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Perspectives

ErbB2 and TGF-β

A Cooperative Role in Mammary Tumor Progression?

Sarah E. Seton-Rogers
Joan S. Brugge*

Harvard Medical School; Department of Cell Biology; Boston, Massachusetts USA

*Correspondence to: Joan S. Brugge; Harvard Medical School; Department of Cell Biology; 240 Longwood Ave.; Boston, Massachusetts 02115 USA; Tel.: 617.432.3974; Fax: 617.432.3969; Email: joan_brugge@hms.harvard.edu

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ABSTRACT

Amplification and overexpression of ErbB2 (HER2/Neu) is one of the most common alterations associated with breast cancer. Activation of ErbB2 via homodimerization in a non-transformed human mammary epithelial cell line, MCF-10A, in basement membrane cultures leads to formation of proliferative structures that share properties with non-invasive early stage lesions. Recently, we have shown that activation of ErbB2 homodimers combined with expression of transforming growth factor (TGF)-β induces invasive and migratory activity in MCF-10A cells. In this system, migration requires inputs from numerous cellular pathways. We discuss this data and a model for migration induced by ErbB2 and TGF-β. Concurrent studies by other groups have also shown that ErbB2 and TGF-β can cooperate to increase metastatic and invasive behavior in murine mammary tumors. Here we discuss these studies and the potential implications of this research on breast cancer therapeutics.

The progression from normal epithelium to a malignant tumor is thought to involve an accumulation of genetic and epigenetic alterations within the neoplastic cells as well as alterations in cells and matrix components of the tumor microenvironment.[1] These alterations allow the cells to escape from normal controls that limit cell proliferation, survival and migration, thus inducing phenotypic effects such as hyperplasia and invasive behavior. Dissecting the molecular events responsible for such changes is critical to understanding tumor progression and to identifying targets for therapeutic intervention.

One of the most common alterations associated with breast cancer is amplification or overexpression of the receptor tyrosine kinase ErbB2 (HER2/Neu). ErbB2 overexpression occurs in up to 85% of comedo-type ductal carcinoma in situ (DCIS), an early non-invasive, yet high grade breast carcinoma, suggesting that ErbB2 plays a role in the induction of this distinct type of DCIS.[2-4] Thus, it is of interest to find alterations that, when present in conjunction with overexpression of ErbB2, enhance invasive behavior and contribute to the progression towards a metastatic tumor.

We have utilized a model system to analyze the effects of oncogenes on mammary epithelial structures. When cultured in basement membrane gels, the immortalized but non-transformed human mammary epithelial cell line, MCF-10A, undergoes a morphogenetic process and forms hollow spherical structures termed acini (Fig. 1A).[5,6] Previously, we engineered MCF-10A cells to express an inducible ErbB2 chimeric receptor such that ErbB2 homodimers are activated in response to a synthetic homodimerizing compound.[7,8] Activation of ErbB2 homodimers in basement membrane cultures of MCF-10A acini led to formation of multi-acinar structures with filled lumen (Fig. 1B), however, ErbB2 activation was not sufficient to induce invasive or migratory activity in MCF-10A cells.[8]

Recently, we performed a screen to identify candidate genes that could potentially induce invasion of tumor cells overexpressing ErbB2 using these MCF-10A cells engineered to inducibly activate ErbB2 homodimers. Two transforming growth factor (TGF)-β family members, TGF-β1 and TGF-β3, scored in this screen. These family members, as well as TGF-β2, were found to induce both migration in transwell chambers and invasion through basement membrane gels in response to inducible activation of ErbB2 homodimers (Fig. 1C).[9]

The most significant clue relating to the mechanism whereby ErbB2 and TGF-β enhance cell migration was the discovery that conditioned medium from cells expressing TGF-β and activated ErbB2 is able to induce migration of naïve parental MCF-10A cells. This result indicated that all of the activities required for motility induced by ErbB2 plus TGF-β are derived from secreted extracellular factors. Investigation of the properties of the secreted factors revealed that both ErbB1 (epidermal growth factor receptor; EGFR)-dependent and -independent soluble motogenic factors are present. To the best of our

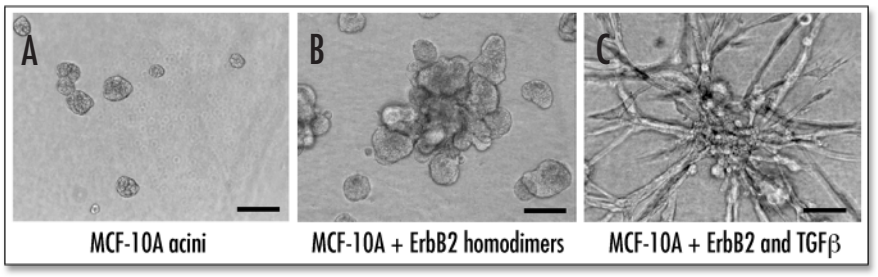

Figure 1. Effects of ErbB2 and TGF-β on MCF-10A epithelial acini. MCF-10A cells expressing the ErbB2 chimeric protein and/or TGF-β1 were seeded in basement membrane cultures. After four days, dimerizing agent was added to activate ErbB2. Eleven days later, the cells were photographed. (A) Normal MCF-10A acini. (B) Phenotype induced by activation of ErbB2 homodimers in MCF-10A acini. (C) Phenotype induced by expression of TGF-β and activation of ErbB2 homodimers in MCF-10A acini. Bars = 100 μm. (Figure reproduced from Seton-Rogers SE, Lu Y, Hines LM, Koundinya M, LaBear J, Muthuswamy SK, Brugge JS. Cooperation of the ErbB2 receptor and TGF-β in induction of migration and invasion in mammary epithelial cells. Proc Natl Acad Sci USA 2004; 101:1257-62. © 2004 National Academy of Sciences, USA).

knowledge this was the first work indicating that the collaborative effects of ErbB2 and TGF-β on migration require the secretion of ErbB1 ligands. However, the collection of soluble, secreted factors induced by ErbB2 and TGF-β, while sufficient to induce motility, was not sufficient to induce invasion of MCF-10A cells.

Several lines of evidence support a role for the Erk pathway in the phenotypic effects of ErbB2 and TGF-β. Activation of these two pathways results in sustained activation of Erk and this hyper-activation is required, but not sufficient, to mediate migration and invasion induced by costimulation of ErbB2 and TGF-β receptors. Erk activation is also required for both secreted factor production and factor-induced motility. These data suggest that Erk is a critical regulator of migration and invasion induced by ErbB2 and TGF-β and is interesting in light of the observation that Erk is both hyper-activated and overexpressed in breast carcinomas.[10]

MCF-10A cells undergo a phenotypic change in response to TGF-β which involves upregulation of mesenchymal markers and a decrease in the levels of detergent insoluble E-cadherin, which may indicate a decrease in E-cadherin linkage to the actin cytoskeleton. While these changes share some features with those commonly induced during epithelial to mesenchymal transitions (EMT), TGF-β does not appear to induce morphological EMT in monolayer cultures of MCF-10A cells. Additionally, the cells do not undergo further mesenchymal changes upon activation of ErbB2, indicating that the EMT-like properties acquired by these cells are not sufficient to induce migration or invasion. However, these findings do not rule out the possibility that these changes are required for migration or invasion induced by TGF-β and ErbB2.

MODEL FOR ERBB2 AND TGF-β INDUCED MIGRATION

Our data suggests a model for migration induced by ErbB2 and TGF-β that involves inputs from numerous cellular pathways (Fig. 2). ErbB2 and TGF-β induce sustained activation of the Erk pathway, which is required for secretion of motogenic factors. At least one of these motogenic factors stimulates ErbB1. However, this alone does not account for the full activity of the secreted motogenic factors. Other ErbB1-independent factors are secreted and account for approximately 40–50% of migratory activity induced by secreted factors. In addition, while Erk plays a critical role in the collaborative induction of migratory activity, other pathways that are activated downstream of TGF-β must contribute to migration, as TGF-β expression significantly enhances migration in cells expressing constitutively active Mek. One possibility is that TGF-β alters the morphology of the cells in a way that renders them competent to migrate in response to the ErbB1 ligands and perhaps ErbB1-independent ligands as well. Uncoupling of E-cadherin from the actin cytoskeleton in response to TGF-β might create a more permissive environment for migration. However, it is possible that other molecular changes in the cells in response to TGF-β, such as upregulation of N-cadherin expression, also contribute. Activation of additional signaling pathways downstream of TGF-β that are not associated with EMT may be involved as well; this possibility has not been ruled out.

ERBB2, TGF-β, AND METASTASIS

During the course of our studies it became clear from work in other laboratories that ErbB2 and TGF-β could cooperate to increase metastasis in murine mammary tumors in vivo.[11-13] Inhibition of TGF-β in mice expressing oncogenic ErbB2 (Neu) under the control of the

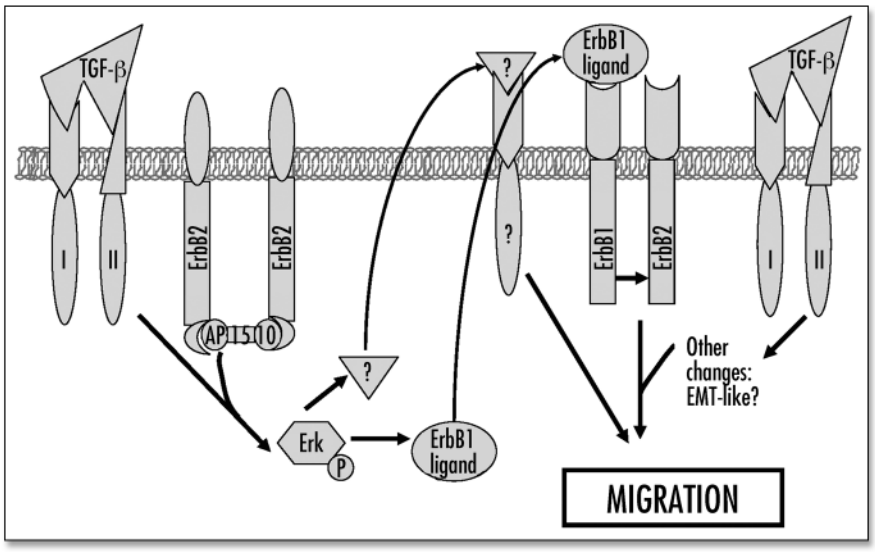

Figure 2. Model for ErbB2 and TGF-β induced migration of MCF-10A cells. TGF-β binds to TGF-β receptors I and II and ErbB2 homodimerization is achieved via treatment of the cells with the dimerizing drug AP1510 (ARIAD Pharmaceuticals). Activation of these two pathways leads to hyper-activation of Erk, whose activity is required for secretion of motility-inducing factors. These factors, both ErbB1-dependent and -independent, are secreted and stimulate their respective receptors, leading to migration. TGF-β is also present in the medium and may further enhance migration via EMT-like changes.

mouse mammary tumor virus (MMTV)-promoter results in a significant reduction in the formation of metastatic lesions.[11] Conversely, animals derived from crossing mice expressing inducible TGF-β with MMTV-Neu transgenic mice display increased numbers of metastatic lesions.[13] In addition, expression of an activated TGF-β receptor increases extravasation of Neu-induced tumor cells from pulmonary vessels.[12] Our findings indicate that TGF-β is capable of exerting its influence on ErbB2-expressing epithelial cells directly and thus raises the possibility that these direct effects on epithelial cells' invasive activity could contribute to the collaborative phenotype observed in mice.

In tracking the basis for TGF-β's enhancement of ErbB2-induced metastasis, the Arteaga and Massague laboratories proposed two different, but not mutually exclusive, mechanisms for ErbB2 and TGF-β collaboration. Arteaga and colleagues present data suggesting that TGF-β enhances the ability of tumor cells from an ErbB2 positive primary cancer to intravasate and thus reach the systemic circulation. To measure intravasation, they cultured tumor cells from the blood of mice with either ErbB2 or ErbB2 plus TGF-β induced tumors and detected significantly more tumor cells in the circulating blood from mice with ErbB2 and TGF-β induced tumors.[13] On the other hand, Massague and colleagues present data suggesting that TGF-β is required for the extravasation of tumor cells from vessels into a site of metastasis. In mice expressing both activated ErbB2 and activated type I TGF-β receptor, the authors do not see increased numbers of metastases in the lungs as compared to controls. However, when these metastases were evaluated further, two classes are observed: those contained within a pulmonary vessel and those that have extravasated from the vessel and have colonized the lung parenchyma. The number of this second class of metastatic lesions, the extravascular metastases, is increased in mice with activated ErbB2 and TGF-β signaling pathways as compared to those with ErbB2 alone.[12]

Given that the molecular mechanisms distinguishing the processes of intravasation and extravasation are not understood, it is difficult to explain the basis for the apparent differences observed in these two models. On the other hand, the differences between these studies may well reflect the timing at which the experimental endpoints were assessed as well as the methods utilized. It is possible that the extent to which the collaborative effects of either TGF-β1 ligand[13] or activated type I TGFβ receptor[12] with ErbB2 are different in the two mouse models employed in these studies and that this accounts for the distinct activities associated with TGF-β-enhanced metastasis. Regardless, as both intravasation and extravasation require invasive activity, both results are consistent with the possibility raised by our studies that at least one aspect of TGF-β and ErbB2 collaboration involves enhanced invasive activity of the tumor cells themselves. This does not rule out the possibility of additional indirect effects of TGF-β on cells in the tumor microenvironment.

While ErbB2 and TGF-β clearly induce migration of MCF-10A cells via secretion of soluble motogenic factors, these factors alone are not sufficient to induce invasion of MCF-10A cells in transwell invasion assays or basement membrane cultures. The insufficiency of the secreted factors to induce invasion suggests that additional changes in the cells that are activated downstream of ErbB2 or TGF-β may be required for invasive activity and supports the concept that induction of migration does not necessarily correlate with invasion. Bigenic mammary tumors expressing neu (ErbB2) and active TGFβ1 exhibit higher local invasiveness and Rac1 activity in situ than tumors expressing neu alone; the same has been shown for MCF-10A cells over-expressing ErbB2 that are treated with TGF-β.[13,14] Another attractive hypothesis is that ErbB2 and TGF-β activate insoluble cell surface proteases that degrade ECM and are required for invasion. Another possibility is that specific adhesive properties of the cells, such as expression of certain integrins or changes in integrin affinity for ECM components, are required for ErbB2 and TGF-β to induce invasion, but these changes are not required for migration.[15] It is also possible that all factors required for invasion are soluble, but that some are unstable and are degraded soon after the conditioned medium is removed from the producer cells.

The invasive activity of cells expressing TGF-β and activated ErbB2 is detected in three-dimensional basement membrane cultures that consist of a mixture of Matrigel™ (a commercial basement membrane preparation) and collagen I; however, no invasive activity is detected when the same cells are grown in cultures comprised of 100% Matrigel™. In vivo imaging of invasive mammary tumors has shown that single cells move along collagen fibers at the edges of a tumor, suggesting that the presence of collagen may be important for tumor cell migration.[16] Laminin and collagen IV are the predominant matrix components in Matrigel™. Collagen I utilizes a different integrin receptor than laminin or collagen IV,[17,18] and thus specific changes in integrin avidity for collagen I may affect the ability of MCF-10A cells to display invasive activity. Additionally, Erk activity has been shown to reduce the avidity of integrins for fibronectin via "inside-out signaling",[19] suggesting the possibility that the sustained Erk activity induced by ErbB2 and TGF-β might modulate the avidity of integrin receptors for collagen and thus promote invasive activity. The presence of collagen I may also change the compliance of the ECM gel and thus increase the ability of ErbB2 and TGF-β to induce migration and invasion, as it has been demonstrated that the degree of compliance of a gel can inversely affect cell motility.[20]

TGF-β has been shown to promote metastatic and invasive behavior in other model systems. For example, TGF-β promotes a fibroblastoid, epithelial-to-mesenchyme (EMT) conversion in cell lines that display constitutive activation of the Ras pathway, thus enhacing invasive activity.[21-27] In addition, TGF-β collaborates with v-Src to induce tumors in chickens infected with Rous Sarcoma Virus.[28] TGF-β has also been observed to enhance metastasis and invasion in other mouse models of tumorigenesis.[29-33]

THERAPEUTIC IMPLICATIONS FOR BREAST CANCERS

The observed collaboration of ErbB2 and TGF-β in promoting metastasis in mice[11-13] suggests that these pathways may be physiologically relevant in the induction of metastatic tumors in humans. It is difficult to establish whether TGF-β contributes to the metastatsis of ErbB2 overexpressing human tumors in vivo. The role of these pathways in human cancers should be examined through analysis of their co-expression in tumors and whether this correlates with prognosis and tumor grade. This will be complicated, as the presence of TGF-β does not indicate it is active, nor does it indicate whether TGF-β receptors are present in the tumor cells. Assessment of the levels of activated TGF-β, as well as activity of the TGF-β signaling pathway via receptor expression and phosphorylation of Smad signal transducers as well as non-Smad signaling programs downstream of TGFβ receptors will be necessary for such studies.

If, indeed, TGF-β and ErbB2 colloborate in human breast tumor metastasis, then TGF-β or the pathways that mediate enhanced invasion and metastasis may represent important points for therapeutic

intervention. Blocking TGF-β systemically through use of recombinant fusion proteins containing the ectodomains of TGF-β receptors II and III has been shown to inhibit metastasis and tumor formation in several mouse models of tumorigenesis and is being pursued as a therapeutic strategy.[11,13,34,35] While such therapy would be expected to block the tumor suppressive activities of TGF-β and potentially cause side effects due to loss of important physiological functions of TGF-β, such effects were not observed in the treated mice.[11] With the advent of technologies that allow profiling of an individual patient's tumor, it may be possible to specifically treat tumors where TGF-β is suspected to promote metastasis with TGF-β antagonists.

Our in vitro data in human mammary epithelial cells combined with the in vivo data in mouse models present striking evidence that supports a role for TGF-β in the progression of breast cancers with activated ErbB2. Confirming the role of these pathways in human tumors and understanding whether the critical downstream signaling events in such tumors parallels those we have seen in vitro may represent the starting point for new therapeutic strategies.

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