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ErbB4/HER4: Role in Mammary Gland Development, Differentiation and Growth Inhibition

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Abstract

The ErbB receptor tyrosine kinase family has often been associated with increased growth of breast epithelial cells, as well as malignant transformation and progression. In contrast, ErbB4/HER4 exhibits unique attributes from a two step proteolytic cleavage which releases an 80 kilodalton, nuclear localizing, tyrosine kinase to a signal transduction mechanism that slows growth and stimulates differentiation of breast cells. This review provides an overview of ErbB4/HER4 in growth and differentiation of the mammary epithelium, including its physiologic role in development, the contrasting growth inhibition/tumor suppression and growth acceleration of distinct ErbB4/HER4 isoforms and a description of the unique cell cycle regulated pattern of nuclear HER4 ubiquitination and destruction.

Keywords

Mammary development; Growth inhibition; HER4 and breast cancer

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Introduction: A Historical Perspective

Almost fifty years ago Stanley Cohen, using a near heroic bioassay, purified a polypeptide from mouse salivary glands whose activity resulted in premature eyelid opening and tooth eruption in neonatal mice [1]. Cohen's isolation of "tooth-lid" factor (quickly renamed epidermal growth factor, or EGF) initiated research in growth factors and their receptors in the modern sense. Biochemical purification, peptide sequencing and cDNA cloning identified the epidermal growth factor receptor (EGFR) as a 170 kDa transmembrane protein [2]; this receptor was the first ligand-activated tyrosine kinase described [3]. The ligands defined by homology to EGF has expanded to include at least ten growth factors across two gene families, which bind to a family of four receptor tyrosine kinases (RTKs) that includes the EGFR [4–9].

These families of ligands and receptors have been firmly linked to proliferative signaling and oncogenesis. The association began with early studies of growth regulation and identification of EGFR family member expression or overexpression in human malignancies, but the link was permanently forged by a surprise arising from the cDNA sequence of the first receptor tyrosine kinase cloned. The EGFR (also known as HER1 or ErbB1) cytoplasmic domain was found to be the human ortholog of the chicken retroviral oncogene, v-erbB. This established this receptor tyrosine kinase's oncogenic potential and identified the EGFR as one of the early proto-oncogenes [2] Isolation of a mutated receptor tyrosine kinase in rat tumors, the neu oncogene, and molecular studies of breast cancer cells led to the identification of a second EGFR/HER2 family member, HER2/ErbB2/Neu [10,11]. Soon labs identified the remaining EGFR family members, HER3/ErbB3 [12,13] and HER4/ErbB4 [14]. Over the years, multiple investigators (see review [8,9,15,16]) have linked the EGFR family to the causation or progression of breast cancer and other epithelial malignancies. Moreover the overexpression is often linked to poor patient outcomes or advanced tumor state. Activation or overexpression of EGFR or HER2/ErbB2 in breast, colon and other tumors has proven to be therapeutically relevant. Small molecule inhibitors of EGFR and HER2/ErbB2 and monoclonal antibodies-targeting EGFR and HER2/ErbB2 have been successfully developed and used to treat human patients with a substantial saving of lives [15,17–19]. For all of these reasons the EGF receptor family is firmly linked to proliferative, prosurvival and oncogenic events.

The first forty years of research into the EGF family were primarily aimed at understanding how the majority of these growth factors stimulate proliferation and survival of epithelial cells, promote tumor formation, and enhance tumor malignancy. However in *Drosophila* and *C. elegans*, a single EGF receptor gene is also linked to differentiation signaling [20] and more recent evidence in mammals indicates that one of the ErbB receptors, HER4/ErbB4, initiates anti-proliferative and pro-differentiation signals. These "non-traditional" actions of ErbB4/HER4 will be the subject of this review.

The ErbB Receptors and Their Ligands

In addition to EGF, other ErbB agonists have been identified that share homology to EGF in a 50-amino acid region known as the EGF-like domain [5,21]. Of these, EGF, transforming growth factor-α and amphiregulin bind exclusively to EGFR/HER1. Betacellulin, epiregulin, and heparin binding EGF-like factor (HB-EGF) bind to both EGFR/HER1 and HER4/ErbB4, whereas heregulin (neuregulin-1) and neuregulin-2 bind to both ErbB3/HER3 and ErbB4/HER4 [5,22–24]. Neuregulin-3 and -4 bind exclusively to ErbB4/HER4. Finally, activities of the ErbB receptors are modified by non-conventional agonists (decorin, tomoregulin, epigen, MUC4, or Cripto-1 [25–27]). The growth factors that bind to the EGF

receptor family members are often expressed in human tumors. Most translational data correlate ligand expression with tumor progression or metastasis in human cancers.

The family's complexity extends beyond these four receptors and ten potential ligands because the various receptors can either homodimerize or heterodimerize in a ligand-dependent way. EGFR and HER4/ErbB4 can homodimerize when bound to ligands, or heterodimerize with any of the other ErbB receptors, with ErbB2/HER2 being the preferred heterodimeric partner. ErbB2/HER2 and ErbB3/HER3 each require heterodimerization in order to transduce signals, because ErbB3/HER3 does not have an intrinsic kinase, while ErbB2/HER2 is not bound by any known ligand [4–6,21,28].

There is perhaps no better example of the biologic complexity of the system and its dependence upon receptor expression and cellular context than the initial studies describing the identification of heregulin/neuregulin-1 [29,30]. One group purified heregulin on the basis of its ability to increase growth of certain cancer cells. Another group isolated NEU differentiation factor based on its anti-proliferative effect in cancer cells. Both factors were encoded by the same gene, underscoring the bidirectional signaling possibility of the same ligand depending upon the cellular context and ErbB family members present.

HER4/ErbB4: Positive Breast Cancer Prognosis, Differentiation, and Growth Inhibitory Signaling

While increased expression and/or activity of EGFR, HER2, and HER3 have been described in human breast cancers, overexpression of HER4/ErbB4 in breast cancer rarely occurs. In fact, HER4/ErbB4 expression is detectable in less than half of breast cancers depending on the technique used [31–35] and correlates in general with increased differentiation and more positive prognostic indicators, consistent with the hypothesis that HER4/ErbB4 signaling promotes differentiation and growth inhibition of breast cells [32,33,35–40]. HER4 expression also predicts a better outcome in patients with carcinoma in situ [41].

Initial studies with several breast cancer cell lines demonstrated heregulin-dependent growth inhibition. For example, in the work that identified and sequenced HER4/ErbB4, Plowman and colleagues demonstrated that activation of HER4/ErbB4 with heregulin slowed growth and produced a more differentiated epithelial phenotype [14]. The initial studies in our lab showed that heregulin slowed the growth of SUM44 breast cancer-derived cells, and induced their morphologic differentiation, including an increase in lipid droplets [42]. HB-EGF also produced this growth inhibitory phenotype, ruling out the role of HER2–HER3 heterodimers in this growth inhibitory response, since HB-EGF is specific for EGFR and HER4/ErbB4 [42]. Intracellular antibody-mediated depletion of cell surface ErbB2/HER2 expression in SUM44 cells did not affect heregulin-mediated growth inhibition, demonstrating that HER4–HER2 heterodimers were not required for growth inhibition in response to heregulin. Because SUM44 cells lack EGFR expression, this suggested that HER4/ErbB4 homodimers inhibited SUM44 cell growth in response to HB-EGF and to HRG. It was also found that exogenous HER4/ErbB4 expression in a HER4-negative breast cancer cell line (SUM102) could confer heregulin-mediated growth inhibition to these otherwise unresponsive cells [42].

HER4/ErbB4 Isoforms, A New Paradigm

Sequencing of HER4/ErbB4 cDNAs revealed that HER4/ErbB4 mRNA could be alternatively spliced in two areas—the first in the extracellular juxtamembrane region (JMa or JMb) [43,44]; and the second in the C-terminus distal to the tyrosine kinase domain (Cyt1 and Cyt2) [45,46]. The JMa isoform introduces an extracellular proteolytic site which is

subject to cleavage by a metalloprotease known as tumor necrosis factor-alpha converting enzyme (TACE) [47]. ErbB4/HER4 cleavage by TACE occurs upon ligand-activation of ErbB4/HER4, and is dependent upon ErbB4/HER4 kinase activity [48]. Metalloprotease-mediated cleavage is seen in other receptor tyrosine kinases. The JMb isoform lacks the TACE cleavage site, and therefore cannot be cleaved.

The importance of ligand-dependent, TACE-mediated cleavage of ErbB4/HER4 became apparent when it was further shown that the JMa isoform, once cleaved by TACE, could undergo a second intramembrane cleavage via a γ-secretase-like activity similar to the genesis of Notch intracellular signaling [49,50]. The secondary cleavage of ErbB4/HER4 releases into the cytoplasm the soluble, 80 kDa intracellular domain of ErbB4, referred to herein as $s80^{HER4}$. Mutation of the critical, γ-secretase cleavage site, the last valine in the transmembrane domain, inhibits the liberation of $s80^{HER4}$ into the cytosol, without impairing the formation of the membrane-tethered m80 isoform [51,52].

HER4/ERBB4 Localizes to the Nucleus

Once liberated into the cytoplasm, $s80^{HER4}$ exhibits nuclear localization. Interestingly, many HER4/ErbB4-positive breast cancers exhibit nuclear HER4/ErbB4. Normal human and mouse mammary tissue, endometrial cancers and normal sensory epithelium all display nuclear HER4/ErbB4 [40,53,54]. Introduction of exogenous GFP-$s80^{HER4}$ by transfection or retroviral infection into breast cancer or mouse mammary cell lines result in the nuclear localization of GFP-$s80^{HER4}$ ([55] and Fig. 1). Nuclear-cytoplasmic translocation of $s80^{HER4}$ may have been predicted based on three putative nuclear localization sequences within the intracellular domain of ErbB4/HER4 [49], one of which has been shown to be required for nuclear localization of the ErbB4 intracellular domain. There are also three putative nuclear export sequences [49], and a D-box motif, an amino acid sequence present in proteins that are targeted by the anaphase-promoting complex, a nuclear E3-ubiquitin ligase [56].

The function of $s80^{HER4}$ in the nucleus, or how its active tyrosine kinase affects nuclear functions is not yet fully understood. It is known, however, that kinase activity of $s80^{HER4}$ is required for its nuclear localization, since kinase-inactive $s80^{HER4}$ is not found in the nucleus [52,55]. Treatment of cells expressing $s80^{HER4}$ with the pan-ErbB small molecule inhibitor GW572016 dramatically reduced the nuclear accumulation of GFP-$s80^{HER4}$, resulting in its cytoplasmic accumulation ([55], Fig. 1), confirming that kinase activity of $s80^{HER4}$ is required for its nuclear localization.

HER4/ErbB4 Alone Can Signal for Growth Inhibition

Guided by these findings and the fact that SUM44 breast cell line expressed the JMa isoform, we examined the role of this unique aspect of HER4/ErbB4 biology. First we used a pan EGF receptor family small molecule tyrosine kinase inhibitor, GW572016, to inhibit the HER4/ErbB4 tyrosine kinase activation, which is necessary for ligand-induced HER4/ErbB4 cleavage and release of $s80^{HER4}$. HER4/ErbB4 tyrosine kinase inhibition blocked the growth inhibitory signal [55]. Next we inhibited $s80^{HER4}$ production by protelysis using a small molecule γ-secretase inhibitor [55]. This too blocked the HER4/ErbB4 anti-proliferative signal; similar experiments were performed by Carpenter and co-workers [49]. Lastly we constructed SUM44 cells stably expressing GFP-$s80^{HER4}$, the GFP-CT^{HER4} (GFP tagged HER4 C-terminus without the tyrosine kinase domain) or GFP alone. GFP-$s80^{HER4}$ translocated to the nucleus and inhibited cell growth. Neither GFP nor GFP-CT^{HER4} had this effect. We have repeated these latter experiments with similar results in multiple breast cell lines including malignant MDA-MB-453 and SUM102 cells as well as non-malignant, MCF-10A, and HC11 (a cell line cultured from pregnant mouse mammary cells). Thus

$s80^{HER4}$ was both necessary and sufficient for HER4-dependent growth inhibition. These results and the majority of clinical translational data from human breast cancer series suggest that HER4/ErbB4 can have tumor suppressor-like activities.

Other studies have demonstrated that HER4/ErbB4 activity can induce apoptosis in breast cancer-derived cells [57]. This work demonstrated mitochondrial accumulation of ErbB4, and interaction between the pro-apoptotic protein BAK and the BH3-like domain within the HER4/ErbB4 intracellular domain. The pro-apoptotic activity of HER4/ErbB4 was counteracted by its interaction with the anti-apoptotic protein Bcl2. These results indicate that ligand-induced HER4/ErbB4 activity may result in death of breast cancer cells, suggesting yet another mechanism by which HER4/ErbB4 limits tumor growth and correlates with a positive prognosis for patients with breast cancer.

ErbB4/HER4 in Embryonic Mammary Bud Specification

Development and maturation of the mammary epithelium requires precise coordination of cellular processes, including cell proliferation, differentiation, and survival. Aberrations in the control of these developmental mechanisms are potentially oncogenic (reviewed in [58]). Normal programming signals involve factors that drive proliferation of the breast epithelium, as well as signals that restrain proliferation. In addition, processes must allow for removal of unneeded cells in a timely fashion. Because the breast epithelium is capable of multiple cycles of robust expansion, for example during puberty and pregnancy, control of these processes is paramount to allow multiple rounds of lactation and for the prevention of tumor formation.

During embryogenesis, mammary gland formation requires an exchange of signals between epithelia and mesenchyme [59]. Signaling molecules from the mesenchyme induce the directional migration, but not proliferation per se, of ectodermally-derived epidermal cells to form the embryonic mammary bud. Within this structure, a population of mammary stem cells exists that will subsequently proliferate to form an arborized epithelium filling the mammary fatpad, and which is capable of milk production [59].

Neuregulin3 (Nrg3), a ligand that binds to ErbB4/HER4, regulates mammary bud specification in mice. Expression of Nrg3 and ErbB4/HER4 in the presumptive mammary region around the future bud site directly precedes the morphological appearance of mammary buds and of bud epithelial markers. Nrg3-soaked beads induced ectopic mammary bud formation and the expression of LEF1, the earliest known mammary bud marker [60]. Furthermore, immunohistochemical localization of Nrg1–4 and ErbB4 occurred at stages prior to morphological appearance of the mammary bud and/or through later embryonic stages of mammary organogenesis [60]. These data suggest that ErbB4/HER4 signaling may directly contribute to inductive events in mammary gland specification.

ErbB4/HER4 in Lactational Differentiation of the Mammary Gland

Aside from specification and formation of the rudimentary mammary epithelium, the majority of mammary gland development occurs postnatal. Prior to puberty, the rudimentary mammary epithelium invaginates from the nipple in the proximal tip of the mammary fat pad. As puberty proceeds, the ductal epithelium proliferates, lengthening and invading the mammary fat pad in a proximal-to-distal direction, until the entire fat pad is permeated with epithelium at the end of puberty, and ductal proliferation ceases. Terminal end buds (TEBs), the club-shaped structures at the distal aspect of the mammary epithelium, represent the most proliferative region of the pubertal mammary gland [59]. Extensive developmental studies in the mouse demonstrate that EGFR/HER1, ErbB2/HER2, and ErbB3/HER3 each

contribute to proliferation and/or survival of MECs, especially during puberty [61]. In the developing mouse mammary gland, expression and activity of ErbB1–3 appear to be highest during phases of epithelial proliferation, such as puberty and pregnancy. Overexpression of ligands that activate EGFR/HER1 signaling results in increased proliferation. For example, increased expression of TGFα in MMTV-TGFα mice results in formation of hyperplastic alveolar nodules (HANs), due to increased proliferation of the mammary epithelium. Similarly, overexpression of an ErbB receptor may increase proliferation of the mammary epithelium [6,7,61]. For example, *MMTV-neu* mice, which overexpress c-Neu (the rat homolog of ErbB2/HER2) in the mammary epithelium, develop hyperplastic glands and metastatic focal carcinomas [6]. Although less is known regarding ErbB3/HER3 in growth and development of the mammary epithelium, recent evidence suggests that loss ErbB3 expression in mice results in impaired proliferation of the mammary epithelium [62], while ErbB3/HER3 is often co-expressed with ErbB2/HER2 in breast cancers, correlating with a poor prognosis, and generating the hypothesis that HER2–HER3 heterodimers may function to simultaneously drive breast epithelial cell proliferation and survival. These data support the proposed role of these members of the ErbB family as stimulators of cellular proliferation in the mammary epithelium.

ErbB4/HER4 signaling is unique among ErbB receptors in the mammary epithelium, in that it is required for the differentiation of mouse mammary epithelial cells during pregnancy and promotes differentiation of murine and human mammary epithelial cells in cell culture, but is not required to drive proliferation of the mammary epithelium. The pituitary peptide hormone prolactin (PRL) controls the differentiation of mammary epithelial cells (MECs) during pregnancy, along with other peptide hormones, steroid hormones, and local growth factors. Upon PRL binding to its receptor, PRLR, expressed on MECs, the intracellular tyrosine kinase Janus kinase 2 (JAK2) is stably recruited to the receptor complex. Tyrosine kinase activation of JAK2 results in tyrosine phosphorylation of PRLR, allowing its association with signal transducer and transcriptional activator 5a (STAT5a) in an SH2-dependent manner. The subsequent phosphorylation of STAT5a by JAK2 triggers activation and nuclear translocation of STAT5a, where STAT5a activates transcription of target genes, including milk protein genes like β-casein [63–66].

Mouse models examining the loss of PRL, PRLR, JAK2, and STAT5a in the mammary epithelium all exhibit an overlapping phenotype of impaired expansion and terminal differentiation of the mammary epithelium during pregnancy, and impaired survival of differentiated mammary epithelial cells, together causing insufficient lactation to support nursing pups [64–66]. Because PRL, PRLR, and JAK2 signals ultimately funnel down to STAT5a, these data strongly suggest that STAT5a mediates indispensable, biologically relevant functions of PRL signaling in normal mammary epithelial cells.

Studies of normal mammary tissue of humans, mice, or rats agree that ErbB4/HER4 expression and activity (measured by tyrosine phosphorylation) is lowest during phases of epithelial cell proliferation (puberty and early pregnancy) and highest during phases of differentiation (late pregnancy and early lactation) [61]. This suggests that ErbB4/HER4 may direct differentiation and growth inhibition of the mammary epithelium. In agreement with this expression data, mammary glands from mice that lack ErbB4 activity by multiple genetic strategies each have lactation defects due an impaired program of differentiation, measured by a decrease in the expression of milk proteins (β-casein and whey acidic protein) and a decrease in activity of the transcription factor STAT5a [67–69]. The phenotype of ErbB4-deficient mammary glands is strikingly similar to what is observed in mammary-specific models of JAK2 [70] and STAT5a-deficiency [71]. Similarly, impaired ErbB4 association with HB-EGF causes a lactation deficiency reminiscent of the phenotype of mammary glands lacking ErbB4, STAT5a, or JAK2 [72]. Furthermore, the ErbB3-ErbB4

ligand Nrg1/HRG induces differentiation of mammary organ cultures and of mammary glands in vivo in a HER2-independent fashion, while disruption of Nrg1/HRG expression impairs late stage differentiation of the mammary epithelium [73]. These data suggest that ErbB4/HER4 directs differentiation of the mammary epithelium.

ErbB4/HER4 and s80^{HER4} Differentiate Normal Mammary Epithelial Cells in Culture

Because ErbB4-deficient mammary epithelial cells fail to demonstrate activation of STAT5a, even in the context of an intact PRL-PRLR-JAK2-STAT5a signaling axis, it was determined that ErbB4 must be an obligate mediator of STAT5a activation in the mammary gland. The mechanism underlying this observation has been elusive, but may relate to the unique proteolytic processing of ErbB4/HER4 following ligand binding that results in liberation of the soluble intracellular domain, s80^{HER4}.

HC11 cells, MECs derived from a mouse at midpregnancy, reliably mimic many of the molecular steps of lactogenic differentiation in culture, including the transcription of the milk protein β-casein, in a STAT5a-dependent manner. Using HC11 cells, it was shown that STAT5a is activated in response to ligand-stimulated ErbB4/HER4 by HRG or HB-EGF, but not in cells expressing kinase-dead ErbB4/HER4. These results suggest that kinase activity of ErbB4/HER4 is required for ErbB4/HER4-mediated STAT5a activation. HC11 cells cultured in three-dimensional Matrigel were induced to form acini, consisting of an organized single epithelial layer surrounding a lumen when cultured in the presence of PRL or ErbB4/HER4 ligands, but not when cultured in the presence of EGF (an EGFR/HER1-specific ligand), nor when cultured in the absence of ligands [52].

Additional studies suggest that formation of s80^{HER4} is required for these aspects of ErbB4/HER4-mediated differentiation [52]. Overexpression of an ErbB4/HER4 variant encoding a point mutation in the γ-secretase cleavage site impaired liberation of s80^{HER4} from full-length ErbB4/HER4, while also inhibiting nuclear translocation of STAT5a in response to HB-EGF. Overexpression of the γ-secretase site mutant of ErbB4/HER4 also impaired PRL-mediated activation of STAT5A activity in response to PRL, suggesting that formation of s80^{HER4} is a critical step in ErbB4/HER4-mediated activation of STAT5a and thus in lactogenic differentiation. Conversely, HC11 cells expressing exogenous s80^{HER4} from a cDNA construct exhibited STAT5a activity even in the absence of PRL, and exhibited rudimentary lumen formation in the absence of PRL. These data suggest that s80^{HER4} formation is sufficient for many aspects of structural and lactogenic differentiation of MECs. It has also been demonstrated that STAT5a interacts directly with the intracellular domain of ErbB4/HER4 in cultured cells. Mutations in the ErbB4 intracellular domain that impair nuclear localization of ErbB4 also impaired nuclear localization of STAT5a [74]. Because STAT5a lacks its own canonical nuclear localization sequences, these observations support the hypothesis that ErbB4/HER4 chaperones STAT5a into the nucleus.

It is also possible that nuclear ErbB4/HER4/ERBB4 influences STAT5a-mediated transcriptional activity in a more direct manner. In support of this idea, ErbB4/HER4 was detected in association with chromatin-bound STAT5a, specifically on sequences located within the β-casein promoter [74]. The idea that nuclear ErbB4/HER4 may regulate the activity of transcriptional complexes is not without precedent. ErbB4/HER4 has been shown to form a complex with transcriptional co-repressors NCoR and TAB2 in vivo. The tripartite complex is translocated to the nucleus in a manner dependent upon s80^{HER4} and its tyrosine kinase. This complex acts as a transcriptional co-repressor in the developing mouse astrocyte, specifically localized to the GFAP promoter regions [75].

HER4/ErbB4 Activity Inhibits Growth of Mammary Epithelial Cells via a G2/M Delay Involving BRCA1

In addition to promoting differentiation of MECs, it has been observed that ErbB4/HER4 activity may decrease their growth. Examination of a panel of human breast cancer-derived cell lines demonstrated that ErbB4/HER4-positive cells were growth inhibited by the ErbB3/ErbB4 ligand heregulin/Nrg1. In contrast, ErbB4/HER4-negative cells displayed increased growth in the presence of heregulin/Nrg1. Heregulin increased the proportion of ErbB4/HER4-positive breast cancer and other cancer cells in the G2/M phase of the cell cycle, but not ErbB4/HER4-negative cells [56,76]. For example, Fig. 2 demonstrates the increase in G2/M phase cells brought about in the HER4 positive SUM44 and BT-474 breast cancer cell lines.

ErbB4-positive cells display a heregulin-mediated increase in the expression of the G2/M checkpoint protein BRCA1 in a HER4/ErbB4-dependent, ErbB2/HER2-independent manner [76], suggesting that perhaps ErbB4/HER4 homodimers were mediating this process, as opposed to HER4-HER2 or HER3-HER2 heterodimers. In a sample of human breast cancers, ErbB4/HER4 mRNA expression showed a positive correlation with BRCA1 mRNA expression [76]. Furthermore, BRCA1 activity is required for heregulin-mediated growth inhibition of breast cells, as knock-down of BRCA1 expression inhibits heregulin-mediated growth inhibition of breast cancer cells, and mammary epithelial cells from a conditional mammary-specific BRCA1 knock-out model were insensitive to heregulin-induced growth inhibition [76]. These results are consistent with a hypothesis that ErbB4/HER4 impairs growth by inducing a G2/M checkpoint, perhaps one involving BRCA1. The signaling mechanism by which ErbB4/HER4 induces growth inhibition or regulates expression of BRCA1 is currently unknown, however all of our evidence supports the concept that production of $s80^{HER4}$ is necessary. Additionally, our data cited earlier that expression of exogenous $s80^{HER4}$ in a panel of breast cancer cells or into “normal” human and mouse mammary epithelial cells (MCF-10A and HC11, respectively) conferred growth inhibition indicates that $s80^{HER4}$ is sufficient for growth inhibition [55]. In some cell lines, this correlated with a delay in G2/M progression and a decrease in the inhibitory phosphorylation of Tyrosine 15 on cdc2 (another molecular marker of late G2 or early M).

These data suggest that $s80^{HER4}$ may engage aspects of the cell cycle machinery at this cell cycle stage. In support of this observation, the intracellular domain of ErbB4/HER4 contains a functional D-Box sequence [56]. These motifs are found in cyclin B and other mitotic targets that are ubiquitylated by the anaphase promoting complex/cyclosome (APC/C) and then destroyed by proteosomal degradation during mitosis. Mutation of the $s80^{HER4}$ D-Box sequence prolongs $s80^{HER4}$ protein half-life and amplifies its ability to produce a G2/M delay (Figs. 3 and 4).

While expression of $s80^{HER4}$ decreased growth of mammary tumor cells in vivo, the expression of the D-box mutant of $s80^{HER4}$ had a more profound effect on the inhibition of polyoma virus middle T-induced mouse breast cancer tumor growth, presumably due to its increased stability [56] (Fig. 5).

This supports the idea that $s80^{HER4}$ may be a tumor suppressor protein, perhaps accessing the cell cycle machinery in some cells. These results further suggest that nuclear $s80^{HER4}$ destruction may be necessary for mitosis to proceed, and that targeted destruction of nuclear $s80^{HER4}$ may represent one mechanism used by cancer cells to escape ErbB4-mediated growth inhibition.

Expression of ErbB4/HER4 at the Cell Membrane and in the Nucleus Correlates with Improved Prognosis in Patients with Breast Cancer

Loss of HER4/ErbB4 activity impairs lactogenic differentiation of the mammary epithelium. This observation is very intriguing in light of the studies demonstrating the protective effects of lactation and involution against tumor formation in the mammary epithelium, due perhaps to the terminal differentiation and clearing of a large population of mammary epithelial cells. Furthermore, the ability of ErbB4/HER4 to induce differentiation of the mammary epithelium may impact tumor grade, and therefore, patient outcome, since the presence of a more differentiated phenotype generally correlates with a more favorable prognosis in human breast cancers. Consistent with this idea, most studies agree that tumors expressing ErbB4/HER4 generally present with a more favorable prognosis [31–34]. It is thus conceivable that ErbB4/HER4 and/or $s80^{HER4}$ may confer a decreased susceptibility to tumor formation or tumor progression, perhaps through growth inhibition or perhaps through enhanced differentiation, or both.

However, conflicting evidence has been reported, in which ErbB4/HER4 expression increases the growth of estrogen-responsive cancer cells in culture [77–78]. Reminiscent of these cell culture studies, there are conflicting reports regarding the prognostic value of ErbB4/HER4 expression in human breast cancers. Some reports demonstrate that HER4/ErbB4 expression in the nucleus correlates with positive prognostic indicators (estrogen receptor expression, lower tumor grade, increased differentiation, lower proliferative index) and greater disease-free and overall survival [40]. However, contrasting reports describe decreased survival in subsets of women whose breast cancers express nuclear HER4/ERBB4 [37]. This observation must be clarified, however. This study indicates that HER4/ErbB4 expression correlates with a more positive prognosis compared to tumors that do not express HER4/ErbB4. It is within this subset of tumors with a good prognosis that this distinction was made between tumors bearing nuclear HER4/ErbB4 and those bearing membrane/cytoplasmic HER4/ErbB4 [34,37].

ErbB4/HER4 Cytoplasmic Isoforms, CYT1 and CYT2: Distinct Signaling Capabilities?

This discrepancy may stem from the different biological properties of the ErbB4/HER4 splice variants, of which there are four. The juxta-membrane isoforms, JMa and JMb, differ within the stalk region of the extracellular domain, with JMa harboring a TACE cleavage site, and JMb lacking this TACE cleavage site [43,44]. Splice variations within the cytoplasmic domain generate the Cyt1 and Cyt2 isoforms. Cyt1 contains a 16-amino acid sequence absent in Cyt2 [45,46]. This stretch of amino acids contains putative binding sites for PI3-kinase [45] and WW domain-containing proteins [78,79]. Much of the work describing growth inhibition and differentiation in response to ErbB4/HER4 signaling was done with the JMa-Cyt1 isoform [42,52,55,56]. Similarly, growth inhibition and lactogenic differentiation in response to $s80^{HER4}$ signaling has been studied primarily with the Cyt1 variant of $s80^{HER4}$ [52,55,56]. In contrast, the JMa-Cyt2 isoform of ErbB4/HER4 was shown to enhance cell viability of 32D cells, while the JMa-Cyt1 did not [81]. Note that both HER4-Cyt1 and HER4-Cyt2 have been previously detected in normal human mammary tissue as well as in many clinical breast cancer specimens and human breast cancer cell lines [37].

There are at least two signaling motifs within the 16 amino acids that are specific to the Cyt1 isoform, and absent from the Cyt2 isoform, including a tyrosine, that when phosphorylated, yields an SH2-binding site for the p85 subunit of PI3 kinase [45]. The second potential

signaling motif is a proline-rich region, PPPAY, which binds WW domain-containing proteins [79] Because HER4-Cyt2 lacks the 16 amino acid insert, it lacks this potential binding site for PI3-kinase and WW-domain proteins. Several WW domain-containing proteins have been shown to interact with $s80^{HER4}$, including Wwox, a known tumor suppressor protein that shuttles between the nucleus and cytoplasm (like $s80^{HER4}$), and is reported to be absent at the genomic level in many breast cancers [79,82]. However, there are two PPPxY motifs outside of the Cyt1 specific motif PPPAY that mediate interactions between Wwox and HER4-Cyt1 or HER4-Cyt2. YAP is another WW domain-containing protein that interacts with ErbB4/HER4 [83], but unlike with Wwox, displayed stronger binding to the Cyt1 than to the Cyt2 isoform of ErbB4/HER4 (Shumang Feng et. al. preliminary data).

Recent studies suggest that HER4-Cyt2 exhibits greater stability as compared to HER4-Cyt1 [84]. Our preliminary studies suggest that the WW domain containing E3 ubiquitin ligase known as WWP1 interacts with HER4-Cyt1 preferentially to HER4-Cyt2, and directs the degradation of HER4-Cyt1 (Feng et. al., preliminary data). Given that E3 ubiquitin ligases often target proteins for proteosomal degradation, this may explain why HER4-Cyt2 exhibits greater stability that HER4-Cyt1. Although the role of PI3 kinase, WWP1, YAP, or Wwox in $s80^{HER4}$-mediated growth control is currently unknown, data from our lab and others indicate that the 16-amino acid Cyt1 domain engages signaling pathways to decrease growth of mammary epithelial cells. The destruction of this powerful signaling agent, HER4/ErbB4 and its products $s80^{HER4}$ and $m80^{HER4}$ and may involve a series of processes and different ubiquitination systems depending on their location in the cell. For example HER4/ErbB4 appears to be subject to degradation by several WW domain containing E3 ligases (Itch and WWP1) [80] and nuclear $s80^{HER4}$ is tagged for ubiquitin dependent degradation by the anaphase promoting complex [56].

Conclusions Regarding HER4/ERBB4 and Future Directions

Despite years of study, mechanistic insights into the action of EGFR/ErbB family members are still being made. Activation at the cell surface targets multiple signaling cascades in the cytoplasm that transmit signals into the nucleus. In addition to this classic model of RTK signaling, recent reports demonstrate that each member of the ErbB family can traffic to the nucleus, opening the possibility that each receptor may have direct nuclear substrates. However, while nuclear localization of the first three members of the receptor family appears to involve full length receptor by translocation mechanisms not yet defined, the release of $s80^{HER4}$, its nuclear localization and export sequences, its role in STAT5a nuclear action, its metabolism by D Box-dependent, APC/C-mediated ubiquitination and its localization in neural transcription complexes, all clearly indicate a nuclear role for this unique transmembrane tyrosine kinase. The molecular pathways accessed by ErbB4/HER4 achieve growth inhibition and differentiation of mammary epithelial cells and strongly support a growth inhibitory/differentiation role for ErbB4/HER4 in the breast.

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Abbreviations

EGF	epidermal growth factor

EGFR	epidermal growth factor receptor
kDa	kilodalton
RTK	receptor tyrosine kinase
HB-EGF	heparin binding epidermal growth factor-like factor
TACE	tumor necrosis factor-alpha converting enzyme
GFP-CT^{HER4}	GFP tagged HER4 C-terminus w/o the tyrosine kinase domain
Nrg3	Neuregulin3
TEBs	terminal end buds
PRL	prolatin
MECs	mammary epithelial cells
JAK2	Janus kinase 2
STAT5a	signal transducer and transcriptional activator 5a
APC/C	anaphase promoting complex/cyclosome
HANs	hyperplastic alveolar nodules

References

1. Cohen S. Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the new-born animal. J Biol Chem USA. 1962; 237:1555–1562.
2. Downward J, Yarden Y, Mayes E, Scrace G, Totty N, Stockwell P, et al. Close similarity of epidermal growth factor receptor and v-erb-B oncogene protein sequences. Nature. 1984; 307:521–527. [PubMed: 6320011]
3. Carpenter G, King L Jr, Cohen S. Rapid enhancement of protein phosphorylation in A-431 cell membrane preparations by epidermal growth factor. J Biol Chem. 1979; 254:4884–4891. [PubMed: 312292]
4. Earp HS, Dawson TL, Li X, Yu H. Heterodimerization and functional interaction between EGF receptor family members: a new signaling paradigm with implications for breast cancer research. Breast Cancer Res Treat. 1995; 35:115–132. [PubMed: 7612898]
5. Yarden Y, Sliwkowski MX. Untangling the ErbB signaling network. Nat Rev Mol Cell Biol. 2001; 2:127–137. [PubMed: 11252954]
6. Olayioye MA, Neve RM, Lane HA, Hynes NE. The ErbB signaling network: receptor heterodimerization in development and cancer. Embo J. 2000; 19:3159–3167. [PubMed: 10880430]
7. Stern DF. ErbBs in mammary development. Exp Cell Res. 2003; 284:89–98. [PubMed: 12648468]
8. Stern DF. Tyrosine kinase signaling in breast cancer: ErbB family receptor tyrosine kinases. Breast Cancer Res. 2000; 2:176–183. [PubMed: 11250707]
9. Hynes NE. ErbB2 activation and signal transduction in normal and malignant mammary cells. J Mammary Gland Biol Neoplasia. 1996; 1:199–206. [PubMed: 10887493]
10. Schechter AL, Hung MC, Vaidyanathan L, Weinberg RA, Yang-Feng TL, Francke U, et al. The neu gene: an erbB-homologous gene distinct from and unlinked to the gene encoding the EGF receptor. Science. 1985; 229:976–978. [PubMed: 2992090]
11. King CR, Kraus MH, Aaronson SA. Amplification of a novel verbB-related gene in a human mammary carcinoma. Science. 1985; 229:974–976. [PubMed: 2992089]
12. Plowman GD, Whitney GS, Neubauer MG, Green JM, McDonald VL, Todaro GJ, et al. Molecular cloning and expression of an additional epidermal growth factor receptor-related gene. Proc Natl Acad Sci USA. 1990; 87:4905–4909. [PubMed: 2164210]

13. Kraus MH, Issing W, Miki T, Popescu NC, Aaronson SA. Isolation and characterization of ERBB3, a third member of the ERBB/epidermal growth factor receptor family: evidence for overexpression in a subset of human mammary tumors. Proc Natl Acad Sci USA. 1989; 86:9193–9197. [PubMed: 2687875]
14. Plowman GD, Culouscou JM, Whitney GS, Green JM, Carlton GW, Foy L, et al. Ligand-specific activation of HER4/p180erbB4, a fourth member of the epidermal growth factor receptor family. Proc Natl Acad Sci USA. 1993; 90:1746–1750. [PubMed: 8383326]
15. Gschwind A, Fischer OM, Ullrich A. The discovery of receptor tyrosine kinases: targets for cancer therapy. Nat Rev Cancer. 2004; 4:361–370. [PubMed: 15122207]
16. Troyer KL, Lee DC. Regulation of mouse mammary gland development and tumorigenesis by the ERBB signaling network. J Mammary Gland Biol Neoplasia. 2001; 6:7–21. [PubMed: 11467454]
17. Anderson NG, Ahmad T. ErbB receptor tyrosine kinase inhibitors as therapeutic agents. Front Biosci. 2002; 7:1926–1940.
18. Hynes NE, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer. 2005; 5:341–354. [PubMed: 15864276]
19. Arteaga C. Targeting HER1/EGFR: a molecular approach to cancer therapy. Semin Oncol. 2003; 30:3–14.
20. Lacenere CJ, Sternberg PW. Regulation of EGF receptor signaling in the fruitfly D. melanogaster and the nematode C. elegans. Breast Dis. 2000; 11:19–30. [PubMed: 15687590]
21. Pinkas-Kramarski R, Alroy I, Yarden Y. ErbB receptors and EGF-like ligands: cell lineage determination and oncogenesis through combinatorial signaling. J Mammary Gland Biol Neoplasia. 1997; 2:97–107. [PubMed: 10882296]
22. Crovello CS, Lai C, Cantley LC, Carraway KL 3rd. Differential signaling by the epidermal growth factor-like growth factors neuregulin-1 and neuregulin-2. J Biol Chem. 1998; 273:26954–26961. [PubMed: 9756944]
23. Daly JM, Olayioye MA, Wong AM, Neve R, Lane HA, Maurer FG, et al. NDF/heregulin-induced cell cycle changes and apoptosis in breast tumor cells: role of PI3 kinase and p38 MAP kinase pathways. Oncogene. 1999; 18:3440–3451. [PubMed: 10376522]
24. Falls DL. Neuregulins: functions, forms, and signaling strategies. Exp Cell Res. 2003; 284:14–30. [PubMed: 12648463]
25. Bianco C, Kannan S, De Santis M, Seno M, Tang CK, Martinez-Lacaci I, et al. Cripto-1 indirectly stimulates the tyrosine phosphorylation of erb B-4 through a novel receptor. J Biol Chem. 1999; 274:8624–8629. [PubMed: 10085099]
26. Carpenter G. ErbB-4: mechanism of action and biology. Exp Cell Res. 2003; 284:66–77. [PubMed: 12648466]
27. Normanno N, Kim N, Wen D, Smith K, Harris AL, Plowman G, et al. Expression of messenger RNA for amphiregulin, heregulin, and cripto-1, three new members of the epidermal growth factor family, in human breast carcinomas. Breast Cancer Res Treat. 1995; 35:293–297. [PubMed: 7579500]
28. Riese DJ 2nd, Stern DF. Specificity within the EGF family/ErbB receptor family signaling network. Bioessays. 1998; 20:41–48. [PubMed: 9504046]
29. Holmes WE, Sliwkowski MX, Akita RW, Henzel WJ, Lee J, Park JW, et al. Identification of heregulin, a specific activator of p185erbB2. Science. 1992; 256:1205–1210. [PubMed: 1350381]
30. Peles E, Bacus SS, Koski RA, Lu HS, Wen D, Ogden SG, Levy RB, et al. Isolation of the neu/HER-2 stimulatory ligand: a 44 kd glycoprotein that induces differentiation of mammary tumor cells. Cell. 1992; 69:205–216. [PubMed: 1348215]
31. Vogt U, Bielawski K, Schlotter CM, Bosse U, Falkiewicz B, Podhajska AJ. Amplification of erbB-4 oncogene occurs less frequently than that of erbB-2 in primary human breast cancer. Gene. 1998; 223:375–380. [PubMed: 9858771]
32. Abd El-Rehim DM, Ball G, Pinder SE, Rakha E, Paish C, Robertson JF, et al. High-throughput protein expression analysis using tissue microarray technology of a large well-characterized series identifies biologically distinct classes of breast cancer confirming recent cDNA expression analyses. Int J Cancer. 2005; 116:340–350. [PubMed: 15818618]

33. Suo Z, Risberg B, Kalsson MG, Willman K, Tierens A, Skovlund E, et al. EGFR family expression in breast carcinomas. c-erbB-2 and c-erbB-4 receptors have different effects on survival. J Pathol. 2002; 196:17–25. [PubMed: 11748637]
34. Witton CJ, Reeves JR, Going JJ, Cooke TG, Bartlett JM. Expression of the HER1–4 family of receptor tyrosine kinases in breast cancer. J Pathol. 2003; 200:290–297. [PubMed: 12845624]
35. Tovey SM, Witton CJ, Bartlett JM, Stanton PD, Reeves JR, Cooke TG. Outcome and human epidermal growth factor receptor (HER) 1–4 status in invasive breast carcinomas with proliferation indices evaluated by bromodeoxyuridine labeling. Breast Cancer Res. 2004; 6:R246–R251. [PubMed: 15084248]
36. Bacus SS, Zelnick CR, Plowman G, Yarden Y. Expression of the erbB-2 family of growth factor receptors and their ligands in breast cancers. Implication for tumor biology and clinical behavior. Am J Clin Pathol. 1994; 102:S13–S24. [PubMed: 7942609]
37. Junttila TT, Sundvall M, Lundin M, Lundin J, Tanner M, Härkönen P, et al. Cleavable ErbB4 isoform in estrogen receptor-regulated growth of breast cancer cells. Cancer Res. 2005; 65:1384–1393. [PubMed: 15735025]
38. Knowlden JM, Gee JM, Seery LT, Farrow L, Gullick WJ, Ellis IO, et al. c-erbB3 and c-erbB4 expression is a feature of the endocrine responsive phenotype in clinical breast cancer. Oncogene. 1998; 17:1949–1957. [PubMed: 9788438]
39. Pawlowski V, Révillion F, Hebbar M, Hornez L, Peyrat JP. Prognostic value of the type I growth factor receptors in a large series of human primary breast cancers quantified with a real-time reverse transcription-polymerase chain reaction assay. Clin Cancer Res. 2000; 6:4217–4225. [PubMed: 11106235]
40. Srinivasan R, Gillett CE, Barnes DM, Gullick WJ. Nuclear expression of the c-erbB-4/HER-4 growth factor receptor in invasive breast cancers. Cancer Res. 2000; 60:1483–1487. [PubMed: 10749108]
41. Barnes NL, Khavari S, Boland GP, Cramer A, Knox WF, Bundred NJ. Absence of HER4 expression predicts recurrence of ductal carcinoma in situ of the breast. Clin Cancer Res. 2005; 11:2163–2168. [PubMed: 15788662]
42. Sartor CI, Zhou H, Kozlowska E, Guttridge K, Kawata E, Caskey L, et al. Her4 mediates ligand-dependent antiproliferative and differentiation responses in human breast cancer cells. Mol Cell Biol. 2001; 21:4265–4275. [PubMed: 11390655]
43. Elenius K, Corfas G, Paul S, Choi CJ, Rio C, Plowman GD, et al. A novel juxtamembrane domain isoform of HER4/ErbB4. Isoform-specific tissue distribution and differential processing in response to phorbol ester. J Biol Chem. 1997; 272:26761–26768. [PubMed: 9334263]
44. Gilbertson R, Hernan R, Pietsch T, Pinto L, Scotting P, Allibone R, et al. Novel ERBB4 juxtamembrane splice variants are frequently expressed in childhood medulloblastoma. Genes Chromosomes Cancer. 2001; 31:288–294. [PubMed: 11391800]
45. Elenius K, Choi CJ, Paul S, Santiestevan E, Nishi E, Klagsbrun M. Characterization of a naturally occurring ErbB4 isoform that does not bind or activate phosphatidyl inositol 3-kinase. Oncogene. 1999; 18:2607–2615. [PubMed: 10353604]
46. Kainulainen V, Sundvall M, Maatta JA, Santiestevan E, Klagsbrun M, Elenius K. A natural ErbB4 isoform that does not activate phosphoinositide 3-kinase mediates proliferation but not survival or chemotaxis. J Biol Chem. 2000; 275:8641–8649. [PubMed: 10722704]
47. Rio C, Buxbaum JD, Peschon JJ, Corfas G. Tumor necrosis factor-alpha-converting enzyme is required for cleavage of erbB4/HER4. J Biol Chem. 2000; 275:10379–10387. [PubMed: 10744726]
48. Vecchi M, Rudolph-Owen LA, Brown CL, Dempsey PJ, Carpenter G. Tyrosine phosphorylation and proteolysis. Pervanadate-induced, metalloprotease-dependent cleavage of the ErbB-4 receptor and amphiregulin. J Biol Chem. 1998; 273:20589–20595. [PubMed: 9685416]
49. Ni CY, Murphy MP, Golde TE, Carpenter G. g-Secretase cleavage and nuclear localization of ErbB-4 receptor tyrosine kinase. Science. 2001; 294:2179–2181. [PubMed: 11679632]
50. Lee HJ, Jung KM, Huang YZ, Bennett LB, Lee JS, Mei L, et al. Presenilin-dependent g-secretase-like intramembrane cleavage of ErbB4. J Biol Chem. 2002; 277:6318–6323. [PubMed: 11741961]

51. Vidal GA, Naresh A, Marrero L, Jones FE. Presenilin-dependent g-secretase processing regulates multiple ERBB4/HER4 activities. J Biol Chem. 2005; 280:19777–19783. [PubMed: 15746097]
52. Muraoka-Cook RS, Sandahl M, Husted C, Hunter D, Miraglia L, Feng SM, et al. The intracellular domain of ErbB4 induces differentiation of mammary epithelial cells. Mol Biol Cell. 2006; 17:4118–4129. [PubMed: 16837552]
53. Long W, Wagner KU, Lloyd KC, Binart N, Shillingford JM, Hennighausen L, et al. Impaired differentiation and lactational failure of Erbb4-deficient mammary glands identify ERBB4 as an obligate mediator of STAT5. Development. 2003; 130:5257–5268. [PubMed: 12954715]
54. Zhang M, Ding D, Salvi R. Expression of heregulin and ErbB/Her receptors in adult chinchilla cochlear and vestibular sensory epithelium. Hear Res. 2002; 169:56–68. [PubMed: 12121740]
55. Feng SM, Sartor CI, Hunter D, Zhou H, Yang X, Caskey LS, et al. The HER4 cytoplasmic domain, but not its C terminus, inhibits mammary cell proliferation. Mol Endocrinol. 2007; 21:1861–1876. [PubMed: 17505063]
56. Strunk KE, Husted C, Miraglia LC, Sandahl M, Rearick WA, Hunter DM, et al. HER4 D-box sequences regulate mitotic progression and degradation of the nuclear HER4 cleavage product s80HER4. Cancer Res. 2007; 67:6582–6590. [PubMed: 17638867]
57. Naresh A, Long W, Vidal GA, Wimley WC, Marrero L, Sartor CI, et al. The ERBB4/HER4 intracellular domain 4ICD is a BH3-only protein promoting apoptosis of breast cancer cells. Cancer Res. 2006; 66:6412–6422. [PubMed: 16778220]
58. Chodosh LA. The reciprocal dance between cancer and development. N Engl J Med. 2002; 347:134–136. [PubMed: 12110743]
59. Howlin J, McBryan J, Martin F. Pubertal mammary gland development: insights from mouse models. J Mammary Gland Biol Neoplasia. 2006; 11:283–297. [PubMed: 17089203]
60. Howard B, Panchal H, McCarthy A, Ashworth A. Identification of the scaramanga gene implicates Neuregulin3 in mammary gland specification. Genes Dev. 2005; 19:2078–2090. [PubMed: 16140987]
61. Schroeder JA, Lee DC. Dynamic expression and activation of ERBB receptors in the developing mouse mammary gland. Cell Growth Differ. 1998; 9:451–464. [PubMed: 9663464]
62. Qu S, Rinehart C, Wu HH, Wang SE, Carter B, Xin H, et al. Gene targeting of ErbB3 using a Cre-mediated unidirectional DNA inversion strategy. Genesis. 2006; 44:477–486. [PubMed: 16991114]
63. Horseman ND. Prolactin and mammary gland development. J Mammary Gland Biol Neoplasia. 1999; 4:79–88. [PubMed: 10219908]
64. Bole-Feysot C, Goffin V, Edery M, Binart N, Kelly PA. Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. Endocr Rev. 1998; 19:225–268. [PubMed: 9626554]
65. Brisken C, Kaur S, Chavarria TE, Binart N, Sutherland RL, Weinberg RA, et al. Prolactin controls mammary gland development via direct and indirect mechanisms. Dev Biol. 1999; 210:96–106. [PubMed: 10364430]
66. Grimm SL, Seagroves TN, Kabotyanski EB, Hovey RC, Vonderhaar BK, Lydon JP, et al. Disruption of steroid and prolactin receptor patterning in the mammary gland correlates with a block in lobuloalveolar development. Mol Endocrinol. 2002; 16:2675–2691. [PubMed: 12456789]
67. Jones FE, Welte T, Fu XY, Stern DF. ErbB4 signaling in the mammary gland is required for lobuloalveolar development and Stat5 activation during lactation. J Cell Biol. 1999; 147:77–88. [PubMed: 10508857]
68. Long W, Wagner KU, Lloyd KC, Binart N, Shillingford JM, Hennighausen L, et al. Impaired differentiation and lactational failure of Erbb4-deficient mammary glands identify ERBB4 as an obligate mediator of STAT5. Development. 2003; 130:5257–5268. [PubMed: 12954715]
69. Tidcombe H, Jackson-Fisher A, Mathers K, Stern DF, Gassmann M, Golding JP. Neural and mammary gland defects in ErbB4 knockout mice genetically rescued from embryonic lethality. Proc Natl Acad Sci USA. 2003; 100:8281–8286. [PubMed: 12824469]
70. Wagner KU, Krempler A, Triplett AA, Qi Y, George NM, Zhu J, et al. Impaired alveologenesis and maintenance of secretory mammary epithelial cells in Jak2 conditional knockout mice. Mol Cell Biol. 2004; 24:5510–5520. [PubMed: 15169911]

71. Liu X, Robinson GW, Wagner KU, Garrett L, Wynshaw-Boris A, Hennighausen L. Stat5a is mandatory for adult mammary gland development and lactogenesis. Genes Dev. 1997; 11:179–186. [PubMed: 9009201]
72. Yu WH, Woessner JF Jr, McNeish JD, Stamenkovic I. CD44 anchors the assembly of matrilysin/MMP-7 with heparin-binding epidermal growth factor precursor and ErbB4 and regulates female reproductive organ remodeling. Genes Dev. 2002; 16:307–323. [PubMed: 11825873]
73. Li L, Cleary S, Mandarano MA, Long W, Birchmeier C, Jones FE. The breast proto-oncogene, HRGalpha regulates epithelial proliferation and lobuloalveolar development in the mouse mammary gland. Oncogene. 2002; 21:4900–4907. [PubMed: 12118369]
74. Williams CC, Allison JG, Vidal GA, Burow ME, Beckman BS, Marrero L, et al. The ERBB4/HER4 receptor tyrosine kinase regulates gene expression by functioning as a STAT5A nuclear chaperone. J Cell Biol. 2004; 167:469–478. [PubMed: 15534001]
75. Sardi SP, Murtie J, Koirala S, Patten BA, Corfas G. Presenilin-dependent ErbB4 nuclear signaling regulates the timing of astrogenesis in the developing brain. Cell. 2006; 127:185–197. [PubMed: 17018285]
76. Muraoka-Cook RS, Caskey LS, Sandahl MA, Hunter DM, Husted C, Strunk KE, et al. Heregulin-dependent delay in mitotic progression requires HER4 and BRCA1. Mol Cell Biol. 2006; 26:6412–6424. [PubMed: 16914727]
77. Tang CK, Concepcion XZ, Milan M, Gong X, Montgomery E, Lippman ME. Ribozyme-mediated down-regulation of ErbB-4 in estrogen receptor-positive breast cancer cells inhibits proliferation both in vitro and in vivo. Cancer Res. 1999; 59:5315–5322. [PubMed: 10537315]
78. Cohen BD, Green JM, Foy L, Fell HP. HER4-mediated biological and biochemical properties in NIH 3T3 cells. Evidence for HER1-HER4 heterodimers. J Biol Chem. 1996; 271:4813–4818. [PubMed: 8617750]
79. Aqeilan RI, Donati V, Palamarchuk A, Trapasso F, Kaou M, Pekarsky Y, et al. WW domain-containing proteins, WWOX and YAP, compete for interaction with ErbB-4 and modulate its transcriptional function. Cancer Res. 2005; 65:6764–6772. [PubMed: 16061658]
80. Omerovic J, Santangelo L, Puggioni EM, Marrocco J, Dall'Armi C, Palumbo C, et al. The E3 ligase Aip4/Itch ubiquitinates and targets ErbB-4 for degradation. Faseb J. 2007; 21:2849–2862. [PubMed: 17463226]
81. Määttä JA, Sundvall M, Junttila TT, Peri L, Laine VJ, Isola J, et al. Proteolytic cleavage and phosphorylation of a tumor-associated ErbB4 isoform promote ligand-independent survival and cancer cell growth. Mol Biol Cell. 2006; 17:67–79. [PubMed: 16251361]
82. Aqeilan RI, Croce CM. WWOX in biological control and tumorigenesis. J Cell Physiol. 2007; 212:307–310. [PubMed: 17458891]
83. Komuro A, Nagai M, Navin NE, Sudol M. WW domain-containing protein YAP associates with ErbB-4 and acts as a co-transcriptional activator for the carboxyl-terminal fragment of ErbB-4 that translocates to the nucleus. J Biol Chem. 2003; 278:33334–33341. [PubMed: 12807903]
84. Sundvall M, Peri L, Määttä JA, Tvorogov D, Paatero I, Savisalo M, et al. Differential nuclear localization and kinase activity of alternative ErbB4 intracellular domains. Oncogene. 2007; 26:6905–6914. [PubMed: 17486069]

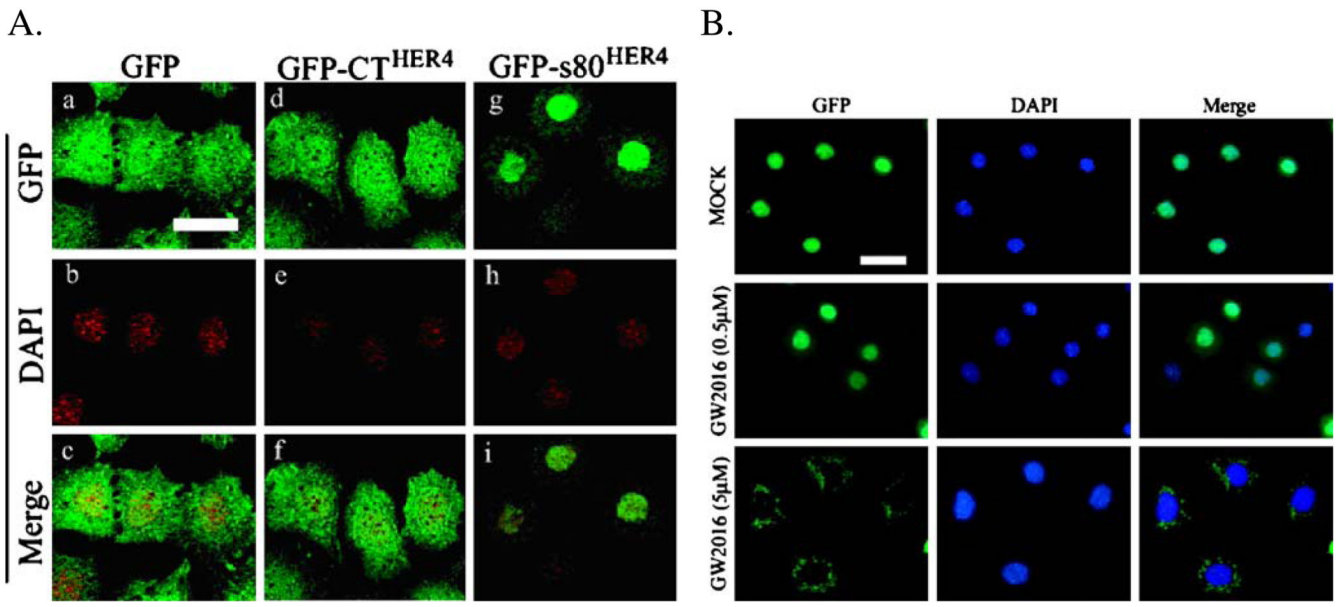

Figure 1.

Nuclear localization of HER4/ErbB4 in mouse HC11 cells. **a** Localization of GFP fluorescence in HC11 cells stably expressing GFP, GFP-CTH^{ER4} (the C-terminal residues 989–1308 of HER4/ErbB4, lacking the kinase domain), or GFP-s80^{HER4}. **b** HC11 cells stably expressing GFP-s80^{HER4} were mock treated or treated for 24 h with 0.5 μM GW572016 (a dose that does not abolish s80^{HER4} tyrosine kinase activity) or 5 μM GW572016 (a dose sufficient to substantially reduce s80^{HER4} tyrosine kinase activity). The counter-stain with DAPI is shown as is the merge of the two images (GFP and DAPI). *Bar*=30 μm, for both (**a**) and (**b**).

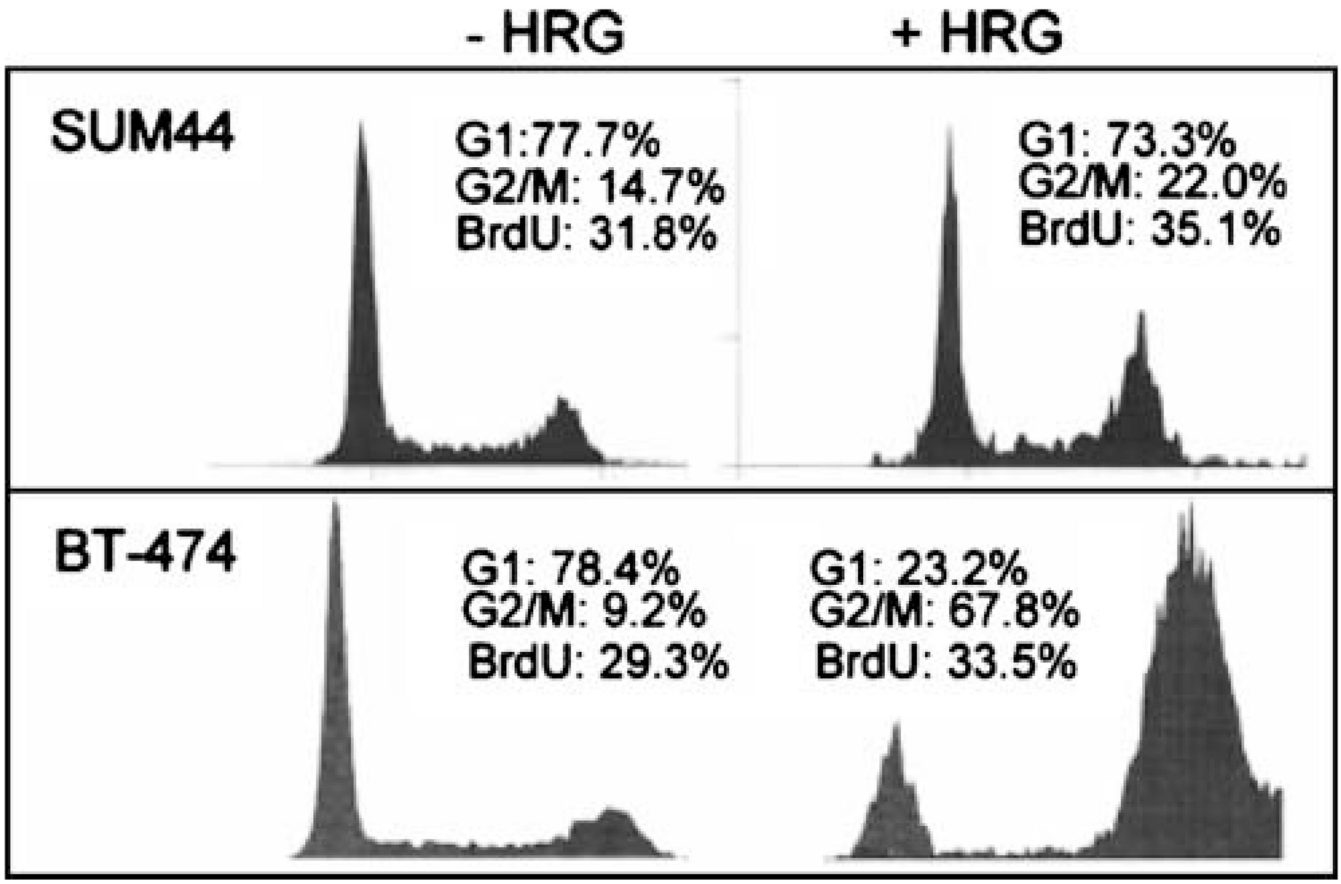

Figure 2.

Heregulin-mediated growth inhibition of HER4 positive breast cancer cells. Representative histograms of human SUM44 or BT-474 cells cultured in serum-free medium with or without HRG for 72 h then stained with propidium iodide. In addition, cells were labeled with BrdU for 6 h, stained with an anti-BrdU antibody, and analyzed by flow cytometry. The percentages of the cell population in G_1, G_2/M phases of the cell cycle and BrdU-positive cells are shown.

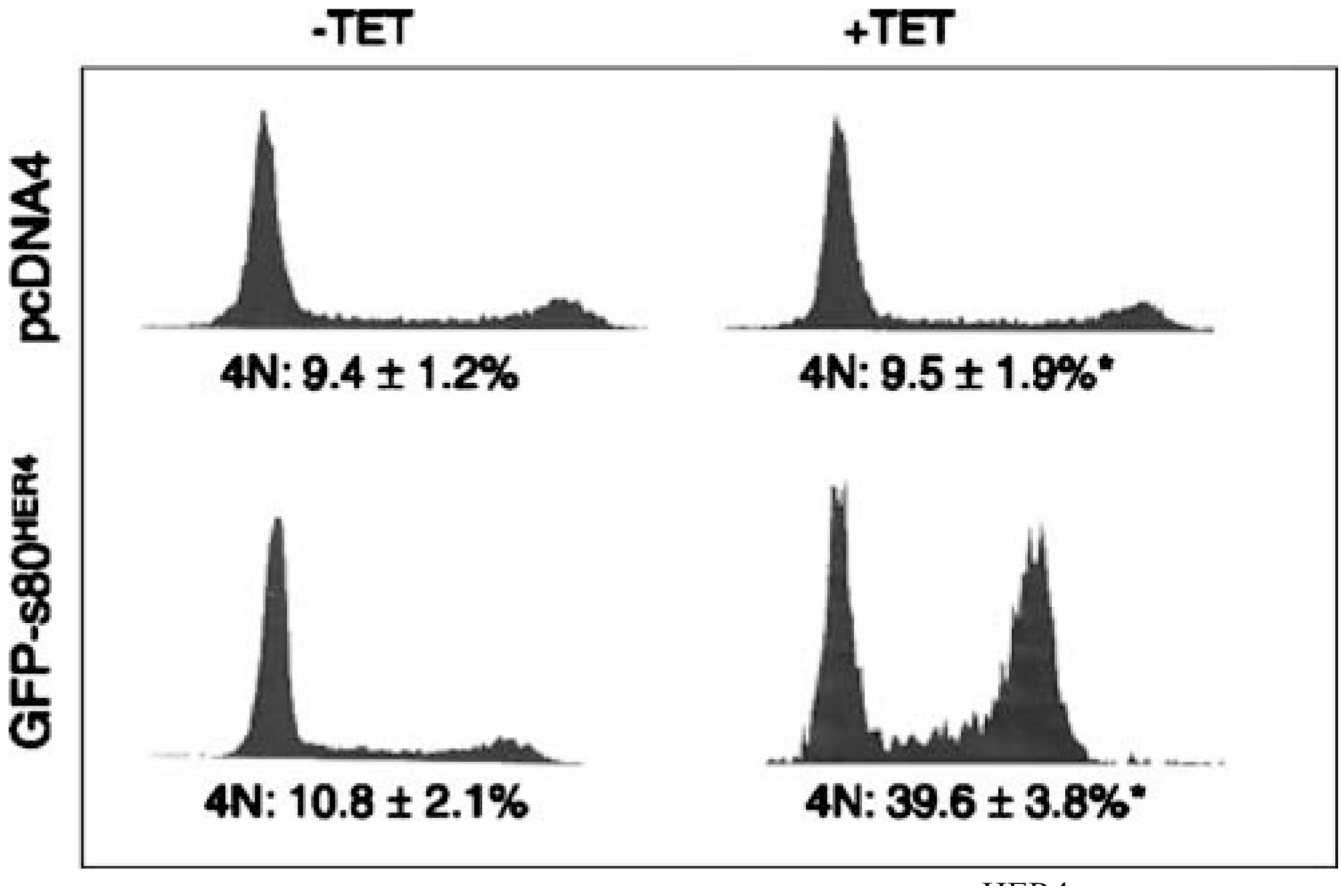

Figure 3.

The intracellular domain of HER4, $s80^{HER4}$, is expressed from a tetracycline inducible promoter in HeLa cells. Induction of $s80^{HER4}$ inhibits growth. Representative histograms of HeLa-pcDNA4 and HeLa-$s80^{Her4}$ cells grown in serum-free medium ± tetracycline (48 h) and stained with propidium iodide and analyzed by flow cytometry. Percentage of cells in the G_2/M phase of the cell cycle are shown, **P*<0.002, Student's unpaired *t* test.

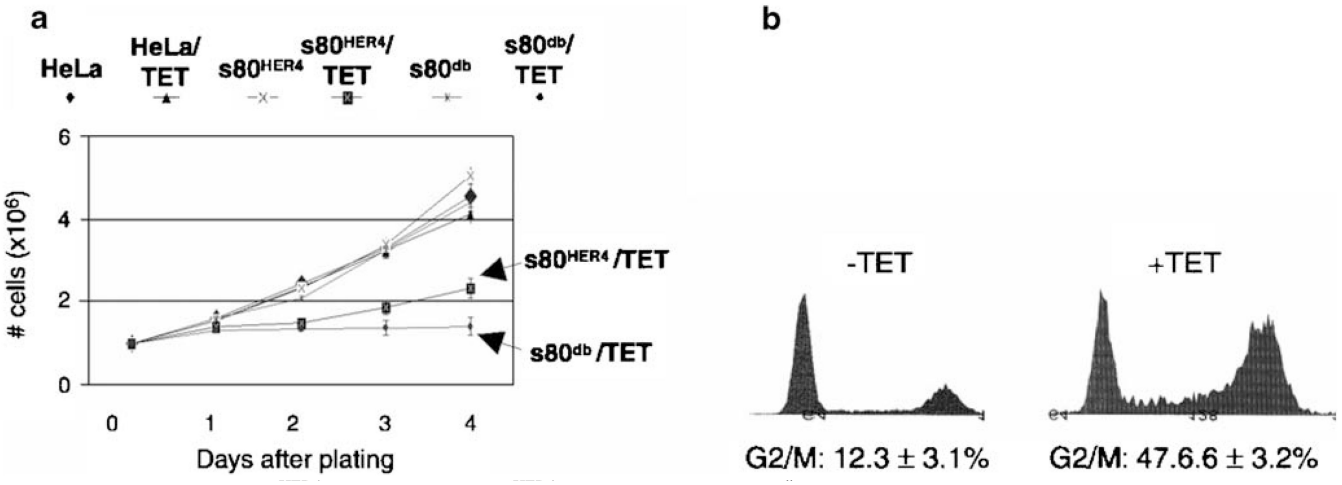

Figure 4.

Mutation of the s80^{HER4} D-box enhances s80^{HER4}-mediated growth inhibition in tetracycline inducible HeLa cells. **a** Equal numbers of cells were plated at day 0 ± tetracycline. Cells were counted at 1-day intervals through day 4. *P*=0.011, comparing cell number of HeLa-s80^{HER4} + tetracycline at day 4 with cell number of HeLa-s80^{db} + tetracycline at day 4, *n*=5, analyzed in triplicate. **b** Analysis of HeLa-s80^{db} cells cultured ± tetracycline (48 h), stained with propidium iodide. Percent of cells in the G_2/M phase of the cell cycle are shown.

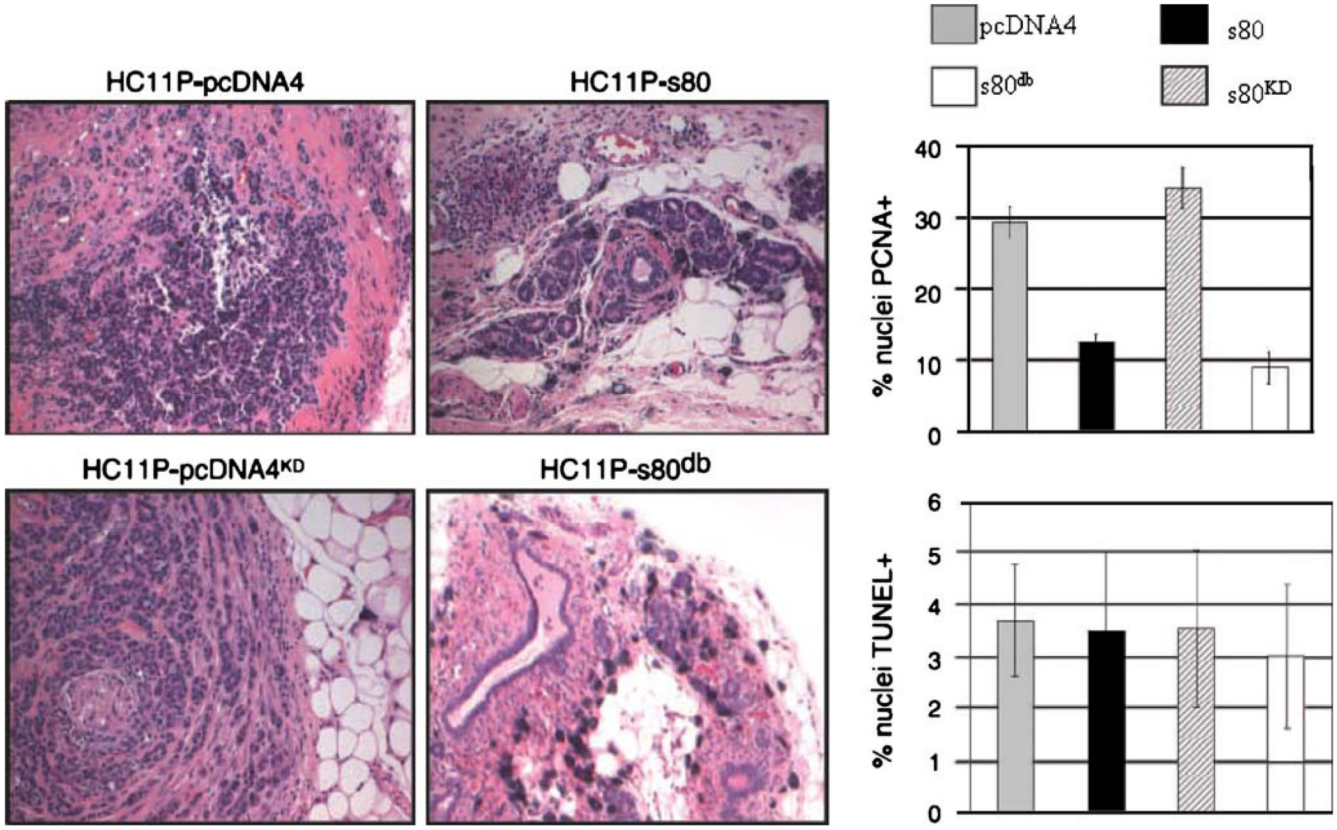

Figure 5.

Decreased tumor formation in PyVmT-transformed HC11 cells (HC11P) expressing $s80^{db}$. **a** HC11P cells expressing $s80^{HER4}$, $s80^{KD}$ (kinase dead), $s80^{db}$ (D-Box mutant), or the empty pcDNA4 vector were injected into the inguinal mammary fat pads of female BALB/c mice. Fifteen weeks after implantation of cells, mice were euthanized and the mammary glands were analyzed histologically. **b** Five tumor samples per group were analyzed by immunohistochemistry. PCNA-positive (*top*) or TUNEL-positive (*bottom*) nuclei. HC11P-$s80^{db}$ percentage of PCNA-positive cells was the lowest of all groups (9.0±2.2) and was also significantly decreased when compared with HC11P-s80 ($P<0.037$).