Metastasis-Associated Protein 1 Short Form Stimulates Wnt1 Pathway in Mammary Epithelial and Cancer Cells

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Abstract

Although Wnt1 downstream signaling components as well as cytoplasmic level of metastatic tumor antigen 1 short form (MTA1s) are elevated in human breast cancer, it remains unknown whether a regulatory cross-talk exists between these two pathways. Here, we provide evidence of a remarkable correlation between the levels of MTA1s and stimulation of the Wnt1 signaling components, leading to increased stabilization of β-catenin and stimulation of Wnt1 target genes in the murine mammary epithelial and human breast cancer cells. We found that MTA1s influences Wnt1 pathway through extracellular signal-regulated kinase (ERK) signaling as selective silencing of the endogenous MTA1s or ERK, or its target glycogen synthase kinase 3β resulted in a substantial decrease in β-catenin expression, leading to the inhibition of Wnt1 target genes. Furthermore, downregulation of β-catenin in cells with elevated MTA1s level was accompanied by a corresponding decrease in the expression of Wnt1 target genes, establishing a mechanistic role for the ERK/glycogen synthase kinase 3β/β-catenin pathway in the stimulation of the Wnt1 target genes by MTA1s in mammary epithelial cells. In addition, mammary glands from the virgin MTA1s transgenic mice mimicked the phenotypic changes found in the Wnt1 transgenic mice and exhibited an overall hyperactivation of the Wnt1 signaling pathway, leading to increased stabilization and nuclear accumulation of β-catenin. Mammary glands from the virgin MTA1s-TG mice revealed ductal hyperplasia and ductal carcinoma in situ, and low incidence of palpable tumors. These findings reveal a previously unrecognized role for MTA1s as an important modifier of the Wnt1 signaling in mammary epithelial and cancer cells.

Introduction

Members of the Wingless (Wnt) family of conserved secreted glycoproteins initiate intracellular signaling pathways that regulate critical cell processes, including cell polarity, normal development, and oncogenesis (1). For example, Wnt1 regulates cellular differentiation and proliferation of the mammary epithelium, and the Wnt/β-catenin pathway is aberrantly activated in human breast cancer (2), and its upregulation in the mammary gland has been shown to induce mammmary hyperplasia and adenocarcinoma (3). Activation of the canonical Wnt signaling pathway results in the stabilization of β-catenin, translocation of β-catenin to the nucleus, formation of a coactivator complex with the T-cell factor/lymphoid enhancer factor DNA-binding protein, and transcriptional stimulation of target genes with roles in proliferation (4). In the absence of Wnt binding to the Frizzled receptor, β-catenin forms a complex with APC and Axin, and is degraded in an ubiquitination-dependent manner (5). However, binding of Wnt to the Frizzled receptor initiates pathways that ultimately phosphorylate glycogen synthase kinase 3β (GSK-3β) and prevent it from phosphorylating β-catenin, leading to β-catenin stabilization. Investigations have shown that several signaling kinases, such as p90 RSK, a kinase downstream of extracellular signal-related kinase (ERK), and Akt phosphorylate GSK-3β and thus regulate its function in the Wnt signaling pathway (6). Wnt signaling can also be regulated by secreted factors such as Dickkopf-1 (DKK1) and disabled-2 (Dab2; ref. 7).

Components of the Wnt signaling pathway also play an essential role in determining cell polarity and morphogenesis in Caenorhabditis elegans. However, mutations of genes other than Wnt signaling components such as egl-27 and lin-40 (also known as egr-1) also adversely affect cell polarity and vulval fate specification and morphogenesis (8), raising the possibility of overlapping phenotypes of either egl-27 or

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Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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doi: 10.1158/0008-5472.CAN-10-0907

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lin-40 and the Wnt signaling pathway. Furthermore, the EGL-27 and LIN-40 proteins are homologues of members of the metastasis-associated protein 1 (MTA1, see below) family in mammals. However, it remains unknown if signaling components of the Wnt1 transforming gene product are influenced by the MTA1 family.

The MTA1 that was initially cloned from highly metastatic mammary adenocarcinomas (9) and its expression correlated with the aggressiveness of several human cancers was later identified to be a component of the vertebrate Mi-2/nucleosome remodeling and deacetylase complex (10). The nucleosome remodeling and deacetylase complex is an abundant deacetylase complex in mammalian cells and have been implicated in chromatin remodelling in normal as well as in cancerous cells (10). MTA proteins represent a family of genes, which is highly conserved through evolution, and involves MTA1, MTA1s, MTA1-I1, MTA2, and MTA3.

MTA1 acts a potent repressor of estrogen receptor and contributes to the invasiveness and anchorage-independent potential of breast cancer cells (11). Subsequently, a naturally occurring variant of MTA1 termed MTA1 short form (MTA1s) was identified, which predominantly localizes in the cytoplasm (12). In addition, MTA1s was found to form a complex with growth factor receptor binding protein 2 (Grb2)-SOS, activate the Ras pathway, and promote ERK stimulation in breast cancer cells (12). The fact that MTA1s (Grb2)-SOS, activate the Ras pathway, and promote ERK stimulation in breast cancer cells (12). The fact that MTA1s stimulates ERK and the ERK has also been shown to play a regulatory role in Wnt1 signaling (13), and cross-talk between ERK and Wnt1 pathways exists and prompted us to investigate the role of MTA1s in Wnt1 signaling in physiologically relevant model systems.

**Materials and Methods**

**Cell line authorization statement**

All the cell lines used in this study are from Dr. Rakesh Kumar’s laboratory and have been previously used in the peer-reviewed articles from the laboratory (11, 12).

**Antibodies and cell culture**

Antibodies against Wnt1 (sc-6280), GSK-3β (sc-9166), ERK (sc-154), β-catenin (sc-7963), cyclin-D2 (sc-181), and poly ADP ribose polymerase (PARP; sc-7150) were purchased from Santa Cruz Biotechnology. Phospho GSK-3β (9336s) and Phospho ERK (9106s) were purchased from Cell Signaling Technology. Paxillin (ms-404-P) and β-casein (MS-935-P) were purchased from NeoMarkers antibodies. Normal mouse IgG, rabbit IgG, and antibodies against actin (A-4700) and vinculin (V-9131) were purchased from Sigma.

**Production of stable cell lines expressing MTA1s**

HC11 cells stably expressing MTA1s were generated by transfecting pcDNA-T7-MTA1s with the use of the FuGENE 6 transfection reagent (Roche Applied Science). Stable clones were selected by using G418 (1 mg/mL) selection. Approximately 40 clones were isolated. Twenty clones were screened for selection of individual clones, and the rest were used as pooled clones. Expression of exogenous MTA1s was verified by immunoblotting with an anti-T7 monoclonal antibody.

**Reverse transcription-PCR analysis, Western blot analysis, TOP-flash assay, and reporter assays**

Reverse transcription-PCR (RT-PCR) analysis, Western blot analysis, TOP-flash, and reporter assays were described in detail in the Supplementary Information.

**Preparation of conditioned medium**

Conditioned medium was collected from cultures of HC11/pcDNA and HC11/MTA1s. Briefly, cells were cultured in 10% serum and then switched to serum-free medium; conditioned medium was harvested after 24 hours. The medium was centrifuged to remove debris and concentrated by using an Amicon Ultra-15 centrifugal filter. The protein concentration was measured, and equal amounts of protein was used for transfection, confocal, and differentiation assays.

**Generation of MTA1s transgenic mice and Southern blot analysis of genomic DNA**

A mouse mammary tumor virus (MMTV)-human MTA1s transgenic (MTA1s-TG) construct was created by subcloning T7-tagged MTA1s cDNA using HindIII-XbaI (blunted) sites into HindIII-EcoRI (blunted) of the MMTV-SV40-BssK vector. The MTA1s transgene was excised from plasmid DNA, and 6.2 linear fragment-containing promoter sequences, MTA1s-coding and 3′-untranslated regions, and SV40 polyadenylation signals were injected into the pronuclei of B6D2F1/J mouse embryos. Southern blot analysis of tail DNA digested with the EcoRI and XhoI restriction enzymes, which are the flanking sites of the 2-kb MTA1s cDNA, was used to identify founder animals. Several MMTV-MTA1s founder mice with the expected 2-kb MTA1s band were identified and kept as lines 3, 11, and 17. These results were confirmed by PCR with a forward primer unique to the T7-epitope-encoding region (5′-CAGCAAATGGGTCGGGATC) and a reverse primer corresponding to MTA1s cDNA (5′-GGACTTGAGCGACTCGGTTCGCTC); these primers only amplify T7-tagged MTA1s and do not recognize endogenous mouse MTA1s. As expected, these primers specifically amplified a 500-bp band in MTA1s-transgene-positive founder mice.

**Human tissue samples**

Sixty surgically resected human breast tumor samples were provided from the M.D. Anderson Cancer Center Breast Pathology Core by Dr. Aysegul A. Sahin per established core procedures and Institutional Review Board approval.

**Mammary gland whole mounts and histology, and bromodeoxyuridine labeling**

For whole-mount analysis, number 4 inguinal mammary glands were stained with carmine alum and bromodeoxyuridine
(BrdUrd) labeling as previously described (14). Detailed experimental procedure was described in the Supplementary Information.

**Statistical analysis and reproducibility**

Statistical analysis of the data was performed using the GraphPad Prism software (GraphPad Software, Inc.). The Spearman’s correlation coefficient test was used to analyze correlation between MTA1s and Wnt1 expression in human breast tumors. Statistical analysis of reporter assays and BrdUrd incorporation was performed using Student’s t test, and the results were presented as mean ± SEM. The presented phenotypic changes in MTA1s-TG mice were documented in MTA1s-TG founder mice in lines 3, 11, and 17.

**Results and Discussion**

**Identification of the Wnt1 pathway as a target of MTA1s**

We first sought to determine whether there was an association between the endogenous expression of MTA1s and the Wnt1 signaling regulator GSK-3β in breast cancer cell lines. We unexpectedly found that breast cancer cells with elevated expression of MTA1s such as SKBR3 and MDA-MB-435 had an increased expression of Wnt1, phosphorylated GSK-3β, and ERK (Supplementary Fig. S1A; Fig. 1A and B). In addition, we observed an increased ratio of cytoplasmic/nuclear β-catenin in cells with high levels of expression of MTA1s (Supplementary Fig. S1B). These observations are indicative of stimulation of the Wnt1 pathway in cells with increased expression of MTA1s. To evaluate the significance of
the noted positive correlation between the levels of MTA1s and Wnt signaling in more physiologic setting, we next searched whether a correlation exists between the status of MTA1s and Wnt1 in human breast tumors. Of the 60 human breast tumors analyzed, we observed an increased nuclear and cytoplasmic accumulation of both MTA1s and Wnt1 (Fig. 1C), and MTA1s and Wnt1 expression were positively correlated (Spearman correlation, \( P < 0.0001 \); Supplementary Fig. S2). These findings raised the possibility that MTA1s might be an upstream activator of the Wnt1 pathway in breast cancer cells.

To directly assess the role of MTA1s in the Wnt1 pathway in mammary gland epithelium, we next generated stable pooled clones of T7-MTA1s in the HC11 murine mammary epithelial cell line, which has been extensively used as a valid model to study the proliferative and differentiating effects of Wnt genes (15). Interestingly, when compared with HC11 cells transfected with pcDNA alone (HC11/pcDNA cells), HC11/MTA1s cells exhibited an increased level of Wnt1 but not Wnt2, Wnt4, and Wnt5a (Fig. 2A). In addition to this, we also found increased levels of \( \beta \)-catenin and decreased levels of phospho-\( \beta \)-catenin in HC11/MTA1s clones compared with HC11/pcDNA stable cells (Fig. 2A). To evaluate the contribution of the observed Wnt1 expression in the HC11/MTA1s cells upon the levels of Wnt target genes, we next performed the RT-PCR analysis for the Wnt1 target genes before and after Wnt1 knockdown by Wnt1-small interfering RNA (siRNA). Results indicated that indeed HC11/MTA1s cells had higher levels of expression of \( \beta \)-catenin as well as several Wnt1 target genes, including c-Myc, WISP-1, WISP-2, PITX2,
and Cyclin D2, and that Wnt1-knockdown in HC11/MTA1s cells was accompanied by a substantial reduction in the expression of Wnt target genes (Fig. 2B). We also noticed a significant increase in the expression of the Cyclin D2 protein by confocal microscopy in HC11/MTA1s cells (Fig. 2C). HC11/MTA1s cells also exhibited enhanced Top-TK luc activity (Fig. 2D). Subcellular fractionation also showed an increased cytoplasmic-to-nuclear ratio of β-catenin in HC11/MTA1s cells compared with HC11/pcDNA cells (Fig. 2E). To closely connect the noticed increased β-catenin to the Wnt1 targets, we show that siRNA-mediated downregulation of β-catenin was accompanied by a corresponding decrease in the expression of the Wnt1 target genes c-myc, Cyclin D2, and WISP-1, whereas there was no effect on Wnt1, which is upstream of β-catenin (Fig. 2F), showing a mechanistic role for β-catenin in supporting the expression of Wnt1 target genes in HC11/MTA1s cells.

This newly discovered connection between the MTA1s and Wnt1 pathway was not restricted to HC11/MTA1s cells, as MTA1s overexpression in ZR-75 human breast cancer cells also resulted in a significant increase in Top-TK luc activity (Fig. 3A), β-catenin protein (Supplementary Fig. S3A), and immunolocalization of Wnt1 and Cyclin D2 (Fig. 3B). In addition, we also observed an increased ratio of cytoplasmic/nuclear β-catenin in the nuclear fractions from ZR-75/MTA1s cells compared with ZR-75/pcDNA cells (Supplementary Fig. S3B).

To test the role of endogenous MTA1s in modifying the Wnt pathway, we selectively silenced endogenous MTA1s by using MTA1s-specific siRNA in MDA-MB-435. Silencing of MTA1s resulted in a substantial decrease in the levels of Wnt1, P-GSK-3β, β-catenin, and Cyclin D2 expression (Fig. 3C). Interestingly, β-catenin expression was found to be upregulated in MTA1s-siRNA–treated cells along with proteosomal inhibitor MG132 compared with MTA1s-siRNA alone–treated cells, further suggesting that MTA1s may play an important role in the stability of β-catenin (Fig. 3D). These observations showed that MTA1s silencing significantly affects the Wnt1 signaling pathway. Collectively, these results suggest a regulatory role of MTA1s in the Wnt1 pathway.

**MTA1s influences Wnt1 signaling through ERK in mammary epithelial cells**

GSK-3β is one of the key regulators of β-catenin stability and is important for β-catenin/T-cell factor–dependent gene transcription (16). Because GSK-3β functionality is governed, in part, by its phosphorylation by ERK (17), we next examined the phosphorylation status and stability of GSK-3β. We observed that overexpression of MTA1s in HC11 cells resulted in increased basal as well as serum-inducible phosphorylation of ERK and GSK-3β (Fig. 4A and B). Interestingly, we found that a small induction of P-ERK in 0% serum condition might be sufficient to bring about such a strong P-GSK-3β (Fig. 4A). To establish a mediatory role for ERK activation in the observed stimulation of Wnt1 target genes in HC11/MTA1s cells, we next inhibited ERK activity in these cells by treating with the pharmacologic ERK inhibitor U0126. We found that suppression of ERK activity was

![Figure 3](image-url). Status of Wnt1 pathway in ZR-75 cells expressing MTA1s and role of MTA1s in modifying Wnt1 pathway. A, ZR-75/pcDNA and ZR-75/MTA1s clones were transfected with either Top-flash or Fop-flash, and luciferase activity was measured after 24 h. B, confocal analysis of Wnt1 and Cyclin D2 expression in ZR-75/pcDNA and ZR-75/MTA1s clones. Scale bar, 10 μm. C, Western blot analysis of Wnt1, P-GSK3-β, GSK3-β, MTA1s, β-catenin, and Cyclin D2 expression in MDA-MB-435 cell line following transfection with MTA1s-specific or control siRNA for 48 h. D, Western blot analysis of β-catenin expression in MDA-MB-435 cell line following transfection with MTA1s siRNA for 48 h and treatment with or without proteosomal inhibitor MG132 (10 μmol/L) for 5 h.
accompanies a decrease in the levels of Phospho-GSK-3β, β-catenin, and the Wnt1 targets Cyclin D2, WISP-1, and WISP-2 and TOP-FLASH luciferase activity in HC11/MTA1s cells (Fig. 4C–E).

To examine the role of ERK pathway in the observed stimulation of Wnt1 signaling, we selectively silenced ERK by using ERK-specific siRNA in HC11/MTA1s cells. Silencing of ERK resulted in a substantial decrease in β-catenin expression (Fig. 4F) as well as TOP-FLASH luciferase activity (Fig. 4G). To our surprise, we also observed a decrease in β-catenin expression following selective silencing of GSK-3β by specific siRNA (Fig. 4F). These results suggest that endogenous levels of GSK-3β are needed for effective Wnt1 signaling in HC11/MTA1s cells. These findings suggested a mechanistic role for the ERK/GSK-3β/β-catenin pathway in the stimulation of expression of Wnt1 target genes by MTA1s in mammary epithelial cells.

MTA1s upregulation promotes Wnt1 secretion

In agreement with noted MTA1s overexpression–linked increased expression of Wnt1 target, we found an increased expression of Wnt1 protein in the conditioned media from cultures of HC11/MTA1s cells (MTA1s-CM) but extremely low expression of Wnt1 protein in conditioned media from
HC11/pcDNA cells (Con-CM; Fig. 5A). This secreted Wnt1 was bioactive because incubation of HC11 cells with MTA1s-CM or Con-CM activated Wnt1-dependent functional assays. In comparison with Con-CM–treated cells, cells treated with MTA1s-CM showed upregulation of Wnt1, phosphorylation of GSK-3β, stabilization of β-catenin and Cyclin D2 (Fig. 5B), and an increase in the promoter activity of the WISP-1 and Cyclin D2 genes (Fig. 5C). In brief, these findings confirmed that overexpression of MTA1s in mammary epithelial cells is accompanied by secretion of biologically active Wnt1. It remains to be investigated in the future whether Wnt1 is autoregulated. These results suggested that Wnt1 expression, secretion, and resulting signaling pathway is upregulated by MTA1s in mammary epithelial and cancer cells.

Activation of the Wnt1 pathway in the MTA1s-TG mice
To determine whether the changes in the levels of expression of Wnt1 and its target genes induced by MTA1s described above are relevant in a physiologically relevant whole-animal system, we generated transgenic mice with expression of MTA1s under the control of the MMTV promoter long-terminal repeat (MTA1s-TG mice; Fig. 6A). Founder mouse lines 3, 11, and 17 showing MTA1s transgene integration were identified by using PCR and confirmed by Southern blot analysis (Fig. 6B). Lines 3, 11, and 17 exhibited expression of three, eight, and two copies of the transgene, respectively, when compared with the intensity of the predetermined positive control (Fig. 6C). Confocal analysis of paraffin-embedded sections of mammary glands obtained from mice in line 17 with an anti-T7 antibody revealed expression of MTA1s primarily in the cytoplasm of epithelial cells. However, in some cases, we also detected epithelial cells with low levels of MTA1s expression in the nucleus (Fig. 6D).

To investigate the effect of MTA1s expression on the development of mammary glands, we examined whole-mount preparations from littermates with matching estrous cycles at different developmental stages. We found that mammary glands of virgin MTA1s-TG mice remarkably resembled extensive hyperbranching phenotype of virgin transgenic mice with elevated Wnt1 expression (3). MTA1s-TG mammary glands exhibited extensive hyperbranching and increased proliferation of ductal and alveolar epithelial cells (Fig. 6E–G). The mammary glands of MTA1s-TG animals also had extensive lateral branching, resembling that of mammary glands in early pregnancy (Fig. 6F, d–f), compared with the smooth surface of ducts from wild-type (WT) mice (Fig. 6E).

![Figure 5. MTA1s expression enhances the expression of biologically active Wnt1. A, Western blot analysis of Wnt1 expression in conditioned medium (CM) from HC11/pcDNA (Con-CM) and HC11/MTA1s cells (MTA1s-CM). B, confocal analysis of Wnt1 (green), P-GSK-3β (green), β-catenin (green), and cyclin D2 (red) expression in HC11/pcDNA clones following treatment with Con-CM or MTA1s-CM for 24 h. Scale bar, 10 μm. C, MTA1s-CM stimulates WISP-1 and Cyclin D2 promoter-luc activity in HC11/pcDNA cells. *, P < 0.05.](image-url)
Figure 6. MTA1s stimulate the Wnt1 pathway in MTA1s-TG mice. A, the MMTV-MTA1s transgene. B, Southern blot detection of the MTA1s transgene in the tail genomic DNA of transgenic (TG) and WT littermates of F1 from lines 3, 11, and 17. C, representative PCR analysis of genomic DNA from founder line mice. Lanes 10 and 1, respective copy number equivalents of control MTA1s cDNA plasmid. D, confocal analysis of T7-MTA1s (red) expression using anti-T7 antibodies in the mammary gland from 12-wk-old virgin MTA1s-TG and WT mice. Scale bar, 10 μm. Note that the transgene is mainly localized in the cytoplasm with few nuclear localization in luminal epithelial cells. E, Carmine Red–stained whole mounts of inguinal mammary glands obtained from WT mice and MTA1s-TG mice at 12 wk of age. The images in the middle and right panel are the images in the left panels at increased magnification. F, H&E-stained sections of mammary glands of 12-wk-old WT mice and MTA1s-TG mice. Dilated ducts (a and b) and lobuloalveolar structures (c) in the mammary glands of the WT mice. Dilated ducts (d and e) and lobuloalveolar structures (f) can be seen in the mammary glands of the MTA1s-TG mice. G, BrdUrd incorporation into the nuclei and its quantitation in mammary epithelial cells obtained from WT and virgin MTA1s-TG mice at 12 wk of age. We counted 5,000 cells per mouse and examined six mice per line. H and I, Western blot showing expression of β-casein, β-catenin, and Cyclin D2 in the mammary glands of 12-wk-old WT and virgin MTA1s-TG mice.
Figure 7. Development of mammary tumors in MTA1s-TG mice. A, example of ductal hyperplasia in mammary glands from virgin MTA1s-TG mice; bottom, higher magnification. B, example of ductal carcinoma in situ in mammary glands from virgin MTA1s-TG mice; bottom, higher magnification. C, example of focal hyperplastic nodules in mammary glands from virgin MTA1s-TG mice; bottom, higher magnification. D, MTA1s-TG mice founder line 3 (top) and 17 (bottom), with large tumor on the thoracic mammary gland. E, H&E staining showing mammary adenocarcinomas in MTA1s-TG (top, a–e) and immunohistochemical analysis of Wnt1 (f–j), β-catenin (k–o), and smooth muscle actin (p–t) in mammary tumors from MTA1s-TG mice. Inset, higher magnification.
and F, a–c). In addition, mammary glands of virgin MTA1s-TG mice contained many lobuloalveolar buds, which is normally associated with hormonal stimulation during pregnancy (Fig. 6F). We detected similar hyperbranching phenotypes in mice from lines 3 and 11. Using BrdUrd pulse labeling, we found that the proliferative index of the alveolar epithelium of 12-week-old virgin MTA1s-TG mice was markedly increased when compared with the WT mice (Fig. 6G).

As expected from the results described above, the noted precocious alveolar development in MTA1s-TG mice was accompanied by functional differentiation as evidenced by increased expression of the milk protein β-casein in the mammary glands of 12-week-old virgin mice (Fig. 6H). As with mammary glands obtained from age-matched WT mice, mammary glands obtained from virgin MTA1s-TG mice had increased expression of β-catenin, a functional indicator of precocious differentiation as well as a critical regulator of the Wnt1 target gene Cyclin D2 (Fig. 6I) in mammary glands from MTA1s-TG mice. These findings identified MTA1s as upstream activator of the Wnt1-β-catenin pathway.

**Upregulation of Wnt1 pathway in mammary tumors from transgenic mice**

To examine the effect of the MTA1s-Wnt1 pathway on tumor formation, we observed a cohort of female MTA1s-TG mice for the potential presence of tumors. Histologic examination of mammary glands from virgin MTA1s-TG mice revealed ductal hyperplasia (Fig. 7A) and ductal carcinoma in situ (Fig. 7B). Furthermore, whole-mount analysis of 26-month-old mice showed the presence of hyperplastic alveolar nodules (Fig. 7C). No such lesions were observed in >60 WT littermates. Palpable tumors developed in the mammary glands of 8.8% (7 of 80) of the MTA1s-TG mice between 15 and 18 months (Fig. 7D). By histopathologic analysis, the tumors in five of the seven mice were identified as infiltrating mammary adenocarcinomas with glandular, acinar, and cribriform patterns (Fig. 7E, a–e), all characteristic of Wnt1-induced mammary tumors (18). Mammary adenocarcinomas from MTA1s-TG (Fig. 7E, a–e) exhibited upregulation of both Wnt1 (Fig. 7E, f–j) and β-catenin expression (Fig. 7E, k–o), and retained myoepithelial differentiation characteristic of Wnt1-induced tumors, as evidenced by smooth muscle actin staining (Fig. 7E, p–t), and compared with age-matched WT mice mammary glands (data not shown).

In brief, for the first time, results presented here have identified MTA1s as novel upstream regulators of the Wnt1 pathway. We found that MTA1s upregulates ERK signaling, which could, in turn, lead to phosphorylation and inactivation of GSK3-β and stabilization of β-catenin. MTA1s has been previously reported to form a complex with Grb2-SOS and activate the Ras-ERK pathway in breast cancer cell lines (12). The existence of a cross-talk and positive feedback loop between ERK and Wnt pathways also strengthens the present findings that MTA1s may feed into Wnt1 signaling, at least in part (12), by inactivating GSK3-β due to ERK activation. In addition, because MTA1s also stimulates the expression and secretion of bioactive Wnt1 due to repression of Six3 corepressor (19), MTA1s contributes to the noted stimulation of the Wnt1 signaling by two mechanisms—one involving ERK-mediated inactivation of GSK-3β and another mechanism involving the upregulation of the Wnt1 itself. As components of the Wnt1 pathway are established contributors to mammary gland tumorigenesis, and considering the fact that MTA1s is upregulated in breast tumors, the newly established dysfunction of the MTA1s-Wnt1 pathway in mammary epithelium may contribute to the pathogenesis of breast tumors. Overall, these findings reveal a previously unrecognized role for MTA1s as an important modifier of the Wnt1 pathway and, consequently, a role in mammary gland development and tumorigenesis.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Acknowledgments**

We thank the following past employees and staff in the laboratory: C.J. Barnes for the confocal imaging, R.R. Singh for Western blotting, L. Clifton Barnes for the confocal imaging, A.J. Levine for generously providing WISP-1-luc reporter, and B.H. Nelson for the cyclooxygenase D2-luc reporter.

**Grant Support**

NIH grant CA 98823 (R. Kumar).

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Received 03/15/2010; revised 05/22/2010; accepted 06/11/2010; published online 08/15/2010.

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Cancer Res 2010;70:6598-6608.

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