Physical and Functional Interactions between the Wwox Tumor Suppressor Protein and the AP-2γ Transcription Factor

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The WWOX gene encodes a tumor suppressor WW domain-containing protein, Wwox. Alterations of WWOX have been demonstrated in multiple types of cancer, and introduction of Wwox into Wwox-negative tumor cells has resulted in tumor suppression and apoptosis. The Wwox protein contains two WW domains that typically bind proline-rich motifs and mediate protein–protein interactions. Recently, we have described functional cross-talk between the Wwox protein and the p53 homologue, p73. To further explore the biological function of Wwox, we investigated other interacting candidates. In this report, we demonstrate a physical and functional association between AP-2γ transcription factor and the Wwox protein. AP-2γ at 20q13.2 encodes a transcription factor and is frequently amplified in breast carcinoma. We show that Wwox binds to the PPPY motif of AP-2γ via its first WW domain. Alterations of tyrosine 33 in the first WW domain of Wwox or the proline-rich motif in AP-2γ dramatically reduce this interaction. In addition, our results demonstrate that Wwox expression triggers redistribution of nuclear AP-2γ to the cytoplasm, hence suppressing its transactivating function. Our results suggest that Wwox tumor suppressor protein inhibits AP-2γ oncogenic activity by sequestering it in the cytoplasm.

INTRODUCTION

WW domain-containing oxidoreductase (WWOX) is a tumor suppressor gene that spans one of the most active fragile sites in the human genome, FRA16D (1, 2). WWOX, also known as FORII, is located at 16q23.3–24.1, a region with a high incidence of loss of heterozygosity and homozygous deletions (3, 4). WWOX expression is altered in several cancer types, including breast, prostate, ovarian, esophageal, lung, pancreatic, and gastric carcinomas (1, 2, 5–9). Similar to FRA16D, WWOX consists of five members, AP-2 (20, 21), and AP-2γ, AP-2α, AP-2β, and the recently discovered AP-2ε (20, 21) and plays important roles in regulating gene expression. The AP-2 transcription factors share a highly conserved COOH terminus that is involved in dimerization and DNA binding. The members of the AP-2 family transcription factors play important roles in orchestrating embryonic development by affecting cell differentiation, proliferation, and survival in multiple organs and tissues (20). Furthermore, AP-2 transcription factors have been implicated in breast carcinogenesis. This notion is supported by the fact that the genomic locus 20q13.2 containing AP-2γ is frequently amplified in breast cancer cell lines and breast carcinomas (22). In addition, AP-2γ has been linked to the regulation of the ERBB2 (23, 24) and ERα (25, 26) genes involved in tumor progression of breast cancer. Altogether, these data suggest that AP-2γ possesses an oncogenic activity in breast cancer. Here, we show negative regulation of AP-2γ by the Wwox tumor suppressor protein through direct protein–protein interaction.

ABSTRACT

The WWOX gene encodes a tumor suppressor WW domain-containing protein, Wwox. Alterations of WWOX have been demonstrated in multiple types of cancer, and introduction of Wwox into Wwox-negative tumor cells has resulted in tumor suppression and apoptosis. The Wwox protein contains two WW domains that typically bind proline-rich motifs and mediate protein–protein interactions. Recently, we have described functional cross-talk between the Wwox protein and the p53 homologue, p73. To further explore the biological function of Wwox, we investigated other interacting candidates. In this report, we demonstrate a physical and functional association between AP-2γ transcription factor and the Wwox protein. AP-2γ at 20q13.2 encodes a transcription factor and is frequently amplified in breast carcinoma. We show that Wwox binds to the PPPY motif of AP-2γ via its first WW domain. Alterations of tyrosine 33 in the first WW domain of Wwox or the proline-rich motif in AP-2γ dramatically reduce this interaction. In addition, our results demonstrate that Wwox expression triggers redistribution of nuclear AP-2γ to the cytoplasm, hence suppressing its transactivating function. Our results suggest that Wwox tumor suppressor protein inhibits AP-2γ oncogenic activity by sequestering it in the cytoplasm.

MATERIALS AND METHODS

Cell Culture. Human embryonic kidney 293 cells, human cervical carcinoma HeLa cells, human MCF-7 breast carcinoma cells, and mouse NIH-3T3 fibroblasts were grown in RPMI supplemented with 10% fetal bovine serum (Gibco-BRL, Invitrogen, Carlsbad, CA) and Gentamicin. Cells were maintained at 37°C in a water-saturated atmosphere of 5% CO2 in air.

Plasmid Constructs and Transient Transfections. The mammalian expression plasmids encoding MYC epitope-tagged WWOX and pCMV-MYC-WWOX133R were described previously (17). Full-length cDNA of AP-2α and AP-2γ were cloned into a hemagglutinin (HA) epitope-tagged pCMV vector (Clontech, Palo Alto, CA) using standard protocols. A diagram of both Wwox and Pin1, and FBPs. Like the SH3 domain, the WW domain is characterized by interaction with proline-containing ligands and mediates protein–protein interactions (13, 14). The short-chain dehydrogenase/reductase domain is usually involved in the metabolism of steroid hormones such as androgens and estrogens (15, 16). Indeed, Wwox expression is up-regulated in endocrine organs such as testis and breast, indicating an important role of Wwox in these tissues (1).

Recently, we performed an enzyme-linked immunosorbent assay (ELISA)-like assay to determine putative interacting proteins with Wwox (17). Peptides containing the WW domain-binding motif PPPY were assayed against WW domains of Wwox expressed as glutathione S-transferase (GST) fusion proteins. Several peptides bound with high affinity to WW domains of Wwox.

We showed that Wwox specifically binds the PPPPY motif of p73, both in vitro and in vivo, via its first WW domain (17). Our data revealed a functional relationship between p73 and Wwox and its role in apoptosis. Another report showed that the murine Wwox ortholog, Wox1, physically interacts with p53 and Jnk1 upon stress conditions (18, 19). Taken together, these observations demonstrate that Wwox is involved in stress and apoptotic responses contributing to the tumor suppressor activity of Wwox.

We aimed to investigate other putative interactions of Wwox to further explore the molecular mechanism of its tumor suppressive activity. Our affinity assay results suggested that the first WW domain of Wwox would bind with high affinity to a peptide derived from the activating protein-2 γ and α (AP-2γ and α). The AP-2 gene family consists of five members, AP-2α, AP-2β, AP-2γ, AP-2δ, and the recently discovered AP-2ε (20, 21) and plays important roles in regulating gene expression. The AP-2 transcription factors share a highly conserved COOH terminus that is involved in dimerization and DNA binding. The members of the AP-2 family transcription factors play important roles in orchestrating embryonic development by affecting cell differentiation, proliferation, and survival in multiple organs and tissues (20). Furthermore, AP-2 transcription factors have been implicated in breast carcinogenesis. This notion is supported by the fact that the genomic locus 20q13.2 containing AP-2γ is frequently amplified in breast cancer cell lines and breast carcinomas (22). In addition, AP-2γ has been linked to the regulation of the ERBB2 (23, 24) and ERα (25, 26) genes involved in tumor progression of breast cancer. Altogether, these data suggest that AP-2γ possesses an oncogenic activity in breast cancer. Here, we show negative regulation of AP-2γ by the Wwox tumor suppressor protein through direct protein–protein interaction.

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and AP-2 binding the functional domains is shown in Fig. 1A. HA-AP-2/Y59, 64A was obtained by site-directed PCR mutagenesis kit (Stratagene, La Jolla, CA), according to the manufacturer’s instruction. Both terminal tyrosines of the PPPY motifs, Y59 and Y64, in AP-2 were mutated to alanine (A), producing the expression vector HA-AP-2/Y59, 64A. The following primers were used: forward, 5′-CGCACCGCGGCTTTCCCTCTCCGGCCACGAGCCGAGGCTG64A; reverse, 5′-GTGCTGGCGGAGGGGGAACGGCGGGTGGGCCCTGATTTGGC. Overexpression of proteins was achieved by transient transfections using Fugene 6 transfection reagent, according to the manufacturer’s instructions (Roche Applied Science, Mannheim, Germany).

**Immunoprecipitation and Immunoblot Analysis.** Cells were lysed using NP40 lysis buffer containing 50 mmol/L Tris (pH 7.5), 150 mmol/L NaCl, 10% glycerol, 0.5% NP40, and protease inhibitors. Immunoprecipitations were carried out overnight as described previously (17). Antibodies used were mouse monoclonal anti-HA (Covance, Princeton, NJ) and mouse monoclonal anti-Myc (Zymed, South San Francisco, CA). Western blotting was performed under standard conditions. Antibodies used for immunoblot were anti-HA horseradish peroxidase (Roche Applied Science), anti-Myc horseradish peroxidase (Santa Cruz Biotechnology), and mouse monoclonal anti-AP2 (Santa Cruz Biotechnology).

**Glutathione S-Transferase Pull-down Assay.** Using standard protocols, we used pGEX6P-2 plasmid (Amersham Biosciences, Uppsala, Sweden) to construct and express GST fusions of the first 50 amino acids of Wwox that we used pGEX6P-2 plasmid (Amersham Biosciences, Uppsala, Sweden) to construct and express GST fusions of the first 50 amino acids of Wwox that we used pGEX6P-2 plasmid (Amersham Biosciences, Uppsala, Sweden) to construct and express GST fusions of the first 50 amino acids of Wwox that we used pGEX6P-2 plasmid (Amersham Biosciences, Uppsala, Sweden) to construct and express GST fusions of the first 50 amino acids of Wwox that we used pGEX6P-2 plasmid (Amersham Biosciences, Uppsala, Sweden) to construct and express GST fusions of the first 50 amino acids of Wwox that we used pGEX6P-2 plasmid (Amersham Biosciences, Uppsala, Sweden) to construct and express GST fusions of the first 50 amino acids of Wwox. A diagram of GST fusion proteins or wild-type GST that was incubated with 500 g of immobilized purified GST (Promega), (4). A diagram of wild-type WW-I and mutated WW-I (WW-I-Y333) is shown in Fig. 2A. Pull-down assays were performed using 5 μg of immobilized purified GST fusion proteins or wild-type GST that was incubated with 500 μg of total cellular proteins, prepared from 293 cells transiently transfected with the indicated plasmids. The lysates were precleared with glutathione-agarose beads and then incubated for overnight at 4°C with the indicated GST fusion proteins. After four washes with immunoprecipitation washing buffer (see above), complexes were resolved by SDS-PAGE and probing with mouse monoclonal anti-HA (Covance) and anti-GST (Santa Cruz Biotechnology, Santa Cruz, CA). Ponceau S solution (Sigma) was used to stain nitrocellulose membranes for protein levels of the different GST fusion proteins.

**Luminescence Assays.** Using standard cloning protocols, we cloned a luciferase reporter downstream of TFP2-binding elements. Three repeats of the AP-2-binding site were cloned upstream of the basic promoter of thymidine kinase (TK) in the pGL3 (Promega), (59PPPY62) (designated as AP2-LUC). A diagram of AP2-LUC is shown in Fig. 4A. Transient transfections of HeLa cells or 293 cells with AP2-LUC, together with the indicated expression vector combination were carried out with the Fugene 6 transfection reagent (Roche Applied Science). Cells were collected 24 hours later, rinsed with PBS, re-suspended in reporter cell lysis buffer (Promega), and incubated for 10 minutes at room temperature. Insoluble material was spun down, and luciferase activity was quantified using a luciferase assay kit (Promega), with the aid of a luminometer. Results are shown as fold induction of the luciferase activity compared with control cells transfected with empty vector alone.

**Immunofluorescence.** Cells were seeded on fibronectin-covered cell culture slides (Becton Dickinson, Franklin Lakes, NJ), fixed for 10 minutes in 3.7% PBS-buffered formaldehyde, and permeabilized with 0.05% Triton X-100 in PBS for 5 minutes. Cells were then incubated for 1 hour in 100% goat serum to block nonspecific bindings (Invitrogen, Carlsbad, CA) and incubated with a primary antibody for 1 hour in 10% goat serum in PBS and with secondary antibody under the same conditions. Antibodies used were anti-HA, anti-Myc rabbit polyclonal antibody (Upstate Biotechnology, Lake Placid, NY), anti-gst-wwox serum (27), antismo Fluoro-conjugated antibody and antirabbit Texas red conjugated antibody (Molecular Probes, Eugene, OR). Cells were examined by confocal microscopy (Bio-Rad, Hercules, CA) under 63 magnification.

**RESULTS**

**Physical Interaction between Wwox and AP-2/γ In vivo and In vitro.** In an attempt to identify candidate Wwox-interacting proteins, we studied the ability of various proline-rich peptides to bind the WW domains of Wwox. Peptides containing the WW domain-binding motif PPPY were assayed against the two WW domains of Wwox expressed as GST fusion proteins in an ELISA-like assay (28). A peptide derived from AP-2/γ (56PPPYFPPPY62) and, to a lesser extent, AP-2/α (56PPPY62) bound with high affinity to the first WW domain of Wwox (results not shown). Based on this in vitro evidence, we proceeded to investigate the ability of Wwox to bind AP-2/α and AP-2/γ in vivo. We transiently cotransfected 293 cells with vectors expressing either HA-AP-2α or HA-AP-2γ and Myc-Wwox. Cell lysates were immunoprecipitated with anti-HA or anti-Myc antibodies followed by immunoblotting with horseradish peroxidase conjugated antibody to HA or Myc. The results demonstrate that Wwox interacts with AP-2/α and with AP-2/γ as determined by immunoprecipitation with anti-Myc and immunoblotting with anti-HA antibody (Fig. 1B, lanes 1 and 4, top panel). As a control, there were no detectable complexes in anti-immunoglobulin G (IgG) immunoprecipitates (Fig. 1B, lanes 2 and 5). The same complexes were detected in reverse when immunoprecipitation with anti-HA and immunoblotting with anti-Myc antibody (Fig. 1B, lanes 3 and 6, bottom panel). In accordance with the affinity assay prediction, Wwox bound AP-2γ more strongly than AP-2α (Fig. 1B), therefore we proceeded to further investigate Wwox-AP-2γ interaction.

To map the region responsible for Wwox-AP-2 interaction, we used site-directed PCR mutagenesis to generate point mutations and determined the ability of these mutants to interact. We had a strong indication from the in vitro affinity assay that the first WW domain of Wwox interacts with the proline-rich motif PPPY in both AP-2α and AP-2/γ. We also showed recently that a point mutation in tyrosine 33 (Y33), Y33R, in the first WW domain of Wwox is sufficient to abolish interaction of Wwox and p73 (17). Therefore, we examined whether this mutant form of Wwox (Myc-WwoxY333) can immunoprecipitate AP-2γ. To this end, we cotransfected 293 cells with expression vectors encoding HA-AP-2γ and MYC-WWOXY33R, and extracts were precipitated with anti-Myc and blotted with anti-HA antibodies. We found that Y33R point mutation in the first WW domain of Wwox dramatically reduces Wwox association with AP-2γ (Fig. 1C, Lane 1 versus 4, top panel). The same interaction was demonstrated when extracts were precipitated with anti-HA and blotted with anti-Myc antibodies (Fig. 1C, Lane 3 versus 6, bottom panel).

In addition, we generated point mutations in the PPPY motif of AP-2γ and determined the ability of these mutants to bind Wwox by coimmunoprecipitation. In the AP-2γ proline-containing motif, terminal tyrosines Y59 and Y64 were mutated to alanine (A), producing the expression vector HA-AP-2γY59,64A. To determine whether Myc-Wwox immunoprecipitate HA-AP-2γY59,64A, we cotransfected 293 cells with both expression vectors and immunoprecipitated with anti-Myc antibody or anti-HA. Fig. 1D shows that point mutations in the proline-containing motif of AP-2γ significantly decreases Wwox-AP-2γ association (Fig. 1D, Lane 1 versus 4, top panel). Much milder, but consistent, difference was observed in reverse coimmunoprecipitation experiment (Fig. 1D, Lane 3 versus 6, bottom panel). The specificity of these interactions was further confirmed when the same result was obtained when cotransfecting 293 cells with Myc-WwoxY333R and HA-AP-2γY59,64A (Fig. 1E, Lane 4, top panel, and Lane 6, bottom panel).

To demonstrate direct binding between Wwox and AP-2γ, we carried out in vitro GST pull-down experiments. Human embryonic kidney 293 cells were transfected with expression vectors HA-AP-2γ or mutated HA-AP-2γY59,64A. Protein lysates were incubated with GST fusion proteins that contain the first 50 amino acids of Wwox sequence coding for the first WW domain (WW-I), either wild-type or harboring a point mutation in Y33 (WW-I-Y333F, Fig. 2A). The pulled down proteins were immunoblotted with anti-HA antibody. A specific complex was pulled down when wild-type WW-I-GST fusion,
but not GST alone or WW-I-Y33F, was incubated with AP-2γ-expressing lysate (Fig. 2B, Lanes 1 and 3 versus 2). In contrast, GST-WW-I or GST-WW-I-Y33F fusions were unable to pull down the mutated AP-2γ (Fig. 2B, Lanes 5 and 6), confirming that the PPPY motif and first WW domain are responsible for Wwox-AP-2γ interaction.

Wwox-AP-2γ Interaction Affects AP-2γ Intracellular Localization. We also examined the effect of Wwox-AP-2γ interaction on the subcellular localization of Wwox and AP-2γ. To address this, we studied the localization of both proteins with the aid of confocal microscopy. HA-AP-2γ or HA-AP-2γ-Y59, 64A alone or transfected with MYC-WWOX was transiently expressed in NIH-3T3 cells. Localization of the HA- or Myc-tagged proteins was then determined by immunofluorescent staining using the appropriate antibodies, as described in Materials and Methods. Although the majority of AP-2γ localizes to the nucleus, some cells also showed mild cytoplasmic staining of transfected NIH-3T3 cells (Fig. 3Aa). In contrast, exogenous Wwox is mainly detected in the cytoplasm (Fig. 3Ab). In cells cotransfected with Myc-Wwox and HA-AP-2γ, 60 to 70% of cells showed cytoplasmic staining of AP-2γ where it colocalizes with Wwox (Fig. 3Ac). Similar results were obtained with MCF-7 cells, which express high levels of endogenous Wwox protein (Fig. 3Ad), upon transfection with HA-AP-2γ (Fig. 3Bc). By contrast, MCF-7 cells transfected with HA-AP-2γ-Y59, 64A show only nuclear localization of AP2γ (Fig. 3Be). Interestingly, coexpression of Wwox-Y33R and AP-2γ showed also a cytoplasmic AP-2γ localization similar to wild-type Wwox (data not shown). These results are consistent with immunoprecipitation results in which a mutation in Y33 did not completely inhibit interaction with AP-2γ (Fig. 1C). Cumulatively, these results indicate that Wwox can modulate the cellular localization of AP-2γ and cause its redistribution to the cytoplasmic compartment.

Wwox Suppresses the Transcriptional Activity of AP-2γ. Because Wwox can alter the nuclear localization of AP-2γ, we examined whether this modulation affects the transcriptional activity of AP-2γ. We constructed a luciferase reporter cloned downstream of TFAP2β binding elements (AP2-LUC, Fig. 4A). Cotransfection of 293 cells with AP2-LUC and HA-AP-2γ alone caused activation of luciferase reporter (Fig. 4B, bar 4). By contrast, cotransfection of AP2-LUC and the MYC-WWOX constructs had no detectable effect on luciferase activity (Fig. 4B, bar 2). Expression of Wwox significantly suppressed the transactivation function of AP2γ (Fig. 4B, bar 3). However, HA-AP-2γ-Y54, 65A (mAP-2γ) expression has less luciferase activation by itself, and its coexpression with Wwox has also lesser effect on AP2γ transactivation activity when compared with the effect on wild-type AP-2 (Fig. 4B, bars 5 and 6 versus 3 and 4). Similar results were observed in HeLa cells, and a dose-dependent effect of Wwox suppression on AP-2γ transcriptional activity was also observed (Fig. 4C). These results indicate that Wwox can suppress AP2γ transactivation activity in a dose-dependent manner.

Fig. 1. Wwox physically interacts with AP-2γ in vivo. A, a schematic diagram showing the functional domains in Wwox and AP-2γ. B, 293 cells were transiently transfected with the expression plasmids encoding Myc-Wwox with HA-AP-2α or HA-AP-2γ (as indicated at the top). Whole-cell lysates were immunoprecipitated (IP) with anti-Myc, anti-IgG, or anti-HA antibodies 36 hours after transfection. The immunoprecipitates were analyzed by immunoblotting (IB) with anti-HA (top panel) or anti-myc (lower panel) antibodies. C, 293 cells were transiently transfected with the expression plasmids encoding Myc-Wwox or Myc-WwoxY33R and treated as in B. D, 293 cells were transiently cotransfected with the expression plasmids encoding Myc-Wwox and HA-AP-2γ or HA-AP-2γ-Y59, 64A. Immunoprecipitation and immunoblotting were as in B. E, 293 cells were transfected with the indicated vectors and lysates were treated as B.
DISCUSSION

In this study, we have shown that Wwox interacts with AP-2γ in vitro and in vivo, and this association involves the first WW domain of Wwox and an AP-2γ region containing the PPPY motif (Fig. 1). The interaction of WW domains with proline-rich ligands is mainly dependent on the structure of these motifs (13, 14). We have recently shown that Wwox also interacts with a p73 region containing a PPPPY motif (17). The first WW domain of Wwox belongs to the first class (class I) of the current classification of the diverse WW domains and binds to ligands containing a PPyY motif (13, 14). Recently, Ludes-Meyers et al. (29) confirmed our observations and showed that Wwox binds to the same proline-rich ligand. Interestingly, a point mutation in the terminal tyrosine of the PPPY motif interferes with Wwox-AP-2γ interaction, emphasizing the importance of this motif. Similar to Wwox-p73 interaction, a point mutation in Y33 of Wwox significantly disrupts Wwox-AP-2γ association. On the other hand, Wwox-Y33R still shows some colocalization with AP-2γ in cytoplasm. Therefore, we cannot exclude that the second WW domain or another domain of Wwox may also contribute to this interaction. Possible involvement of other Wwox domains is also consistent with the fact that full-length Wwox shows visible coimmunoprecipitation with mutated AP-2γ, whereas GST pull-down assay using only the first WW domain of Wwox does not show any interaction. Y33 is a phosphorylation target of Src kinase (17). The function, activity, stability, or subcellular localization of many proteins can be altered after post-translational modifications such as phosphorylation. We showed that Y33 phosphorylation enhances Wwox-p73 association (17), and this phosphorylation is also essential for pro-apoptotic activity of Wwox (19). Therefore, Y33 seems to harbor an important function in regulating Wwox activity.

We have also shown, using confocal microscopy, that Wwox causes the redistribution of AP-2γ to the cytosol. Interestingly, Pelikainen et al. (30), showed an increased cytoplasmic expression of AP-2γ in breast cancer tumor samples, suggesting a functional significance of such redistribution.

Our data suggest that Wwox suppresses AP-2γ transcriptional activity, as demonstrated by luciferase reporter assay (Fig. 4). Elorentza et al. (31) recently reported that AP-2γ interacts with the SUMO-conjugating enzyme UBC9 and is sumoylated in vivo. By exogenously increasing cellular levels of free SUMO-1, AP-2γ transcriptional activity was suppressed. Because sumoylation is known to

Fig. 2. Direct interaction between Wwox and AP2γ in vitro. A, a diagram showing GST fusion proteins, GST-WW-I and GST-WW-IY33F, of the first 50 amino acids of Wwox. B, 293 cells were transfected with HA-AP-2γ or HA-AP-2γ59,64A, and lysates were mixed with the indicated GST fusions or GST alone. Complexes were captured with glutathione-Sepharose and bound proteins were detected by anti-HA immunoblot. The bottom panel shows a staining of the membrane with Ponceau S solution for GST fusion protein levels.

Fig. 3. Subcellular localization of Wwox and AP-2γ. A, NIH-3T3 cells were cotransfected with the plasmids indicated in each panel. After fixation and permeabilization, the cells were coimmunostained with anti-HA and anti-Myc antibodies, followed by fluorescein isothiocyanate-conjugated antimouse IgG (green; AP-2γ) and Texas red–conjugated antirabbit IgG (red; Wwox), and cells were examined by confocal microscopy. Colocalization of Wwox and AP-2γ is shown in yellow as a result of merging the two colors. B, MCF-7 cells were transfected as indicated. Antibodies used were polyclonal anti-wwox (red) and monoclonal anti-HA (green).
alter the subcellular localization of many target proteins such as PML (32) and p73 (33). Eloranta et al. (31) suggested that suppression of AP-2γ transactivation function after sumoylation is due to its localization into an inactive subcellular compartment. Similarly, our findings suggest that the reduced AP-2γ transcriptional activity is likely to be due to its cytoplasmic colocalization with Wwox.

The function of AP-2γ transcription factor in tumorigenesis is intriguing. AP-2 genes have been shown to regulate proliferation (reviewed in ref. 20). AP-2γ null mice displayed an embryonic lethal phenotype due to the decreased proliferation of placental cells (34). In addition, AP-2γ stimulates proliferation and apoptosis and impairs differentiation in a transgenic mouse model (35). Interestingly, the ERBB2 gene, a molecule known for its oncogenic potential, is a direct transcriptional target of AP-2γ (23, 24). Indeed, in AP-2γ transgenic mammary gland, the expression of ErbB2 protein is up-regulated (35). Altogether, this implies a role of AP-2γ transcription factor in the maintenance of a proliferative and undifferentiated state of cells, characteristics not only important during embryonic development but also in tumorigenesis. Our results suggest that by sequestering AP-2γ in the cytoplasm, Wwox suppresses its transactivating and, therefore, oncogenic activity.

The function of AP-2α and AP-2γ seems to be different in cancer. AP-2α was shown to inhibit cancer cell growth and activate p21WAF1/CIP1 expression in cell culture models (36). In addition, AP-2α interacts with p53 and augments p53-mediated transcriptional activation (37). On the other hand, AP-2γ stimulates proliferation and apoptosis in a transgenic mouse model, although no development of mammary carcinomas in the AP-2γ transgenic mice was observed. This indicates that AP-2γ may be a gene involved in tumor progression rather than tumor initiation (35). In addition, analysis of primary tumor samples revealed significant overexpression of AP-2γ in 80% of breast tumors (38), and high expression of AP-2γ was correlated with low survival rate (39). Recently, Guler et al. (27) have shown that the Wwox protein is lost or reduced in more than 60% of breast primary tumors. Therefore, it seems likely that aggressive progression of breast carcinoma needs an increase of expression of AP-2γ targets either by induction of AP-2γ levels or Wwox inactivation and, therefore, translocation of AP-2γ to the nucleus. It was also shown that ectopic expression of Wwox in breast cancer cells inhibits tumor growth both in vitro and in vivo (12). Because Wwox behaves as a tumor suppressor protein, it is therefore possible that interaction with AP-2γ suppresses proliferation and thus suppresses tumorigenicity in breast cancer. Additional studies will investigate these possibilities. In summary, this study describes a possible mechanism by which Wwox suppresses tumorigenicity through down-regulation of the AP-2γ transcription factor by protein–protein interaction.

REFERENCES


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