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Co-opted integrin signaling in ErbB2-induced mammary tumor progression

Although almost two decades of study point to a central role for aberrant ErbB2 activation in breast cancer, many cellular and biochemical mechanisms underlying ErbB2-induced tumor initiation and progression remain to be resolved. A study by Guo et al. published recently in *Cell* indicates that the signaling function of β4 integrin actively contributes to the initiation, growth, and invasion of ErbB2-induced mammary tumors in transgenic mice by promoting the activation of c-Jun and STAT3. These observations offer novel mechanistic insight into ErbB2 action and highlight the notion that ErbB2 co-opts the functions of other signaling proteins to elicit tumor progression.

Overexpression of the ErbB2 (HER2/neu) receptor tyrosine kinase is observed in a variety of solid tumor types, including 20%–30% of breast tumors. Overexpression correlates with poor patient prognosis and resistance to some therapies. In vitro, expression of a constitutively active point mutant of ErbB2 is sufficient to mediate the transformation of cultured cells, suggesting that aberrant kinase activation is sufficient to initiate tumorigenic processes. Likewise, expression of an activated form in the mammary epithelium of transgenic mice gives rise to the rapid emergence of invasive tumors, pointing to a central role for ErbB2 activation in breast cancer malignancy. Coupled with reports that ErbB2 overexpression is sufficient to activate its tyrosine kinase activity, such observations have prompted the development of strategies to interfere with ErbB2 activity in breast tumors. Indeed, the humanized anti-ErbB2 monoclonal antibody Herceptin has been in clinical use for over a half dozen years, and other therapies are under development.

While it is clear that aberrant ErbB2 activation actively contributes to the genesis and progression of breast tumors, cellular and biochemical mechanisms underlying ErbB2-mediated proliferation and invasion remain to be fully elucidated. Of particular interest are the mechanisms connecting activated ErbB2 to the breakdown of mammary epithelial cell-cell interactions. ErbB2 activation dissolves interepithelial cell interactions mediated by tight junctions or by adherens junctions through E-cadherin, leading to a loss of cell polarity and the initiation of invasion. An insightful study by Guo et al. (2006) points to a key role for cellular signaling mediated by β4 integrin in ErbB2-mediated proliferation and invasiveness of breast tumor cells and underscores an unappreciated role for STAT3 signaling in mediating the loss of mammary epithelial adhesion.

Hemidesmosomal α6β4 integrin contributes to the anchoring of mammary epithelial cells to the basement membrane through its intracellular interactions with the cytoskeleton and extracellular interactions with the matrix component laminin-5. Several studies suggest that the large intracellular domain of the β4 subunit is also involved in cellular signaling. For example, expression of β4 integrin in β4-deficient cultured breast cancer cells augments cellular invasive properties (Shaw et al., 1997). This effect requires the β4 intracellular domain and is suppressed by inhibitors of PI3 kinase activity, suggesting that β4 signaling to the PI3 kinase pathway augments the invasiveness of these cells. Moreover, blocking antibodies to either α6 or β4 integrin subunits suppress the formation of apoptosis-resistant acinar structures in Matrigel by mammary epithelial cells (Weaver et al., 2002), suggesting a role for β4-mediated cellular polarity in mediating antiapoptotic signaling. Finally, β4 has been demonstrated to physically interact with ErbB2 in some cultured breast tumor cells, and the two proteins synergize in promoting cellular proliferation and invasion (Falcioni et al., 1997). Taken together, these and other in vitro studies strongly point to a potential role for β4 signaling in promoting breast tumor progression.

To examine the role of β4 integrin signaling in ErbB2-induced mammary tumors in vivo, Guo et al. employed a knockin mouse where expression of the endogenous *β4* gene was replaced with a variant (called 1355T) lacking the carboxy terminal ~450 amino acids. This form is capable of interacting with laminin-5 and the keratin cytoskeleton, thus maintaining the ability

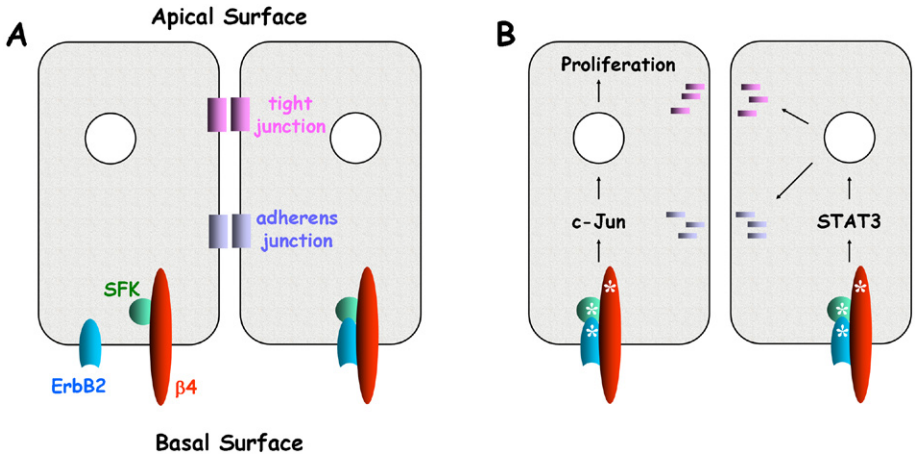

Figure 1. Activated ErbB2 co-opts β4 integrin to promote mammary tumor progression

A: In normal mammary epithelium, basally localized ErbB2 and β4 signal minimally.
B: Activated ErbB2 forms a complex with β4 integrin and a src family kinase (SFK), leading to the tyrosine phosphorylation (asterisks) of all three components and signaling to c-Jun and STAT3. c-Jun signaling mediates cellular proliferation, while STAT3 signaling leads to cell-cell junction breakdown and the initiation of invasion.

to mediate adhesion, but lacks five tyrosine phosphorylation sites (Nikolopoulos et al., 2004). Female knockin animals exhibit normal gross mammary gland morphogenesis and nursing, suggesting that β4 signaling does not play a major role in mammary gland development.

These mice were crossed into a mouse model of mutationally activated ErbB2-induced mammary tumors (Dankort et al., 2001), and the properties of tumors expressing the 1355T variant were compared with tumors expressing wild-type β4 integrin. Loss of the signaling domain delayed the median time for tumor onset and inhibited tumor multiplicity and size, suggesting that signaling by integrin β4 facilitates both ErbB2-induced tumor formation and growth. Moreover, tumors expressing the 1355T form exhibited significantly fewer lung metastases, suggesting that β4 signaling also facilitates ErbB2-mediated tumor invasion and metastasis. These observations confirm previous reports that β4 integrin may synergize with ErbB2 in augmenting mammary epithelial tumorigenic properties and focus attention on the signaling functions of the integrin.

Interestingly, histological analysis of tumors at various stages of development began to reveal mechanistic insights into the role of β4 signaling in mammary tumor progression. In general, tumors from mice expressing the 1355T form tended to be more differentiated, characterized by a glandular appearance, while tumors from mice expressing wild-type β4 were poorly differentiated solid masses. Tumors from mice expressing wild-type β4 exhibited a higher degree of proliferation and a lower degree of apoptosis than tumors from 1355T animals. Moreover, staining for basal plasma membrane and tight junction markers pointed to a loss of cell polarity in tumors from wild-type β4 mice but less so in tumors from 1355T mice. Importantly, treatment of cultured cells from the wild-type β4 tumors with Iressa, a small molecule inhibitor of EGF receptor that can also act toward ErbB2, restored polarity, suggesting that β4 effects on cell polarity were the outcome of signaling events rather than secondary mutations in the tumors. Collectively, these observations suggest that ErbB2 and β4 integrin cooperatively signal the disruption of epithelial adhesion and polarity, contributing to tumor progression by promoting epithelial invasiveness.

To elucidate the molecular mechanisms by which β4 integrin and ErbB2 might cooperate in signaling tumor progression, Guo et al. developed an in vitro knockin system, where expression of the endogenous mouse β4 gene in ErbB2-induced tumor cells was suppressed by RNAi and replaced with either wild-type or 1355T human integrin β4. This system recapitulated most phenotypic aspects of the in vivo tumors, including the augmentation of cellular proliferation and the disruption of adhesion in cells expressing wild-type β4 relative to 1355T. Through a series of coimmunoprecipitation, phosphorylation, and inhibitor studies, the authors developed a model whereby the β4 signaling domain assembles a ternary complex of ErbB2, a src family kinase, and β4 integrin. Thus, its signaling domain permits β4 integrin to interact with ErbB2 and expand its signaling potential.

The abundance of phosphorylated forms of the transcriptional regulators c-Jun and STAT3 in cells expressing wild-type β4 relative to 1355T suggested that these factors could play central roles in mediating synergistic cellular responses to the ErbB2/β4 complex in cells expressing wild-type β4. Indeed, inhibition of c-Jun function in cells suppressed tumor cell proliferation in vitro and tumorigenicity but was unable to restore the assembly of cell-cell junctions or inhibit invasion. Conversely, inhibition of STAT3 function restored assembly of tight and adherens junctions and inhibited invasion but had little impact on proliferation or tumorigenicity. In summary, the signaling domain of β4 integrin allows its physical association with activated ErbB2, leading to the activation of c-Jun and STAT3, and resulting in the independent stimulation of tumor cell proliferation and invasion (Figure 1).

These studies provide novel insight into processes leading to the breakdown of cellular adhesion upon ErbB2 activation. The ErbB2/β4 complex activates STAT3, presumably to regulate the expression of genes that contribute to the structural integrity of cell-cell junctions, particularly tight junctions. This is consistent with a very recent report that STAT3 activation downstream of the EGFR is necessary but not sufficient for prostate tumor cell migration and invasion (Zhou et al., 2006), suggesting a common role for STAT3 involvement in growth factor receptor-induced invasiveness. These observations in turn point to the ErbB2/β4 complex and activated STAT3 as potential targets for therapeutic suppression of breast tumor progression.

These studies additionally underscore the concept that otherwise quiescent signaling molecules may be co-opted by ErbB2 in promoting tumor progression. While β4 signaling contribution to normal mouse mammary gland development is minimal, it is clearly required to fully promote the malignancy of tumors in an activated ErbB2 mouse model. It will be interesting to determine whether the functions of other signaling-related proteins such as ErbB3, CD44, or Muc4 are similarly recruited by ErbB2 to promote the initiation or progression of

mammary tumors. Again, the necessity of such accessory proteins in tumor progression offers novel avenues for therapeutic intervention.

Kermit L. Carraway III[1,*] and Colleen Sweeney[1]

[1]University of California, Davis, Cancer Center, Sacramento, California 95817
*E-mail: klcarraway@ucdavis.edu

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pVHL's kryptonite: E2-EPF UCP

E2-EPF ubiquitin carrier protein (UCP) is a member of an E2 family of enzymes that catalyzes the ligation of ubiquitin to proteins targeted for destruction by the proteasome. UCP is overexpressed in common human cancers, suggesting its involvement in oncogenesis, but a physiologic target of UCP has not been identified. In a recent report published in *Nature Medicine*, Jung et al. identified von Hippel-Lindau (VHL) tumor suppressor protein, which targets the α subunit of hypoxia-inducible factor (HIF) for ubiquitin-mediated destruction, as a bona fide substrate of UCP and demonstrated a potential pVHL-HIF pathway-dependent role for UCP in cancer development.

von Hippel-Lindau disease (OMIM 193300) is a rare hereditary cancer syndrome that is characterized by the development of hypervascular tumors in multiple, and yet specific, organs, including the cerebellum, retina, adrenal gland, and kidney. VHL disease is caused by the inheritance of a faulty *VHL* gene, and the tumors arise when the remaining wild-type *VHL* allele is lost or inactivated via mutation, deletion, or promoter methylation in a susceptible cell. Biallelic inactivation of *VHL* has also been associated with the development of sporadic clear cell renal cell carcinoma (CC-RCC), the most common form of kidney cancer (Kaelin, 2002).

pVHL is a substrate-recruiting component of an E3 ubiquitin ligase called ECV (Elongins/Cul2/pVHL) that is structurally and functionally analogous to the SCF (Skp1/Cdc53/F box protein) complex. Crystal structure of the pVHL/elongin B/elongin C complex revealed two functional domains on pVHL: α and β (Stebbins et al., 1999). The α domain binds elongin C, which acts as a bridge connecting pVHL to the scaffold component Cul2, which binds Rbx1 and a cognate E2 ubiquitin-conjugating enzyme, Cdc34 or UbcH5. The β domain acts as a substrate-docking site. The majority of tumor-associated *VHL* mutations map to the surface residues on either domain, suggesting that these domains are functionally important for the tumor suppressor activity of pVHL.

To date, several cellular proteins have been identified as pVHL binding proteins that are subjected to ECV-dependent ubiquitylation. However, the most convincing substrate that continues to shed significant insight into the tumor suppressor function of pVHL is HIFα (see Figure 1). HIF is a major heterodimeric transcription factor consisting of α and β subunits that transactivates 60 or more hypoxia-inducible genes, including *vascular endothelial growth factor* (*VEGF*; also known as *vascular permeability factor*), *erythropoietin* (*EPO*), and *glucose transporter-1* (*GLUT1*) to promote angiogenesis, oxygen-carrying erythrocyte production, and anaerobic metabolism, respectively, in adaptation to compromised oxygen availability. While the HIFβ subunit (also known as aryl-hydrocarbon receptor nuclear translocator [ARNT]) is abundantly expressed irrespective of oxygen tension, the HIFα subunit is oxygen labile. In the presence of oxygen, HIFα is hydroxylated on conserved prolines within the oxygen-dependent degradation (ODD) domain by prolyl hydroxylase domain-containing enzymes (PHDs). Prolyl hydroxylation is both necessary and sufficient for the binding of HIFα by pVHL and subsequent ubiquitylation via ECV. Accordingly,

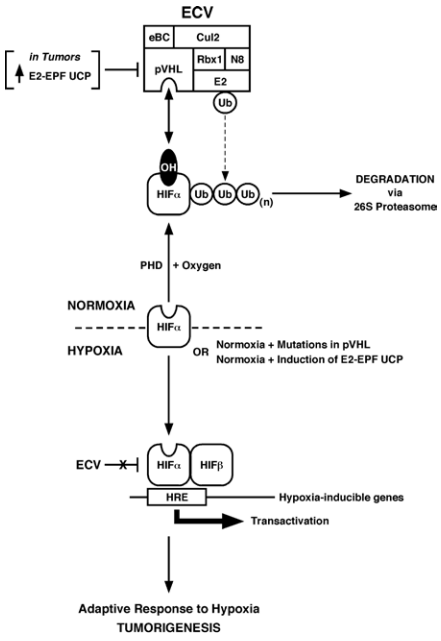

Figure 1. The UCP-pVHL-HIF pathway in cancer

See text for details. eBC, elongins B and C; N8, NEDD8; Ub, ubiquitin; OH, hydroxyl group.

hypoxia or mutation in pVHL leads to the stabilization of HIFα. HIFα then dimerizes with HIFβ to form an active transcriptional complex, which engages the 5′-RCGTG-3′ hypoxia-responsive elements (HREs) within the promoter/enhancer of hypoxia-