located on chromosome 7 in a region (7q22.1) that is frequently amplified in breast cancer, and EphB4 gene amplification has indeed been detected in several breast cancer cell lines (13).
Surprisingly, despite the substantial levels of EphB4 expression, EphB4 tyrosine phosphorylation is much lower in breast cancer cell lines compared with nontransformed MCF-10A epithelial cells (6). Furthermore, in breast cancer cells, Abl kinase activity is lower, and Crk is less tyrosine phosphorylated and therefore better able to function as an adaptor protein (Fig. 1C). The silencing of EphB4 signaling in breast cancer cells is consistent with the low expression of ephrin-B2 in these cells. Loss of ephrin-B2 has also been reported in mouse mammary tumor models (4), and ephrin-B2 is down-regulated by the Wnt/catenin/Tcf4 pathway in colorectal cancer cells (14). Furthermore, allelic losses have been described in various cancers for the chromosome 13q33 region, where the ephrin-B2 gene is located (16).

The low EphB4 tyrosine phosphorylation in mammary tumor cells suggested that ligand-stimulated signaling through the EphB4 cytoplasmic domain may be detrimental to tumor development. Indeed, EphB4 inhibits breast cancer cell tumorigenicity both in vitro and in vivo when its tyrosine kinase activity is stimulated by a soluble form of the ligand, ephrin-B2 Fc (6). Treatment of several breast cancer cell lines with ephrin-B2 Fc inhibited proliferation and increased apoptosis. Furthermore, ephrin-B2 Fc inhibits breast cancer cell motility and invasion, concomitant with decreased expression of the matrix metalloprotease MMP2. Increased activity not only of Abl but also the related Arg kinase contributes to Crk phosphorylation in breast cancer cells activated with ephrin-B2 Fc. A series of experiments using Abl and Crk mutants, RNA interference, and the Abl/Arg kinase inhibitor Gleevec showed that restoration of the EphB4/Abl/Crk pathway is responsible for the anti-oncogenic effects of ephrin-B2 Fc in cell culture and in vivo in a breast cancer mouse xenograft model (6).

Oncogenic forms of Abl, such as BCR-Abl, have dysregulated kinase activity and aberrant subcellular localization and thus mediate inappropriate signaling pathways (17). In contrast, the activities of cellular Abl downstream of EphB4 in breast cancer cells are consistent with previous reports that Abl can inhibit cell motility and invasion as well as promote apoptosis through inhibitory phosphorylation of Crk (18, 19). A likely mediator of the effects of Crk in breast cancer cells is the Rac1 GTPase (refs. 7, 18; Fig. 1C). Crk can promote Rac1 activation by forming signaling complexes with the scaffolding protein Cas and the Rac1 exchange factor DOCK180, and ephrin-B2 Fc treatment does indeed disrupt Crk/Cas complexes in breast cancer cells (6, 18). Additional
pathways that are operational in epithelial cells involve complexes
of Crk with the scaffolding protein paxillin, the ARF-GAP GIT2, and
the Rac1 exchange factor β-PIX or with the scaffolding protein
Gab1. Thus, a decrease in EphB4 activation and the resulting
up-regulation of Crk downstream signaling pathways leading to
Rac1 activation may contribute to breast cancer initiation and
progression (Fig. 1C). In contrast, the Rap1 GTPase, which is also
activated downstream of Crk in complex with Cas, does not seem
to play a critical role (7, 19).

Increasing evidence shows that oncogenic signaling pathways
also up-regulate the expression of other Eph receptors, including
EphA2, EphB2, and EphB3, in cancer cells (9, 10, 14, 15). However,
like EphB4, these receptors have been shown to inhibit tumori-
genesis in at least some cancer types. For example, EphA2 inhibits
cell proliferation and promotes epithelial cell morphology when
activated by the ligand ephrin-A1 (8–10). In the case of EphA2 in
skin tumors and of several EphB receptors in colorectal tumors, it
has also been proposed that interactions between the Eph
receptor–positive tumor cells and the surrounding ephrin-positive
normal epithelial cells compartmentalizes the tumor and prevents
tumor cells from expanding and infiltrating the normal tissue
(10, 14). Interestingly, there is evidence to suggest that the tumors
overexpressing Eph receptors may be the less malignant ones, and
that Eph receptor expression is lost in the more advanced stages
(e.g., through promoter methylation or gene mutations; refs. 2, 9, 10,
14, 15, 20). One study indicates that this may also be the case for
EphB4 in breast cancer (2). Thus, cancer cells may elude the tumor
suppressor activities of Eph receptors by down-regulating ephrin or
Eph receptor expression. Additionally, mutations that impair Eph
receptor signaling ability or up-regulation of tyrosine phosphatases
desphosphorylate Eph receptors may also promote tumorigen-
esis (20, 21). Taken together, the available information suggests that
Eph-ephrin interactions and signaling in normal epithelial tissues
help maintain tissue homeostasis, and that their disruption may be
a factor in the development and progression of cancer.

Tumor-Promoting Effects of EphB4

In addition to its tumor suppressor activity in breast cancer, the
EphB4 receptor can also promote tumorigenesis through different
mechanisms. The EphB4 extracellular domain can induce angiog-
genic responses by stimulating ephrin-B2 reverse signaling in
cultured endothelial cells (11). In agreement with this, EphB4
expressed on the surface of breast cancer cells has been shown to
promote angiogenesis in tumor xenografts by activating ephrin-B2
reverse signaling in the vasculature, thus increasing tumor growth
(11). Tumor angiogenesis may also have contributed to the
increased tumor growth observed in a mouse mammary tumor
model with transgenic overexpression of EphB4 in epithelial cells
of the mammary gland (4). EphB4 expressed in tumor endothelial
cells has also been shown to predominantly function by stimulating
reverse signaling through endothelial ephrin-B2 (13, 22).

Additional tumor-promoting effects of EphB4 that are indepen-
dent of angiogenesis have also been identified by down-regulating
EphB4 using RNA interference and antisense oligonucleotide
approaches. EphB4 knockdown was found to reduce survival,
proliferation, migration, and invasion of breast cancer cells and
many other types of cancer cells (ref. 13 and other articles by the
same group). In these cells, EphB4 was generally found to be poorly
tyrosine phosphorylated, suggesting that the tumor-promoting
ability of this receptor is independent of ligand-mediated kinase
activation (1). Similarly, the EphA2 receptor overexpressed in
MCF-10A cells is poorly tyrosine phosphorylated and promotes
oncogenic transformation, an effect that is reversed by treatment
of the cells with the ligand ephrin-A1 Fc (8). In addition, the low
molecular weight tyrosine phosphatase promotes transformation
of MCF-10A cells by dephosphorylating EphA2 (21). Whether
signaling pathways that are independent of ephrin-mediated
Eph receptor phosphorylation and crosstalk with oncogenic or
apoptotic signaling pathways may explain the tumor-promoting
effects of Eph receptors remains to be determined.

Further adding to the complexity of EphB4 function in cancer
cells, it has also been reported that in some types of cancers, such
as melanoma, ephrin-B2–dependent EphB4 signaling enhances the
migratory and invasive ability of the cells (23). These effects require
EphB4 signaling and activation of the RhoA GTPase. In addition,
signaling by another Eph receptor (EphB2) has been shown to
promote the invasive ability of human glioma cells through
phosphorylation of the R-Ras GTPase (24). Interestingly, however,
the EphB2/R-Ras pathway inhibits glioma cell proliferation. Hence,
the cellular context also seems to play an important role in
determining the tumor-promoting or tumor-suppressing effects of
Eph receptors in cancer.

EphB4 as a Breast Cancer Target

The widespread expression of EphB4 and other Eph receptors in
tumors has stimulated interest in exploring these receptors as
targets for the development of new cancer therapies. Given the
different activities of EphB4 in breast cancer cells, the most
effective design for EphB4-based breast cancer treatments should
be to inhibit EphB4 binding to endothelial ephrin-B2 while at the
same time promoting EphB4 downstream signaling. The soluble
form of the ligand (ephrin-B2 Fc) can promote EphB4 activation
and, at higher concentrations, inhibit endogenous EphB4/ephrin-
B2 interaction. Ephrin-B2 Fc administered systemically at low
concentrations has already been shown to inhibit the growth of
breast cancer xenografts in nude mice by activating the EphB4/Abl/Crk
pathway (6). It will be interesting to examine whether higher
doses of ephrin-B2 Fc may be even more effective by also
inhibiting tumor angiogenesis. However, ephrin-B2 Fc can bind all
the EphB receptors and also EphA4 (1), increasing the potential for
unwanted effects. More selective reagents, such as EphB4-
activating antibodies, might be more suitable to specifically inhibit
the interaction of EphB4 with endothelial ephrin-B2 and also
activate EphB4 downstream anti-oncogenic signaling pathways.

A soluble monomeric form of the EphB4 extracellular domain,
which can inhibit both EphB4 signaling and ephrin-B2 reverse
signaling, has also been useful for decreasing tumor growth in
several mouse tumor xenograft models, including a breast cancer
model (25, 26). This suggests that peptides and small molecules
that inhibit EphB4/ephrin-B2 interaction (27), which may have
more desirable therapeutic properties and cost-effectiveness than
the large EphB4 extracellular domain, represent promising agents
to inhibit tumor angiogenesis. Furthermore, they could inhibit
EphB4 tumorigenic signaling pathways in certain cancers such as
melanoma (23). Down-regulation of EphB4 expression with
antisense oligonucleotides has also been an effective strategy to
inhibit the growth of breast and other types of tumor xenografts
expressing high levels of EphB4 (13). This approach can both
inhibit EphB4-dependent angiogenesis and counteract possible
kinase-independent EphB4 signaling pathways.
Inhibition of EphB4 kinase activity using ATP analogues will be useful against those tumors, such as melanoma, where EphB4 kinase activity promotes tumorigenesis. Kinase inhibitors may instead be ineffective or even detrimental for the treatment of breast cancer and other types of cancer where EphB4 signaling suppresses tumorigenesis. This is also the case for the Ab1 kinase inhibitor Gleevec, although Gleevec has proven to be a very effective therapy for targeting oncogenic BCR-Ab1 in chronic myelogenous leukemia patients (17). Tumor xenograft studies show that Gleevec can counteract the anti-oncogenic effects of EphB4 agonists in breast cancer and should therefore not be used in combination with them (6). On the other hand, chemotherapeutic agents that target ErbB receptors or taxol may enhance the effects of EphB4-targeted therapies (13, 28). Finally, antibodies, peptides, and small molecules that bind to EphB4 but lack intrinsic biological activity could be coupled to toxic substances to selectively kill tumor cells that overexpress the receptor.

Our understanding of the complex roles of EphB4 and other Eph receptors in cancer is still evolving, and more information is needed to resolve the many confusing and controversial issues. Future research will determine whether EphB4-based therapeutic strategies can be effective for the treatment of cancers that overexpress EphB4 and in which types of cancer different therapeutic approaches may be most appropriate. It will also be important to examine the effects of EphB4-targeting agents on normal epithelial cells in vivo. New insights into Eph signaling pathways in normal and tumor cells will be important not only for the development of new cancer therapies but also for the optimal use of existing therapies.

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