Loss of Nrdp1 Enhances ErbB2/ErbB3–Dependent Breast Tumor Cell Growth

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Abstract

Dysregulation of ErbB receptor tyrosine kinases is thought to promote mammary tumor progression by stimulating tumor cell growth and invasion. Overexpression and aberrant activation of ErbB2/HER2 confer aggressive and malignant characteristics to breast cancer cells, and patients displaying ErbB2-amplified breast cancer face a worsened prognosis. Recent studies have established that ErbB2 and ErbB3 are commonly co-overexpressed in breast tumor cell lines and in patient samples. ErbB2 heterodimerizes with and activates the ErbB3 receptor, and the two receptors synergize in promoting growth factor–induced cell proliferation, transformation, and invasiveness. Our previous studies have shown that the neuregulin receptor degradation protein-1 (Nrdp1) E3 ubiquitin ligase specifically suppresses cellular ErbB3 levels by marking the receptor for proteolytic degradation. Here, we show that overexpression of Nrdp1 in human breast cancer cells results in the suppression of ErbB3 levels, accompanied by the inhibition of cell growth and motility and the attenuation of signal transduction pathways. In contrast, either Nrdp1 knockdown or the overexpression of a dominant-negative form enhances ErbB3 levels and cellular proliferation. Additionally, Nrdp1 expression levels inversely correlate with ErbB3 levels in primary human breast cancer tissue and in a mouse model of ErbB2 mammary tumorigenesis. Our observations suggest that Nrdp1-mediated ErbB3 degradation suppresses cellular growth and motility, and that Nrdp1 loss in breast tumors may promote tumor progression by augmenting ErbB2/ErbB3 signaling. (Cancer Res 2006; 66(23): 11279-86)

Introduction

The ErbB family of receptor tyrosine kinases regulates a number of cellular processes, such as proliferation, differentiation, cell survival, migration, and invasion (1). Members of this family include the epidermal growth factor receptor (ErbB1/HER1), ErbB2 (HER-2/neu), ErbB3 (HER3), and ErbB4 (HER4). Aberrant expression of these receptors is commonly found in human cancers (1–3) and is associated with aggressive disease (1). The expression of multiple ErbB family members has been observed in a number of cancers, including breast (4, 5), and co-overexpression of ErbB2 or ErbB3 is significantly associated with decreased survival (6–10).

Both the diversity and hence complexity of the ErbB signaling network is mediated by the existence of multiple ligands, each with specificity towards distinct members of the ErbB family. The ErbB receptors take part in a complex process of combinatorial interactions through the formation of ligand-induced homodimers and heterodimers between the different family members (11–13), which in turn activate distinct signaling pathways (11, 12). The ErbB3 receptor has impaired kinase activity (14), whereas the ErbB2 receptor has no known ligand. In this respect, both receptors must signal in the context of a receptor heterodimer. Binding of the neuregulin-1 (NRG1) growth factor to its cognate ErbB3 receptor results in preferential activation of ErbB2/ErbB3 heterodimers (15), thus initiating extracellular-related kinase/mitogen-activated protein kinase (ERK/MAPK) and phosphatidylinositol 3-kinase (PI3K) pathway activation (16–19). Both the ERK (20–22) and PI3K (23–26) pathways have been implicated in cellular motility and invasion, cell survival, and proliferation; thus, they are critical mediators of the aggressive and invasive breast cancer phenotypes resulting from ErbB receptor activation. Indeed, it is now clear that the ErbB2/ErbB3 receptor pair forms the most potent mitogenic (27) and transforming (17, 28) receptor complex.

ErbB2-positive tumors were found to have elevated levels of ErbB3 phosphorylation (17), suggesting that ErbB2 recruitment of ErbB3 contributes to malignant growth. Furthermore, in tumors from transgenic mice generated by expressing an active mutant of Neu/ErbB2 (29) or in cell lines derived from tumors borne of transgenic mice overexpressing wild-type Neu/ErbB2 (30), ErbB3 overexpression and activation is also observed. Moreover, ErbB2 and ErbB3 coexpression has been found in human breast cancer cells lines (4, 19) and primary human breast cancer (6, 31), with coexpression correlating with even further reduced patient survival compared with expression of ErbB2 or ErbB3 alone (10). ErbB2/ErbB3 heterodimers have also been shown to be involved in NRG-mediated motility and invasion (32–34). Taken together, these observations suggest that there may be an advantage for both receptors to be activated in tumor cells to promote breast tumor growth and progression.

Although the mechanisms by which overexpressed and aberrantly activated ErbB receptors contribute to tumor progression are coming into focus, cellular mechanisms controlling receptor protein levels in tumors remain largely unexplored. For example, the loss of specific protein degradation pathways could play a significant role in augmenting ErbB receptor levels in tumors (35). The RING finger E3 ubiquitin ligase neuregulin receptor degradation protein-1 (Nrdp1) associates with ErbB3 in an activation-independent manner (36) and is believed to be involved in its trafficking or localization. Nrdp possesses ubiquitin ligase activity towards ErbB3 in vitro (36, 37), suggesting that it functions in the
maintenance of normal ErbB3 levels by mediating the degradation of overexpressed receptors. Hence, loss of Nrdp1 function may lead to receptor overexpression and disease progression.

As very little is known regarding the expression and role of Nrdp1 in cancer, this study seeks to determine the effect of Nrdp1 expression on growth factor–induced biological responses. Herein, we provide evidence that modulation of Nrdp1 results in alteration of NRG-stimulated motility and proliferation of cultured ErbB3-expressing human breast cancer cells likely through altered growth factor–mediated activation of ERK and PI3K signal transduction. We also show that decreased Nrdp1 expression in tumors correlates with enhanced expression of ErbB3 in both an in vivo transgenic mouse model of ErbB2-induced mammary tumorigenesis and in a panel of primary breast cancer specimens. Together, our observations suggest that the suppression of ErbB3 levels by Nrdp1 inhibits cellular growth and invasive properties, and that loss of Nrdp1 expression may contribute to breast tumor progression through up-regulation of ErbB3 signaling.

Materials and Methods

Cell lines and cell culture. Human breast adenocarcinoma cell lines MCF7, T47D, and MDA-MB-435 were obtained from the American Type Culture Collection (Manassas, VA) and maintained in the supplier-specified media (Mediatech, Washington, DC) supplemented with 10% fetal bovine serum and penicillin-streptomycin antibiotics. The 293GP expressing human breast cancer cells likely through altered growth factor–mediated activation of ERK and PI3K signal transduction. We also show that decreased Nrdp1 expression in tumors correlates with enhanced expression of ErbB3 in both an in vivo transgenic mouse model of ErbB2-induced mammary tumorigenesis and in a panel of primary breast cancer specimens. Together, our observations suggest that the suppression of ErbB3 levels by Nrdp1 inhibits cellular growth and invasive properties, and that loss of Nrdp1 expression may contribute to breast tumor progression through up-regulation of ErbB3 signaling.

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Primary human tissue specimens. Frozen human tissues from clinical breast cancer patients were provided by the University of California, Davis School of Medicine, Department of Pathology, the University of California, Davis Cancer Center Specimen Repository and the National Cancer Institute Cooperative Human Tissue Network, Western Division at Vanderbilt University Medical Center (Nashville, TN). All the samples were de-identified, and the study was approved by the institutional review board of the School of Medicine, University of California, Davis. Frozen tissues were homogenized in T-PER Tissue Protein Extraction Reagent (Pierce, Rockford, IL) with 4 µg/mL leupeptin, 4 µg/mL pepstatin, 4 µg/mL aprotonin, 1 mM/L ZnCl, 1 mM/L NaF, 1 mM/L Na3, 5% SDS, 5% β-mercaptoethanol, 10% glycerol, 0.05% bromophenol blue) and boiled for 10 minutes at 95°C. Protein lysates were resolved by SDS-PAGE, transferred to nitrocellulose (Pall Life Sciences, Pensacola, FL), blocked overnight in TBS-T containing 5% dried milk, then detected with antibody against phosphoerlotroisine (PY20, BD Transduction Labs, San Jose, CA). Chemiluminescence detection was done using SuperSignal detection reagents (Pierce Biotechnology, Rockford, IL) and imaged using an Alpha Innotech Digital Imaging Station (Alpha Innotech Corp., San Leandro, CA). Blots were then stripped with Restore stripping buffer (Pierce) and immunoblotted with antibodies specific for ErbB3 (clone C-17; Santa Cruz Biotechnology, Santa Cruz, CA), ErbB2 (A-8; clone 3B5; Calbiochem, San Diego, CA), phospho-Akt (Ser473; Cell Signaling Technology, Danvers, MA), phospho-ERK (Thr202/Tyr204, Cell Signaling Technology), Bag epitope (M2; Sigma), FLB/Frdp1 (BL1123; Bethesda, Inc., Montgomery, TX), or actin (AC15; Sigma). Quantification of ErbB3 levels was done by densitometric analysis using Scion Image software (Scion Corp., Frederick, MD).

Motility assay. Transduced MCF7 cells were seeded in six-well plates and grown to confluence. A scratch was then made on the monolayer using a sterile 10-µL pipette tip. The monolayer was rinsed thrice with PBS then cultured in serum-starved medium with or without NRG. At the initiation of the experiment (t = 0), a digital image of the scar was taken at a magnification of ×10. After 48 hours (t = 48), the same region of the scar was imaged again. The images were imported into the AlphaEaseFC imaging program (Alpha Innotech), and quantification of the two-dimensional movement of the cells was assessed by comparing the surface area of the scar at t = 0 with the surface area at t = 48. The assay was repeated three individual times, with five replicates each.

Proliferation assay. Exponentially growing cells (1-3 × 104 per 100 µL) were seeded in 96-well plates and incubated for 16 hours. Next, cells were starved for 18 hours in serum-starved medium and then treated continuously with NRG or control starved media for 72 hours. Cell proliferation was evaluated using the 3-(4,5-dimethylthiazolo-2-y1)-2,5-diphe- nyltetrazolium bromide (MTT) assay. Each assay was done five times, with six replicates for each.

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Statistical analysis. Values were expressed as mean ± SD and compared by two-tailed Student's t test. P < 0.05 were considered statistically significant.

Results

**Nrdp1 expression in breast cancer cells suppresses endogenous ErbB3 receptor levels.** To assess the effect of Nrdp1 overexpression on ErbB3 in breast cancer cells, we used three human breast adenocarcinoma cell lines. MCF7, T47D, and MDA-MB-453 cells were transduced with high-titer retroviral particles and subsequently selected for resistance to puromycin. As shown in Fig. 1A, GFP fluorescence reveals that the transduction efficiency was >90% for MCF7 cells infected with Nrdp1 retrovirus. Similar efficiencies were found in T47D- and MDA-MB-453–transduced cells (data not shown). Expression of Flag-Nrdp1 in all three cell lines resulted in a reduction in the endogenous levels of ErbB3 receptor compared with the control pMXpie-transduced cells (Fig. 1B, right lanes). In contrast, stable expression of the dominant-negative form of Nrdp1 (Flag-dnNrdp1) lacking the RING finger domain (36) in all three cell lines caused an enhancement of ErbB3 receptor levels (Fig. 1B, middle lanes). Although overexpression of exogenous proteins may lead to the down-regulation of their corresponding endogenous counterparts, the effects on ErbB3 levels seen upon overexpression of Flag-Nrdp1 or Flag-dnNrdp1 are not due to changes in endogenous Nrdp1 levels (Fig. 1B).

**Nrdp1 inhibition of breast cancer cell proliferation and ErbB3 signaling.** Because ErbB3 may play a key role in the proliferation of breast cancer cells (19), we sought to determine the effect of Nrdp1 on NRG1-dependent cellular proliferation using an MTT assay. We observed a significant reduction in the proliferation of MCF7 cells expressing Nrdp1 compared with control transduced cells (Fig. 2A). To understand the mechanism of Nrdp1 inhibition of cell growth, we evaluated the effect of Nrdp1 expression on the activation of downstream signaling in response to NRG1. Similar to that seen in Fig. 1B, cells overexpressing Nrdp1 exhibit lowered basal ErbB3 expression (Fig. 2B). Stimulation with NRG1 leads to efficient phosphorylation and activation of ErbB3 in both control and Nrdp1-overexpressing cells, as evidenced by Western blot analysis of tyrosine phosphorylation (phospho-Y; Fig. 2B). NRG1-induced activation of ErbB3 is a well-established stimulator of the ERK/MAPK pathway (41, 42), which is a potent promoter of cell proliferation. As shown in Fig. 2B, NRG1 treatment caused a robust activation of ERK1/2, as determined by phospho-specific ERK antibody, with maximal activation occurring within 10 minutes. In control MCF7 cells, sustained activation of ERK1/2 was seen beyond 1 hour after NRG1 stimulation. In contrast, NRG1-induced activation of ERK1/2 in MCF7 cells overexpressing Nrdp1 was more transient, with ERK1/2 phosphorylation returning to basal levels within 30 to 45 minutes. Additionally, this NRG1 time course revealed that Nrdp1 expression in MCF7 cells also resulted in decreased activation kinetics of Akt (Fig. 2B), a known PI3K effector. NRG1-induced Akt phosphorylation remained robust after 60 minutes in control MCF7 cells, whereas strong activation was seen for only 20 minutes in cells transduced with Nrdp1. Thus, it is

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Regulation of endogenous ErbB3 receptor levels in breast cancer cells by Nrdp1 forms. A, proliferating MCF7 cells were infected with concentrated pMXpie-Nrdp1 retroviral particles for 48 hours and visualized by phase-contrast and GFP fluorescence microscopy. B, MCF7, T47D, and MDA-MB-453 cells were transduced with full-length Nrdp1 (pMXpie-Nrdp1), dominant-negative Nrdp1 (pMXpie-dnNrdp1), or control GFP (pMXpie) retroviral particles. Lysates were immunoblotted with antibodies to ErbB3, Flag epitope, Nrdp1, or actin.
likely that Nrdp1 inhibits NRG1-dependent breast cancer cell growth via inhibition of ERK or PI3K signaling.

Inhibition of cell motility by Nrdp1. Because the PI3K signaling pathway is an important mediator of cell motility, we next sought to determine if Nrdp1 expression would modulate motility in a scratch wound-healing assay. Consistent with its inhibition of PI3K activation, overexpression of Nrdp1 in MCF7 cells resulted in an impairment of NRG1-induced migration into the scarred wound area, compared with control GFP-transduced cells (Fig. 3). Furthermore, overexpression of the dominant-negative form of Nrdp1 in MCF7 cells led to an augmentation of migration in response to NRG1.

siRNA against Nrdp1 or inducible dominant-negative Nrdp1 augments ErbB3 expression and promotes cell proliferation. The inhibitory effect of Nrdp1 on cell growth was further shown by knockdown of endogenous Nrdp1 expression using siRNA introduced by retroviral transduction. As shown in Fig. 4A, transduction of MCF7 cells with pSuper-siNrdp1 efficiently reduced Nrdp1 protein levels compared with control GFP-transduced cells. This reduction in Nrdp1 expression brought about an ~6-fold increase in ErbB3 expression levels (Fig. 4A), with a significant enhancement of NRG1-mediated cell proliferation (Fig. 4B). Furthermore, the importance of Nrdp1 in NRG1-induced cell growth was shown using an inducible dominant-negative Nrdp1 (Fig. 5). MCF7 cells were stably transfected with a construct to allow reversible ecdysone-inducible expression of dominant-negative Nrdp1. As illustrated in Fig. 5A, treatment with ecdysone analogue resulted in very good induction of dominant-negative Nrdp1. This was accompanied by an ~2.4-fold up-regulation of the endogenous levels of ErbB3 protein. We next used this model to show that induction of dominant negative Nrdp1 expression enhanced both the basal rate of cell growth as well as the NRG1-stimulated cellular proliferation (Fig. 5B).

Inducible dominant-negative Nrdp1 potentiates NRG1-induced signaling. To further explore the manner by which dominant-negative Nrdp1 expression effects cell growth, we examined the downstream signaling events following NRG stimulation. As shown in Fig. 5C, stimulation with NRG1 resulted in efficient receptor activation, as revealed by the increased receptor...
phosphotyrosine content. ERK1/2 activation was seen as early as 5 minutes following NRG1 treatment in both the control uninduced and ec dysone-induced cells. However, induction of dominant-negative Nrdp1 enhanced this ERK activation, as shown by the markedly increased phosphorylation of ERK. Additionally, whereas NRG1-induced AKT activation in uninduced cells peaked at 20 minutes before tapering off, Akt activation continued to increase well past 45 minutes in cells expressing dominant-negative Nrdp1. Moreover, the maximal Akt activation seen in cells expressing the dominant-negative Nrdp1 was much greater than was seen in the control cells. Thus, the induction of dominant-negative Nrdp1 expression in MCF7 cells seems to markedly enhance the activation of both MAPK/ERK and Akt signaling. Interestingly, induction of dominant-negative Nrdp1 expression resulted in increased basal expression of not only ErbB3 but also ErbB2 receptor levels (Fig. 5C, top). These increased basal expression levels still allowed for normal receptor turnover upon stimulation with NRG1 (Fig. 5C, top). Taken together, our results reveal that wild-type and dominant-negative Nrdp1 have reciprocal effects on ErbB2/3-mediated breast tumor cell signaling and proliferation.

**Figure 4.** Nrdp1 siRNA augments ErbB3 levels and promotes NRG1-stimulated cell growth. A, lysates from MCF7 cells stably transduced with retrovirus for Nrdp1-directed siRNA (pSuper-siNrdp1) or control (pSuper) were collected and blotted with antibodies to detect endogenous Nrdp1, endogenous ErbB3, or actin. B, serum-starved MCF7 cells transduced with siNrdp1 retrovirus were treated without or with NRG1, and proliferation was measured by MTT assay. Columns, mean for six independent replicates; bars, SD. ***, $P < 0.001$.

**Nrdp1 is lost in a mouse model of ErbB mammary tumorigenesis.** In tumors from transgenic mice generated by expressing an activating deletion mutation of Neu/ErbB2 (termed Neu-deletion or NDL), ErbB3 overexpression and activation is also observed (29). Interestingly, ErbB3 overexpression in these mouse mammary tumors seems to be at the protein level; ErbB3 message remains constant in normal and tumor tissue (29). This finding alludes to the notion that the up-regulation of ErbB3 upon breast tumor formation may be the result of elevated translation of or increased stability of the receptor protein. Because Nrdp1 was found to mediate a decrease in ErbB3 protein levels, we next sought to determine if there exists a correlation between the ErbB3 up-regulation seen in the mouse mammary tumors and a possible loss of Nrdp1 (Fig. 6A). Western blot analysis of transgenic mouse tissue revealed that Nrdp1 protein is present in the normal mammary gland. In tumor specimens, Nrdp1 expression is lost, whereas ErbB3 expression occurs. Therefore, loss of Nrdp1 expression may contribute to the ErbB3 overexpression that accompanies tumor development. Although the concomitant up-regulation of both ErbB3 and ErbB2 in tumor tissue is consistent with previous reports (29), it is also noteworthy that an induction of dominant negative Nrdp1 expression also resulted in an up-regulation of both ErbB2 and ErbB3 receptors (Fig. 5C). Thus, a loss of Nrdp1 expression or function may contribute to the progression of a subset of tumors, particularly those with overexpression of ErbB2.

**Nrdp1 is lost in primary human breast cancer.** Based on the above *in vivo* and *in vitro* results showing that a down-regulation in Nrdp1 occurs at the same time as the up-regulation of ErbB expression, we next sought to determine whether Nrdp1 expression is aberrantly affected in samples from breast cancer patients. To that end, we surveyed the expression levels of Nrdp1 and ErbB3 in a panel of primary human breast carcinomas and their matched normal breast tissues (Fig. 6B). ErbB3 was found to be elevated in 62.9% of breast cancer specimens (22 of 35) compared with their matched normal tissue. This is in agreement with reports showing ErbB3 overexpression in primary breast cancer (6, 9, 10, 31, 43). Decreased Nrdp1 expression was found in 57.1% (20 of 35) of breast tumors compared with their matched normal tissue. Among the breast tumors with ErbB3 overexpression, decreased expression of Nrdp1 was found in ~68% of those tumors (15 of 22), whereas among all breast tumors surveyed, 42.9% (15 of 35) displayed both overexpression of ErbB3 and decreased expression of Nrdp1. Additionally, 54.3% of all tumors (19 of 35) showed a reciprocal relationship between ErbB3 and Nrdp1 expression. Notably, all tumors with overexpression of ErbB2 (12 of 35) had concomitant overexpression of ErbB3. Among the ErbB2-overexpressing tumors, ~58% of those tumors (7 of 12) showed decreased Nrdp1 expression. Hence, this preliminary survey revealed a potential link between the overexpression of both ErbB3 and ErbB2 receptors with down-regulation of Nrdp1 in primary human breast cancer.

**Conclusions**

Much effort over the past two decades has gone into understanding the mechanisms by which overexpressed ErbB receptors contribute to tumor aggressiveness, and these studies in turn have led to the development of ErbB-directed small molecule and antibody therapies. However, very little is known about the mechanisms leading to ErbB protein overexpression in tumors. Certainly, the ErbB2 gene is amplified in a subset of breast tumors (7), and the resulting elevated message levels contribute to elevated protein expression. However, observations with transgenic mouse models of ErbB2-induced tumors indicate that augmentation of ErbB gene expression in the mammary gland is not sufficient to give rise to ErbB protein overexpression (29). ErbB2 and ErbB3
proteins are very highly expressed in mammary tumors of transgenic mice relative to normal tissue, despite similar transcript levels. Hence, it seems that tumor cells must inactivate very potent endogenous posttranscriptional mechanisms, such as protein degradation pathways, that keep ErbB receptor protein levels in check (35). The effect of such ErbB-negative regulatory mechanisms in cancer is presently unknown.

We have previously shown that the E3 ubiquitin ligase Nrdp1 is a key regulatory component of an endogenous cellular pathway responsible for the degradation of the ErbB3 tyrosine kinase (36), a growth factor receptor whose overexpression is found to be upregulated in breast cancer (6, 9, 31). This study sought to determine whether Nrdp1 could be exploited to suppress the growth and invasive properties of ErbB3-expressing breast cancer cells. In addition, to assess if Nrdp1 may be involved in tumor progression in the in vivo setting, we sought to examine the expression of Nrdp1 protein as it relates to ErbB3 levels in a mouse mammary tumor model and in tumors from clinical breast cancer patients.

Our observations have shown that overexpression of Nrdp1 potently suppresses the endogenous cellular levels of ErbB3 in a variety of breast cancer cell lines, whereas overexpression of dominant-negative Nrdp1 results in enhanced ErbB3 levels. Our analysis of spontaneous mammary tumors induced in a transgenic mouse model of active ErbB2/neu expression revealed a loss of Nrdp1 expression concomitant to a substantial up-regulation of both ErbB3 and ErbB2 receptors. Previous studies using this mouse model of ErbB2 overexpression have shown that the ErbB2 and ErbB3 co-overexpressing mammary tumors frequently metastasize to the lung (29), thus arguing that the two receptors function in concert to promote motility and metastasis. Furthermore, a number of studies have shown that the two receptors synergize in mediating increased motility and invasiveness induced by the NRG1 growth factor in breast cancer cell lines (32–34, 44–46).

One can therefore postulate that an agent that reduces ErbB3 levels in breast tumor cells would be expected to suppress cancer progression. Evidence that Nrdp1 may play such a role was shown in our analysis of Nrdp1 expression in a panel of primary breast cancer tissue. Our data showed that human breast tumors presenting with either overexpression of ErbB3 or dual overexpression of ErbB2 and ErbB3 exhibited decreased expression of Nrdp1. Based on these results, large-scale analysis of this phenomenon using a large sampling of human tumor specimens is warranted and is the subject of ongoing studies. For further evidence of dysregulated Nrdp1 expression in cancer, we queried the ONCOMINE database (47), which comprises microarray data compiled from >300 published cancer gene expression studies encompassing over 16,000 human microarrays.4

In silico analysis of Nrdp1 expression

revealed a correlation between underexpression of Nrdp1 in breast carcinomas with increasing stage (48).

We have shown that the down-regulation of ErbB3 by Nrdp1 overexpression was accompanied by decreased growth factor–mediated cellular proliferation and motility, which was due in part to attenuation of ERK and PI3K signaling. Conversely, we have shown that the enhancement of ErbB3 expression, by either siRNA against Nrdp1 or expression of dominant-negative Nrdp1, resulted in increased cellular proliferation and migration, likely via enhanced activation of the PI3K and ERK signal transduction pathways. Indeed, a number of studies show that ErbB3 activation in ErbB2-overexpressing breast cancer cells contributes to their progression by activating ERK/MAPK and/or PI3K, thereby promoting proliferation and invasion (19, 32, 49). Although Nrdp1 seems to mediate a decrease in breast cancer proliferation via altered activation of ERK and PI3K, it is possible that Nrdp1 also contributes to inhibition of growth by up-regulating apoptotic mechanisms. For example, a recent study has shown that Nrdp1 promotes the ubiquitination and degradation of the inhibitor of apoptosis, BRUCE (50).

Taken together, these results suggest that a biological function of Nrdp1 is in the maintenance of steady-state levels of ErbB3, perhaps as a means to prevent excessive growth factor–mediated cell signaling. Our observations raise the possibility that whereas ErbB2 and ErbB3 collaborate in the breast cancer progression, loss of Nrdp1 expression may also be a factor in mediating receptor up-regulation. In this regard, it is possible that Nrdp1 acts as an endogenous suppressor of tumor cell growth and invasion. Because inactivation of ErbB receptors as a method of cancer treatment has been the subject of intense investigation, our findings may pave the way for the ultimate development of therapeutic methods to introduce Nrdp1 into tumors of patients presenting with ErbB2- and ErbB3-positive breast cancer. Furthermore, additional clinical studies into the relationship between Nrdp1 loss and cancer development could lead to the development of Nrdp1 loss as a marker of invasive breast tumors.

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Figure 6. Nrdp1 loss is associated with enhanced ErbB3 and ErbB2 levels in tumors. A, tumor specimens (T) and normal mammary fat glandular (MG) tissue were collected from 5.5-month-old NDL transgenic mice. Lysates from these tissues were blotted with antibodies to detect the levels of endogenous ErbB3, ErbB2, or Nrdp1. Cytokeratin-18 (CK18) levels were also determined to normalize for epithelium. B, top, lysates from primary human breast tumors (T) and their matched normal breast tissue (N) were analyzed by Western blotting to detect ErbB3, Nrdp1, or actin. Representative samples. Bottom, pie chart depicting the frequency of ErbB3 overexpression and Nrdp1 underexpression in primary human breast tumors.


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