Association of Wwox with ErbB4 in Breast Cancer

Rami I. Aqeilan,1 Valentina Donati,1 Eugenio Gaudio,1 Milena S. Nicoloso,1 Maria Sundvall,2,3 Anna Korhonen,4 Johan Lundin,5 Jorma Isola,6 Marius Sudol,7 Heikki Joensuu,5 Carlo M. Croce,1 and Klaus Elenius2,4

1Department of Molecular Virology, Immunology and Medical Genetics, Human Cancer Genetics Program, Comprehensive Cancer Center, Ohio State University, Columbus, Ohio; 2MediCity Research Laboratory, and Department of Medical Biochemistry and Molecular Biology, University of Turku, Turku, Finland; 3Turku Postgraduate School of Biomedical Sciences; 4Department of Oncology, Turku University Central Hospital, Turku, Finland; 5Department of Oncology, University of Helsinki, Helsinki, Finland; 6Laboratory of Cancer Biology, Institute of Medical Technology, Tampere University, and Tampere University Hospital, Tampere, Finland; and 7Weiss Center for Research, Geisinger Clinic, Danville, Pennsylvania

Abstract

WWOX, WW domain-containing oxidoreductase, is a tumor suppressor that is altered in many human cancers, including breast cancer. Wwox interacts with the ErbB4 receptor, reduces nuclear translocation of the cleaved intracellular domain of ErbB4, and inhibits its transactivation function mediated through Yes-associated protein. Here, we assessed the clinical significance of the Wwox-ErbB4 association. We determined Wwox protein expression by immunohistochemistry in a series of 556 breast cancers. Wwox expression was absent in 36% of the cancers, and loss of Wwox expression was associated with unfavorable outcome (P = 0.02). Membranous location of ErbB4 was associated with favorable survival compared with women whose cancer lacked such ErbB4 expression (P = 0.02). Wwox expression was strongly associated with membranous ErbB4 localization (P = 0.0003) and with overall ErbB4 expression (P = 0.0002). Coexpression of membranous ErbB4 and Wwox was associated with favorable outcome compared with cases with membranous ErbB4 and no Wwox immunoreactivity (P = 0.002). In vitro, Wwox associated with the two ErbB4 isoforms, JMa-CYT-1 and JMa-CYT-2, expressed in breast cancer. Moreover, expression of Wwox both in vitro and in vivo led to accumulation of total full-length membrane-associated ErbB4. These results suggest that expression of Wwox is associated with ErbB4 expression and that their coexpression has prognostic significance in breast cancer. [Cancer Res 2007;67(19):9330–6]

Introduction

The WWOX gene encodes a protein with tumor suppressor function, a WW domain-containing oxidoreductase. WWOX spans the second most common chromosomal fragile site 16D (FRA16D) and is frequently inactivated very early in breast cancer (1). Loss of heterozygosity, homozygous deletion, and chromosomal translocation affecting WWOX have been reported in several types of cancer, including breast, ovarian, esophageal, and stomach carcinoma and multiple myeloma (2, 3). We and others have shown that ectopic expression of WWOX in cancer cells lacking endogenous Wwox results in significant growth inhibition and prevents the development of tumors in athymic nude mice (4). In addition, we have reported that restoration of Wwox expression results in caspase-3-mediated apoptosis (4). Recently, we have shown that targeted deletion of the Wwox gene in the mouse results in increased tumor incidence and multiplicity, indicating that WWOX is a bona fide tumor suppressor (5).

We have previously shown that Wwox interacts with ErbB4 receptor kinase (CYT-2 isoform) via its first WW domain (6). ErbB4 is expressed as four alternatively spliced isoforms. Alternative splicing occurs within the intracellular cytoplasmic domain (isoforms CYT-1 and CYT-2; ref. 7) and within the extracellular juxtamembrane region (isoforms JMa-a and JMa-b; ref. 8), which generates receptors with different signaling capabilities (9, 10). The only JM isoform expressed in breast cancer is JMa-a, whereas both CYT isoforms are present (11). JM-a isoforms can be cleaved by the tumor necrosis factor-α-converting enzyme, which triggers a secondary cleavage event by γ-secretase that releases a soluble intracellular domain (ICD) fragment. The ICD can translocate into the nucleus and regulate gene transcription (10, 12–15). Wwox binds ErbB4 at the cell membrane and in the cytoplasm and hence prevents ICD translocation into the nucleus (6). We have also previously reported that both WW domain-containing proteins, Wwox and Yes-associated protein (YAP), compete for interaction with ErbB4, thus determining its transcriptional transactivation activity. Whereas YAP coactivates ErbB4 transactivation, Wwox suppresses this function (6).

Recently, we reported that high ErbB4 protein expression level at the cell membrane associates with a favorable outcome in estrogen receptor (ER)-positive breast cancer cases (11). By contrast, nuclear ErbB4 associates with poor survival compared with women whose cancer has membranous ErbB4 localization. Furthermore, overexpression of cleavable ErbB4 isoform enhances nuclear ErbB4 accumulation, proliferation, anchorage-independent growth, and estrogen response element–mediated transcriptional activity, thus suggesting a tumor-promoting role for ErbB4 ICD in the nucleus (11).

In this study, we set to determine the relevance of Wwox-ErbB4 interaction and its association with breast cancer survival. To this end, we investigated the expression of Wwox and its association with ErbB4 expression and subcellular localization in clinical breast cancer samples. Our findings suggest that expression of Wwox associates with overall expression of ErbB4 and membranous ErbB4 localization and that Wwox-ErbB4 association results in favorable patient survival. Moreover, results from in vitro experiments indicate that Wwox interacts with ErbB4 isoforms present in...
breast cancer tissues and that this association up-regulates membrane-associated full-length ErbB4. These findings suggest that Wwox-ErbB4 association has a significant implication on the clinical outcome of breast cancer patients.

Materials and Methods

Patients. Tissue microarrays, including representative 0.6-mm-diameter breast tumor samples from 556 patients, with a median of 10 years of clinical follow-up after the diagnosis of breast cancer, were analyzed. The preparation of the microarrays and histopathology of the tumors have been described elsewhere (16).

Immunohistochemistry. Immunohistochemical analysis of the breast cancer tissue array for ErbB4 expression has been described (11). Wwox expression was detected using a polyclonal rabbit anti-Wwox (17) diluted at 1:5,000. Unstained 5-μm sections, mounted on glass slides, were deparaffinized through serial baths in xylene and rehydrated in a graded series of alcohol and water. To remove any endogenous peroxidase activity, the sections were soaked in absolute methanol containing 1% hydrogen peroxide for 15 min at room temperature. After being washed with PBS for 5 min, the slides were blocked with normal goat serum diluted 1:40 in PBS (pH 7.2) containing 1% bovine serum albumin for 20 min to inhibit nonspecific background staining. Antigen retrieval was done by heating sections in 0.01 mol/L sodium citrate buffer (pH 6.0) using a microwave oven (620 W) for three 5-min periods. Subsequently, sections were incubated with anti-Wwox primary antibody for 60 min at room temperature in a humidified box and detection was done with streptavidin-biotin complex using the LSAB2 System (DakoCytomation). Finally, sections were incubated with PBS containing 6 mg 3,3′-diaminobenzidine tetrahydrochloride (Sigma) and 100 μL 1% hydrogen peroxide per 10 mL and then lightly counterstained with Mayer’s hematoxylin for 30 s, washed in running water, dehydrated, and mounted with Canadian balsam. A section of normal human breast, previously proven to be Wwox positive, was used as a positive control, whereas an invasive ductal carcinoma case that was negative for Wwox was included as a negative control.

To evaluate Wwox expression, the staining was scored by extent of stained cancer tissue area. Extent of staining was evaluated as the percentage of cells with cytoplasmic immunoreactivity in the evaluated compartment and was scored in three classes as follows: 0% to 25%, 25% to 50%, and >50%.

Statistical analyses. The χ2 test was used to test for associations between factors. Life tables were calculated according to the Kaplan-Meier method. Distant disease-free survival was calculated from the date of the diagnosis to the occurrence of metastases outside the locoregional area or death from breast cancer, whichever came first. Survival curves were compared with the log-rank test and hazard ratios (HR) were calculated using Cox regression. All P values are two tailed.

Cell culture and transfection. COS-7 fibroblasts and MCF-7 breast cancer cells were maintained in DMEM supplemented with 10% FCS (Bioclear) and 1% L-glutamine-penicillin-streptomycin solution (Sigma). Tissue cultures, including representative 0.6-mm-diameter breast tumor samples from 556 patients, with a median of 10 years of clinical follow-up after the diagnosis of breast cancer, were analyzed. The preparation of the microarrays and histopathology of the tumors have been described elsewhere (16).

RNA interference. RNA interference was performed using small interfering RNA (siRNA) duplexes directed against WWOX or GFP. Cells were transfected with pcDNA3.1-ErbB4M-αCYT-1 with or without pcMV-MYC-WWOX. Cells were starved without serum for 2 h followed by addition of protein synthesis inhibitor cycloheximide (100 μg/mL) for 2, 4, or 6 h. After lysis, 30 μg samples of total protein were analyzed by Western blotting using an anti-ErbB4 antibody. Expression of Myc-tagged Wwox protein was detected with an anti-Myc antibody.

Results

Loss of Wwox expression associates with nodal metastases. To assess the clinical significance of Wwox protein expression in breast cancer, a tissue array including 556 breast cancer samples was analyzed by immunohistochemistry and compared with samples of normal mammary gland tissue. The Wwox immunoreactivity in cancer tissue sample was scored as normal if >50% of the cells were Wwox positive, reduced if 25% to 50% of cells were positive, or absent if Wwox expression was present in 0% to 25% of cells. Representative examples of breast cancer sections stained with anti-Wwox antibody are shown in Supplementary Fig. S1. Normal Wwox expression was detected in 50% of patients, reduced expression in 14% of the patients, whereas 36% of patients were negative for Wwox immunoreactivity.

Associations of Wwox immunoreactivity with clinical and histopathologic characteristics are listed in Supplementary Table S1. Loss of Wwox expression significantly associated with the number of metastatic axillary lymph nodes (P = 0.02). However, molecular markers reflecting cancer cell survival or proliferation (i.e., loss of p53 staining or high Ki-67 expression) did not associate with Wwox expression (P = 0.94 or 0.67, respectively).

Loss of Wwox expression associates with poor survival. In Kaplan-Meier survival analysis, absent Wwox expression (Wwox present in 0–25% of cells) significantly associated with poor distant disease-free survival when compared with normal or reduced Wwox expression [Wwox in >25% of cells; HR, 1.51; 95% confidence interval (95% CI), 1.01–2.51; P = 0.019; Fig. 1A]. Because Wwox expression has previously been shown to correlate with ER negativity in breast cancer (20), the analysis was repeated after stratifying the patients according to their ER status. In women with ER-negative cancer, loss of Wwox expression was significantly associated with shorter survival (HR, 1.78; 95% CI, 1.01–3.15; P = 0.045; Fig. 1C). However, association between Wwox expression and survival did not reach significance in the subgroup of ER-positive patients (HR, 1.32; 95% CI, 0.79–2.22; P = 0.29; Fig. 1B). A similar tendency for an association of lost Wwox expression with unfavorable survival was observed in the subgroup of progestosterone receptor (PgR)-negative (HR, 1.67; 95% CI, 0.99–2.80; P = 0.05) but not in PgR-positive (HR, 1.26; 95% CI, 0.70–2.25; P = 0.43) patients (Supplementary Fig. S2A and B). Because absent Wwox expression tended to associate with cases without ErbB2 amplification
(Supplementary Table S1), the prognostic significance of Wwox expression was further analyzed in subgroups negative or positive for ErbB2 amplification. Again, loss of Wwox expression was associated with unfavorable survival of ErbB2-negative (HR, 1.65; 95% CI, 1.08–2.54; P = 0.02) but not of ErbB2-positive (HR, 1.55; 95% CI, 0.71–3.36; P = 0.27) patients (Supplementary Fig. S2C and D). These results indicate that loss of Wwox expression is associated with unfavorable outcome, particularly in hormone receptor–negative and ErbB2-negative early breast cancer.

Presence of Wwox associates with favorable survival in patients with cell membrane ErbB4 expression. Because ErbB4 expression at the cell membrane was shown to be associated with favorable survival using the same series of breast cancer samples (P = 0.024; ref. 11), and Wwox regulates ErbB4 localization and function (6), we set to determine whether presence or absence of Wwox immunoreactivity affected the prognostic value of ErbB4. Patients whose cancer expressed Wwox and had ErbB4 localized at the cell membrane had longer survival than those whose cancer did not express Wwox (94% versus 70% alive at 5 years of follow-up, respectively; HR, 0.15; 95% CI, 0.04–0.62; P = 0.016; Fig. 2A). In contrast, the expression of Wwox was not associated with survival in the subset of patients who had cancer with ErbB4 localized in the nucleus (HR, 1.53; 95% CI, 0.57–4.08; P = 0.39; Fig. 2B).

Similarly, in the subgroup of patients with cancer with detectable Wwox expression (either normal or reduced expression), presence of ErbB4 immunoreactivity at the cell membrane was associated with favorable survival compared with cases having no detectable ErbB4 expression at the cell membrane (HR, 0.29; 95% CI, 0.10–0.83; P = 0.013; Fig. 2C). In contrast, presence or absence of ErbB4 immunostaining in the cancer cell nuclei was not associated with survival in this subset of patients (HR, 1.17; 95% CI, 0.54–2.57; P = 0.68; Fig. 2D), and no significant associations between the subcellular ErbB4 localization and survival were found in the subset of patients whose cancer did not express Wwox (HR, 1.30; 95% CI, 0.46–3.68; P = 0.61 and HR, 0.94; 95% CI, 0.43–2.06; P = 0.89 for membranous versus nonmembranous and nuclear versus nonnuclear ErbB4 expression, respectively). Taken together, our data indicate that patients whose cancer has ErbB4 localized at the cell membrane generally have a favorable outcome, and presence of Wwox expression in cancer cells accentuates this effect.

Presence of Wwox is associated with ErbB4 localization at the cell membrane but not with ErbB4 nuclear expression. The majority of breast cancers that expressed Wwox (186 of 283, 66%) were also positive for ErbB4 staining (in any subcellular compartment), whereas only 29% (17 of 58) of ErbB4-negative patients showed Wwox immunoreactivity (P = 0.0002; Table 1). Because Wwox expression has been suggested to prevent nuclear accumulation of ErbB4 in vitro (6), we investigated whether Wwox expression is associated with ErbB4 subcellular localization in vivo. In particular, we set to examine whether normal expression of Wwox is associated with retained membranous ErbB4 localization and whether loss of Wwox expression is linked with nuclear ErbB4 localization. Immunohistochemical analyses indicated that Wwox expression was positively associated with ErbB4 localization at the cell membrane (P = 0.0003; Table 1). In contrast, no significant association was observed between Wwox expression and nuclear ErbB4 immunoreactivity (P = 0.82). These data show that expression of Wwox is associated with localization of ErbB4 at the cell membrane in breast cancer.

Wwox binds both ErbB4 isoforms present in breast cancer tissues. Different ErbB4 isoforms are generated as a result of tissue-specific alternative splicing of the extracellular juxtamembrane and the intracellular cytoplasmic domains (11, 21), resulting in different molecular interactions (7). We reported previously that Wwox binds the JM-a CYT-2 isoform of ErbB4 (6). Real-time reverse transcription-PCR analyses showed that transcripts encoding the

Figure 1. Association of Wwox protein expression with survival of breast cancer patients. Kaplan-Meier survival (distant disease-free survival) curves comparing Wwox expression status analyzed by immunohistochemistry are shown. The analysis included all patients (A), ER-positive patients (B), or ER-negative patients (C).
JM-a domain are the only mRNAs present in breast cancer samples, together with both CYT-1 and CYT-2 types of cytoplasmic domains (11). Therefore, we did immunoprecipitation experiments to examine whether Wwox interacts similarly with both ErbB4 JM-a CYT-1 and ErbB4 JM-a CYT-2. To this end, COS-7 cells were transiently cotransfected to express Myc-Wwox or mutated Myc-Wwox-Y33R [containing a WW1 domain mutation that abrogates Wwox interaction with PPxY motifs (6, 19)] together with ErbB4 JM-a CYT-1 or ErbB4 JM-a CYT-2. Twenty-four hours after transfection, the cells were treated or not with the phorbol ester PMA to stimulate ErbB4 cleavage generating ICD. Cells were immunoprecipitated using anti-ErbB4 or anti-Myc antibodies and

**Figure 2.** Association of Wwox protein expression analyzed simultaneously with ErbB4 protein localization with survival of breast cancer patients. Kaplan-Meier survival (distant disease-free survival) curves comparing immunohistochemical Wwox expression status and subcellular localization of ErbB4 immunoreactivity are shown.

**Table 1.** Association of Wwox immunoreactivity with ErbB4 expression and subcellular localization

<table>
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<tr>
<th>Wwox expression</th>
<th>Overall ErbB4 expression</th>
<th>ErbB4 localization at cell membrane</th>
<th>ErbB4 localization in the nucleus</th>
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<tr>
<td>Wwox expression</td>
<td>P</td>
<td>P</td>
<td>P</td>
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<td>Reduced (25–50%)</td>
<td>Absent (0–25%)</td>
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<td>13 (20%)</td>
<td>11 (17%)</td>
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<tr>
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<tr>
<td>No</td>
<td>130 (48%)</td>
<td>39 (14%)</td>
<td>103 (38%)</td>
</tr>
</tbody>
</table>

NOTE: Numbers of patients (%) are indicated.

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Research.
immunoblotted with anti-Myc or anti-ErbB4 antibodies. As shown in Fig. 3, both CYT-1 and CYT-2 isoforms interacted with the wild-type (wt) Wwox but much less with the mutated Wwox-Y33R. In addition to full-length ErbB4, soluble ICD forms of ErbB4 coimmunoprecipitated with Wwox (data not shown). These results indicate that Wwox, via its first NH₂-terminal WW domain, associates with both ErbB4 isoforms expressed in breast cancer.

Expression of Wwox up-regulates levels of membrane-anchored full-length ErbB4. As Wwox expression was associated with ErbB4 localization at the cell membrane in vivo (Table 1), and Wwox expression in association with ErbB4 localization at the cell membrane also influenced patient outcome (Fig. 2), we next addressed whether expression of Wwox affected the levels of membranous ErbB4 in vitro. Transient expression of Myc-Wwox in COS-7 cells impaired degradation of exogenously expressed membrane-anchored full-length ErbB4 JM-a CYT-1 in the presence of the protein synthesis inhibitor cycloheximide (Fig. 4A). To assess the effect of endogenous Wwox on ErbB4 naturally expressed by breast cancer cells, MCF-7 cells previously shown to express both JM-a CYT-1 and JM-a CYT-2 (11) were used. Transient expression of MCF-7 cells with siRNAs specifically targeting human Wwox mRNA resulted in reduced expression of full-length endogenous ErbB4 protein (Fig. 4B). Consistent with this finding, MEF clones established from null mice with targeted Wwox alleles showed less endogenous full-length ErbB4 expression when compared with MEFs isolated from wt littermate mice (Fig. 4C). These data indicate that Wwox expression is causally related to increased stability of membrane-anchored full-length ErbB4.

Discussion

Here, we show that Wwox expression strongly associates with membranous ErbB4 localization as well as with overall ErbB4 expression in a series of 556 breast cancers in vivo. The data also suggest that expression of intact Wwox, together with localization of ErbB4 at the cell membrane, is associated with generally favorable survival. Moreover, Wwox expression enhances stability of membrane-anchored full-length ErbB4 in breast cancer cells in vitro and in vivo. These findings represent the first evidence that Wwox association with ErbB4 is of prognostic and clinical significance in breast cancer.

Consistent with previous studies (17, 20), we observed that a significant proportion of breast cancer samples exhibited either reduced (14%) or total loss (36%) of Wwox expression. Absence of Wwox expression was associated with the number of positive axillary nodes as well as with poor survival in the unselected series of 556 patients. Interestingly, the association of Wwox loss with unfavorable outcome was preserved in the subgroup of ER-negative but not in ER-positive patients. Similarly, Wwox loss was associated with poor survival in PgR-negative but not in PgR-positive patients. Finally, an association was found between Wwox loss and poor survival in the subset of ErbB2-negative breast cancer but not in ErbB2-positive disease. These observations imply that loss of Wwox signaling may be biologically significant in the triple-negative (ER⁻, PgR⁻, and ErbB2⁻) high-risk breast cancer (22).

In a previous study, we showed that Wwox, via its first WW domain, associates with the PPₓᵧ motif of ErbB4 and functions as a regulator of ErbB4 ICD subcellular localization (6). Wwox inhibits nuclear translocation of ICD and hence suppresses its trans-activating ability. Here, we extend these findings by showing that Wwox equally binds both of the ErbB4 isoforms present in breast cancer tissue in vivo (i.e., ErbB4 JM-a CYT-1 and JM-a CYT-2) and that expression of Wwox correlates positively with expression of ErbB4.

Figure 3. Wwox interacts with ErbB4 isoforms present in breast cancer tissues. COS-7 cells were transfected with plasmids encoding ErbB4 isoforms JM-a CYT-1 or JM-a CYT-2 and either empty vector, wt Myc-Wwox, or Myc-Wwox-Y33R with mutated NH₂-terminal WW1 domain. To stimulate ErbB4 cleavage, cells were treated for 30 min with or without 100 ng/mL PMA. Cell lysates were precipitated with anti-ErbB4 antibody (A) or with an anti-Myc antibody (B) and subsequently analyzed by reciprocal Western blotting using an anti-Myc (A) or anti-ErbB4 antibody (B). Original loading of ErbB4 and Wwox proteins was also controlled by Western blot analyses as indicated.
ErbB4 and localization of ErbB4 at the cell membrane. In addition, we found a significant association between coexpression of Wwox and ErbB4 at the cell membrane and favorable survival compared with patients whose cancer expressed ErbB4 at the membrane but lacked Wwox or patients with detectable Wwox but no ErbB4 signal at the membrane. Importantly, survival of the 17% of patients with ErbB4 immunoreactivity at the membrane is favorable regardless of the Wwox status (5-year survival rate of 91% as opposed to 79% in the whole population of 2,459 patients included in the tissue microarrays used; refs. 11, 16), whereas combining the Wwox status to the prognostic evaluation improves the prognosis even further. Indeed, the 5-year survival rate is 94% for those 16% of patients with normal or reduced Wwox (>25% positive cells) and ErbB4 at the membrane and 98% for those 12% with normal Wwox (>50% positive cells) and ErbB4 at the membrane.

The WWOX gene spans a fragile genomic region that is frequently altered in preneoplastic and invasive breast carcinoma (1–3, 17, 20). Recent observations by the Huebner laboratory suggest that Wwox expression can also be down-regulated by DNA hypermethylation of the promoter region of WWOX (23). Importantly, our results from Wwox knockout mice indicate that Wwox heterozygous mice exhibit a higher incidence of spontaneous tumors compared with the wt control mice (5). The molecular mechanism underlying the tumor suppressor function of Wwox, however, still remains to be fully elucidated. Our present study suggests that Wwox association with ErbB4 may contribute to Wwox tumor suppressor function in breast cancer cells. We have also previously shown that Wwox binds AP2 transcription factors (α and γ) in the cytoplasm and suppresses their transactivation potential (24), suggesting that, in addition to ErbB4, Wwox loss in breast cancer can affect other signal transduction pathways contributing to tumorigenicity.

Our data indicate that the mechanism by which Wwox regulates ErbB4 function involves more than one mechanism. In addition to suppressing the nuclear activity of ErbB4 (6), interaction between Wwox and ErbB4 may contribute to Wwox tumor suppressor function in breast cancer cells. We have also previously shown that Wwox binds AP2 transcription factors (α and γ) in the cytoplasm and suppresses their transactivation potential (24), suggesting that, in addition to ErbB4, Wwox loss in breast cancer can affect other signal transduction pathways contributing to tumorigenicity.

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Figure 4. Wwox expression stimulates accumulation of full-length ErbB4. A, COS-7 cells transiently expressing ErbB4 JM-a CYT-1 alone (lanes 1–4) or together with Wwox (lanes 5–8) were starved without serum for 2 h followed by addition of protein synthesis inhibitor cycloheximide (CHX; 100 μg/mL) for 2, 4, or 6 h. Cell lysates were analyzed by Western blotting using an anti-ErbB4 antibody. Expression of Myc-Wwox was controlled with an anti-Myc antibody. B, effect of RNA interference directed against endogenous Wwox on endogenous ErbB4 protein level. MCF-7 cells were transfected with 100 nmol/L siRNAs as indicated. Forty-eight hours after treatment with siRNA, cells were harvested and whole-cell extracts were resolved by Western blot analysis with anti-ErbB4, anti-Wwox, and anti-Gapdh antibodies. C, MEFs were isolated from 13.5 d Wwox-null embryos and their corresponding wt control embryos. Whole-cell extracts from the different clones were resolved by SDS-PAGE, and Western blot analysis was done using anti-ErbB4, anti-Wwox, and anti-Gapdh antibodies.

Figure 5. A schematic model of Wwox-ErbB4 association in breast cancer. A, when Wwox is expressed normally, it physically associates with ErbB4 stabilizing full-length ErbB4 at the cell membrane. In addition, it may bind ErbB4 ICD and other PPxY motif-containing proteins, such as AP2 transcription factors, in the cytosol, preventing their transactivation activity in the nucleus. B, when Wwox is lost, full-length ErbB4 is down-regulated at the cell membrane and PPxY motif-containing proteins, including ErbB4 ICD and AP2 transcription factors, are free to translocate into the nucleus.
proteasomal pathways. For example, membrane-coupled HECT-type of ubiquitin ligases that also include WW domains for protein-protein interactions could compete with Wwox for an interaction with full-length ErbB4 within the membrane environment (25, 26).

Whereas Wwox is a relatively well-defined tumor suppressor (2, 5), the role of ErbB4 in breast cancer is complicated by the existence of functionally different ErbB4 isoforms and the extent to which some of these isoforms are processed into intracellular signaling fragments (21, 27, 28). Most clinical studies have shown a significant association between total ErbB4 expression and favorable prognostic indicators, particularly ER positivity (11, 29, 30), but reports on association with survival have been contradictory (28, 31). Our recent observations, however, indicate that ErbB4 has differential prognostic significance when functioning in different subcellular localizations: whereas full-length ErbB4 at the cell membrane associates with favorable survival, localization of ErbB4 ICD in the nucleus associates with worse prognosis (11). Similar findings about the association of nuclear ErbB4 with unfavorable survival have recently been reported by Tovey et al. (32). We found in our prior study that in vitro overexpression of a cleavable ErbB4 isoform in breast cancer cells results in enhanced proliferation, anchorage-independent growth, and estrogen response element-mediated transcriptional activity (11). ErbB4 ICD is a direct coregulator of ER in the nucleus and is required for estrogen-stimulated proliferation of the T-47D breast cancer cell line (33).

Therefore, neutralizing nuclear ErbB4 and stabilizing intact membrane-anchored ErbB4 could both be independent mechanisms to suppress breast cancer cell growth.

In conclusion, we propose a model (Fig. 5) in which normally expressed Wwox binds the COOH-terminal fragment of ErbB4, which may both prevent translocation of ErbB4 ICD into the nucleus and stabilize the full-length ErbB4 at the cell membrane. This favors signaling via the full-length ErbB4 as opposed to the nuclear ErbB4 and is associated with favorable survival in early breast cancer. When Wwox is absent or its function is lost, this balance is disturbed promoting tumor progression. Simultaneous expression of Wwox and ErbB4 localization on the cell membrane may be a novel means to define a subgroup of breast cancer patients with a favorable outcome.

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