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β4 Integrin Amplifies ErbB2 Signaling to Promote Mammary Tumorigenesis

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SUMMARY

Amplification of the *ErbB2* locus, which encodes a receptor tyrosine kinase, is common in aggressive breast tumors and correlates with poor prognosis. The mechanisms underlying ErbB2-mediated breast carcinoma progression remain incompletely defined. To examine the role of the signaling and cell-adhesion receptor β4 integrin during ErbB2-mediated tumorigenesis, we introduced a targeted deletion of the β4 signaling domain into a mouse model of ErbB2-induced mammary carcinoma. Loss of β4 signaling suppresses mammary tumor onset and invasive growth. Ex vivo studies indicate that β4 forms a complex with ErbB2 and enhances activation of the transcription factors STAT3 and c-Jun. STAT3 contributes to disruption of epithelial adhesion and polarity, while c-Jun is required for hyperproliferation. Finally, deletion of the β4 signaling domain enhances the efficacy of ErbB2-targeted therapy. These results indicate that β4 integrin promotes tumor progression by amplifying ErbB2 signaling and identify β4 as a potential target for molecular therapy of breast cancer.

INTRODUCTION

Breast cancer is the most common malignancy in women and causes over 400,000 deaths per year (Parkin et al., 2005). About 30% of all breast cancers carry amplifications of the *ErbB2* locus, which encodes the receptor tyrosine kinase (RTK) ErbB2 (Slamon et al., 1987). These tumors have an aggressive behavior, high rates of relapse, and poor prognosis (Berger et al., 1988). In agreement with the hypothesis that ErbB2 signaling initiates mammary tumorigenesis, the levels of ErbB2 increase at the onset of ductal carcinoma in situ (DCIS) (van de Vijver et al., 1988). Furthermore, dimerization of ErbB2 induces proliferation and suppresses apoptosis in MCF-10A mammary epithelial acini, yielding solid multiacinar structures resembling DCIS (Muthuswamy et al., 2001). Finally, transgenic mice expressing constitutively activated ErbB2 (*Neu*) in their mammary glands develop invasive ductal carcinomas (Muller et al., 1988), directly implicating ErbB2 signaling in mammary oncogenesis.

Oncogene-induced hyperproliferation and suppression of apoptosis are sufficient to induce luminal filling and expansion of carcinoma in situ in glandular epithelia (Debnath et al., 2002). Progression to invasive carcinoma, however, requires that cancer cells disassemble intercellular junctions, reorientate their cytoskeletons, and finally invade into the interstitial matrix. Certain oncogenic mutations and combinations thereof can cause this gain in motility in vitro, and studies with various cellular models of tumor progression have identified a plethora of putative pathogenic pathways (Thiery, 2002). However, the mechanisms by which *ErbB2* amplifications or other oncogenic lesions promote breast carcinoma progression in vivo remain poorly understood.

Current insight into this problem derives largely from studies on E-cadherin. E-cadherin mediates assembly of adherens junctions and operates to restrain carcinoma invasion. Lobular carcinomas of the breast often exhibit loss-of-function mutations of *E-cadherin* or methylation of its promoter (Cavallaro and Christofori, 2004). Furthermore, they often express elevated levels of the bHLH transcription factor Twist, which silences the expression of E-cadherin as part of its proinvasive epithelial-to-mesenchymal transition (EMT) program (Yang et al., 2004). However, common ductal carcinomas of the breast, including ErbB2-positive tumors, often retain expression of E-cadherin (Gamallo et al., 1993), suggesting that other mechanisms can disrupt epithelial adhesion and polarity in these tumors.

Studies of in vitro models suggest that the α6β4 integrin—a component of hemidesmosomes—contributes to oncogenesis by sustaining RTK signaling. β4 integrin signaling proceeds through Src family kinase (SFK) mediated phosphorylation of the cytoplasmic domain of β4, recruitment of Shc, and activation of Ras and PI-3K (Mainiero et al., 1997; Shaw et al., 1997). The RTKs ErbB2, EGF-R, and Met associate with α6β4, and there is evidence suggesting that they promote invasive signaling through phosphorylation of β4 (Falcioni et al., 1997; Mariotti et al., 2001; Trusolino et al., 2001). Accordingly, wild-type, but not signaling-defective, β4 causes a gain in invasive ability in a breast carcinoma cell line expressing Met (Shaw et al., 1997). In spite of this body of work, the hypothesis that α6β4 has a protumorigenic function remains controversial. Expression of wild-type, but not signaling-defective, β4 activates p53 and induces cell-cycle arrest and apoptosis in rectal carcinoma cells (Bachelder et al., 1999). Furthermore, antibody blockage of α6β4 disrupts mammary epithelial polarity and promotes hyperproliferation in 3D Matrigel (Weaver et al., 1997). These complex and apparently contrasting effects of α6β4 may reflect physiologically distinct roles of this integrin in different cancer cells or intrinsic limitations of these models.

Given the complexity of tumor progression, it is important to use experimental models that closely mimic the evolution of human breast cancer while allowing molecular manipulation. DNA microarray data reveal that the basal-like ductal breast carcinomas and related ErbB2-positive tumors often express high levels of β4 (Sorlie et al., 2001). To examine whether β4 signaling plays a role in ErbB2-mediated mammary tumorigenesis, we have introduced a targeted deletion of the β4 signaling domain in MMTV-*Neu* mice. By combining in vivo analysis with an ex vivo RNAi knockdown/reconstitution approach, we provide evidence that β4 combines with ErbB2 and amplifies its signaling ability, enabling mammary tumor progression.

RESULTS

Deletion of the β4 Signaling Domain Suppresses ErbB2-Driven Mammary Gland Tumorigenesis

Mice carrying a targeted deletion of the β4 signaling domain ($\beta4^{1355T/1355T}$; see Figure S1 in the Supplemental Data available with this article online) exhibit defects in wound healing and postnatal angiogenesis, consistent with the observation that this mutation does not impair α6β4-dependent adhesion or assembly of hemidesmosomes but suppresses β4 signaling (Nikolopoulos et al., 2004, 2005). Whole-mount analysis of ductal outgrowth and immunostaining studies suggest that $\beta4^{1355T/1355T}$ female mice undergo normal mammary gland morphogenesis (Figure S2). In addition, these mice are able to nurse their progeny effectively. These results indicate that loss of β4 signaling does not cause obvious defects in mammary gland development.

To examine the role of β4 signaling in mammary tumorigenesis, we introduced the β4-1355T mutation in MMTV-*Neu(YD)* mice, which progress rapidly from ductal carcinoma in situ to invasive and metastatic carcinoma (Dankort et al., 2001), allowing an examination of the role of β4 signaling during tumor progression. MMTV-*Neu(YD)*;$\beta4^{1355T/1355T}$ (henceforth Neu(YD)/β4-1355T) mice developed palpable tumors significantly later as compared to control MMTV-*Neu(YD)*;$\beta4^{+/+}$ (Neu(YD)/β4-WT) mice: Median tumor onset was delayed by approximately 50% in β4 mutant mice (Figure 1A). Moreover, they displayed significantly fewer tumors as compared to control mice (Figure 1B). Finally, Neu(YD)/β4-1355T mice exhibited greatly reduced cumulative tumor burden at 17 and 22 weeks of age (Figure 1C). Heterozygous MMTV-*Neu(YD)*;$\beta4^{+/1355T}$ mice exhibited an intermediate phenotype (Figure 1A; cumulative tumor burden at 22 weeks: 7.1 g ± 2.1 SD, n = 6), suggesting that β4 signaling is haploinsufficient for mammary tumor onset and growth. Together, these results indicate that deletion of the β4 signaling domain inhibits ErbB2-initiated mammary tumorigenesis.

β4 Signaling Promotes Tumor Cell Proliferation and Suppresses Apoptosis

Neoplastic cells in mammary intraepithelial neoplasia (MIN) lesions of Neu(YD)/β4-WT mice had significantly elevated levels of β4 as compared to normal luminal cells (Figure S3A). β4 was no longer concentrated at the basement-membrane junction but was instead diffusely distributed over the cell surface. The levels of its basement-membrane ligand, laminin-5, were severely reduced. As MIN lesions progressed to invasive carcinomas, laminin-5 became undetectable, but β4 remained elevated (Figure S3B). The tumors of Neu(YD)/β4-1355T mice exhibited a similar upregulation of β4 and downregulation of laminin-5. However, mutant β4 remained in part concentrated at the basement-membrane junction in MIN lesions of these mice (Figure S3A), suggesting that β4 signaling may disrupt epithelial polarity.

Since established tumors of Neu(YD)/β4-1355T mice grew only at a modestly reduced rate (approximately 70% of control value; see Figure 1G and below) and were vascularized to the same extent as those of control Neu(YD)/β4-WT mice (Figure S4), we examined whether loss of β4 signaling inhibits mammary tumor induction or initial growth. Neu(YD)/β4-1355T mice exhibited significantly fewer MIN lesions at 13 weeks of age (2.1 ± 2.4 per median longitudinal section, n = 7 mice) as compared to Neu(YD)/β4-WT mice (10.1 ± 6.6, n = 8 mice, p = 0.01), indicating that loss of β4 signaling inhibits mammary tumor onset and initial growth.

To dissect the mechanism by which loss of β4 signaling suppresses mammary tumorigenesis, preneoplastic and MIN lesions were stained with anti-Ki-67. As shown in Figure 1D, activated ErbB2 induced robust epithelial cell proliferation prior to overt morphological transformation in the ducts and lobules of Neu(YD)/β4-WT mice (age-matched wild-type mice contained only scattered Ki-67$^+$ cells). By contrast, cell proliferation was only modestly