Priority Report

Silencing of TMS1/ASC Promotes Resistance to Anoikis in Breast Epithelial Cells

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Abstract

Ductal carcinoma *in situ* (DCIS) is characterized by ductal epithelial cells that have filled the luminal space of the breast duct and survive despite loss of extracellular matrix contact. In normal epithelial cells, the loss of such contact triggers a form of apoptosis known as detachment-induced apoptosis or "anoikis." TMS1/ASC is a bipartite adaptor molecule that participates in inflammatory and apoptotic signaling pathways. Epigenetic silencing of *TMS1* has been observed in a significant proportion of human breast and other cancers, but the mechanism by which *TMS1* silencing contributes to carcinogenesis is unknown. Here, we examined the role of TMS1 in anoikis. We found that TMS1 expression is induced in response to loss of substratum interactions in breast epithelial cells. siRNA-mediated knockdown of TMS1 leads to anoikis resistance, due in part to the persistent activation of extracellular signal-regulated kinase and an impaired ability to up-regulate the BH3-only protein Bim. We further show that the detachment-induced cleavage of procaspase-8, a newly described mediator of cellular adhesion, is significantly inhibited in the absence of TMS1. These data show a novel upstream role for TMS1 in the promotion of anoikis, and suggest that silencing of *TMS1* may contribute to the pathogenesis of breast cancer by allowing epithelial cells to bypass cell death in the early stages of breast cancer development. This conclusion is supported by *in vivo* data showing that TMS1 is selectively down-regulated in the aberrant epithelial cells filling the lumen of the breast duct in a subset of primary DCIS lesions. [Cancer Res 2009;69(5): 1706–11]

Introduction

Epithelial cells require adhesion to the extracellular matrix (ECM) for survival. Adhesion maintains proper tissue architecture by dictating cellular properties such as shape, polarity, and proliferation. Molecularly, these properties are mediated by integrins, which bind components of the ECM and induce downstream signaling through cytosolic adaptor molecules. Detachment from the substratum and loss of integrin-mediated survival signals induce a form of programmed cell death termed "anoikis" (1). Anoikis is important for organogenesis and tissue homeostasis, and for ensuring that cells remain in their proper cellular context. One of the hallmarks of cancer is the acquired ability to invade basement membranes and neighboring tissues, which requires that cells survive in an anchorage-independent state and develop the means to resist anoikis. Thus, understanding how epithelial cells avoid anoikis is a critical task in understanding, preventing, and treating human cancer.

The molecular events that mediate anoikis are incompletely understood and can be cell type dependent. Both the intrinsic/mitochondrial apoptotic pathway, which is governed by the initiator caspase-9, and the extrinsic/death receptor–mediated pathway of apoptosis, which is governed by the initiator caspase-8, have been implicated. The involvement of the mitochondrial pathway is supported by studies showing that overexpression of the antiapoptotic molecules Bcl-2 or Bcl-x_L inhibit anoikis (2). In addition, the proapoptotic Bcl-2 family members, Bax, Bid, and Bim, translocate to the mitochondrion during anoikis, where they may function to promote cytochrome *c* release and cell death (3, 4).

There is also evidence supporting a role for caspase-8 in anoikis. Extracellular death ligands [e.g., Fas, TRAIL, tumor necrosis factor α (TNFα)], promote caspase-8 activation through a multimeric complex called the death inducing signaling complex (5). However, recent evidence indicates that anoikis is dependent on caspase-8, independent of death receptor ligation, although the mechanism for its activation remains unknown (2, 6). Upregulation of the caspase-8 inhibitor c-Flip has been observed in many transformed cell lines that are resistant to anoikis (6), and inhibition of c-Flip in these cells restores sensitivity to anoikis (6). Furthermore, several studies have proposed a nonapoptotic role for caspase-8 as a mediator of cellular adhesion and motility (7–10).

TMS1 (also known as ASC and PYCARD) is a bipartite signaling molecule that participates in apoptosis and inflammation. Although the precise function of TMS1 in apoptosis is unclear, overexpression or forced oligomerization of TMS1 in epithelial cells is sufficient to induce apoptosis via a mechanism that is dependent on caspase-8 (11–13). There is also evidence to suggest that TMS1-induced apoptosis is dependent on the intrinsic mitochondrial pathway in some cell types (11, 14, 15). Importantly, *TMS1* is subject to aberrant DNA methylation and epigenetic silencing in a number of different tumor types (16–20), suggesting that loss of TMS1 confers a survival advantage to tumor cells. However, the mechanism by which silencing of *TMS1* contributes to carcinogenesis remains unclear. Here, we examined the effect of TMS1 expression on anoikis in breast epithelial cells, and discovered a novel role for TMS1 in detachment-induced cell death and breast cancer progression.

Materials and Methods

Cell culture and anoikis assays. MCF10A cells were obtained from the Karmanos Cancer Institute and cultured as described (13). MCF10A/pBabe

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and MCF10A/Bcl-2 cells were a kind gift from Dr. Joan Brugge (Harvard Medical School, Boston, MA; ref. 21). For anoikis assays, cell culture dishes were coated twice with 20 mg/mL poly-HEMA (Sigma) in 95% ethanol and dried overnight. poly-HEMA–coated dishes were washed twice with $1\times$ PBS and seeded with 2.5×10^6 MCF10A cells per 10-cm dish in complete medium.

Immunohistochemistry. Archived specimens of paraffin-embedded normal breast tissue ($n = 4$) and infiltrating ductal carcinoma ($n = 20$) with associated ductal carcinoma *in situ* (DCIS) were obtained from the Avon/Grady Memorial Hospital Breast Tumor Bank. Sections were deparaffinized, subjected to antigen retrieval, and incubated with primary antibody. The TMS1 antibody was an affinity-purified rabbit polyclonal antibody (EU107) raised against a peptide encompassing amino acids 182 to 195 of human TMS1, and has been described (19). The E-cadherin

antibody is from Invitrogen (#15068). Immunocomplexes were detected by the avidin-biotin complex method, using diaminobenzidine as the chromogen (DAKO). Sections were counterstained with hematoxylin.

Immunoblotting. Protein lysates were harvested and analyzed as described (13). The antibodies used were as follows: anti-ASC/TMS1 (MBL or Protein Tech), β-tubulin (Sigma), Bim (Stressgen), caspase-8, Erk1/2, Phospho-Erk1/2 (pThr202/pTyr204), IκBα (Cell Signaling Technologies), glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Abcam), nuclear factor-κB (NF-κB) p65, Bcl2 (Santa Cruz), PARP (Alexis Biochemicals), and PARP p85 (Promega).

siRNA transfection. MCF10A cells (5.5×10^5) were seeded in 10-cm dishes and transfected the following day with 200 nmol/L of the indicated siRNA using Oligofectamine (Invitrogen). siRNA duplexes were from
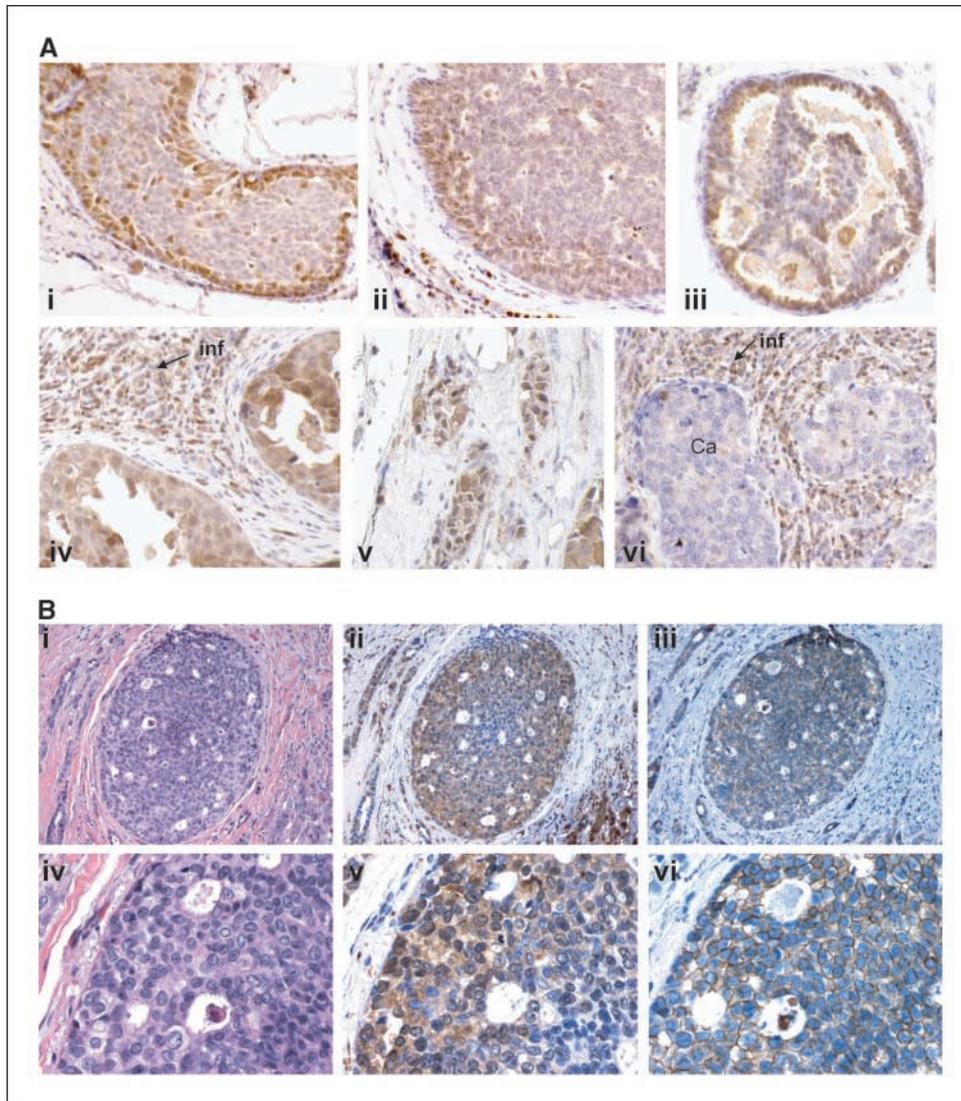

Figure 1. *A,* TMS1 expression in primary DCIS and invasive ductal carcinomas. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded breast tumor tissue samples. The TMS1 antibody was an affinity-purified rabbit polyclonal antibody (EU107) raised against a peptide corresponding to amino acids 182 to 195 of human TMS1, and was used at a dilution of 1:400 as described (18, 19). Immunocomplexes were detected by the avidin-biotin complex method, using diaminobenzidine as the chromogen. Slides were counterstained with hematoxylin (*blue*). *i* to *iii,* TMS1 expression in DCIS. Note the unusual staining pattern in DCIS lesions with retention of TMS1 expression in the cells closest to the myoepithelium and basement membrane and loss of expression in the more dysplastic cells filling the luminal space. This pattern was most often observed in solid-form DCIS (*i* and *ii*) and cribriform DCIS (*iii*) lesions. *iv* to *vi,* TMS1 expression in invasive ductal carcinomas. Whereas some infiltrating glands retain TMS1 expression (*iv* and *v*), 3 of 19 invasive carcinomas showed complete loss of TMS1 expression (*vi*). Note that even in ductal carcinoma cases that lose TMS1 expression (*vi*; *ca*), surrounding normal inflammatory cells (*iv* and *vi*; *inf*) retain TMS1 expression. *B,* relationship between TMS1 and E-cadherin expression in primary DCIS. Parallel sections were stained with H&E (*i*) or analyzed for the expression of TMS1 (*ii*) or E-cadherin (*iii*). The TMS1 antibody (EU107) was used at a dilution of 1:400, and the E-cadherin antibody (Invitrogen #15068) was used at a dilution of 1:50. Note the unusual TMS1 staining pattern in DCIS lesions with retention of TMS1 expression in the outermost cells and loss of expression in the more dysplastic cells filling the luminal space. The same cells that lose TMS1 expression retain E-cadherin expression. *iv* to *vi,* higher power (×600) magnifications of the same lesion shown in *i* to *iii* (×100). Shown is a representative DCIS lesion from a single individual. An independent example from a second individual is shown in Supplementary Fig. S1.
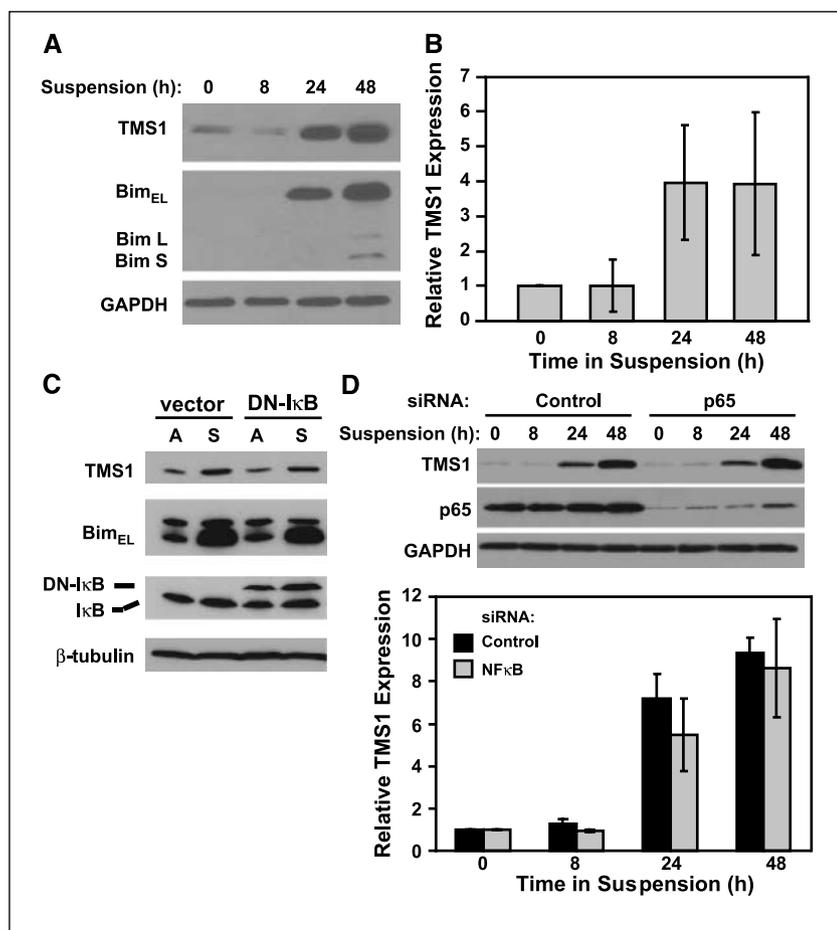
Figure 2. TMS1 protein and mRNA are induced during anoikis. *A* and *B,* expression of TMS1 after detachment. MCF10A cells were suspended in growth medium containing 0.5% methylcellulose and plated onto poly-HEMA coated plates, and harvested at the indicated time points. *A,* protein lysates were subjected to western blot analysis using antibodies against TMS1, Bim, and GAPDH. *B,* total cellular RNA was isolated and TMS1 mRNA expression levels were quantified by reverse transcription and real-time PCR analysis as previously described (13). Relative starting quantities were determined by comparison to MCF7 cDNA standard curve included in each run. Shown is the relative increase in TMS1 mRNA compared with time zero after normalization to an 18s rRNA internal control. *Columns,* mean of three independent experiments performed in triplicate; *bars,* SD. *C* and *D,* detachment-induced up-regulation of TMS1 is not dependent on NF-κB signaling. *C,* MCF10A cells were infected with 20 multiplicity of infection of a dominant-negative IκBα adenoviral construct the night before plating onto poly-HEMA plates. Cells were left attached (*A*) or plated on poly-HEMA–coated dishes (*S*) for 24 h. Protein lysates were harvested and subjected to western blot analysis with the indicated antibodies. *D, top panel,* MCF10A cells were transfected with either 200 nmol/L scrambled siRNA (*control*) or siRNA targeting the p65 subunit of NF-κB (*p65*). After 48 h, cells were suspended in growth medium containing 0.5% methylcellulose and plated onto poly-HEMA–coated 10-cm plates. Lysates were collected and analyzed by Western blot with antibodies to TMS1, the p65 subunit of NF-κB, and GAPDH. *D, bottom panel,* total cellular RNA was isolated and TMS1 mRNA expression levels were determined and analyzed by real-time PCR as described in *B*. Shown is the relative expression of TMS1 mRNA compared with time zero after normalization to an 18s rRNA internal control. *Columns,* mean of three independent experiments performed in triplicate; *bars,* SD.

Dharmacon and had the following sequences: TMS1 siRNA#1, 5′-CGAGGGUCACAAACGUUGAdTdT-3′ (sense); TMS1 siRNA#2, 5′-GCAA-GAUGCGGAAGCUCUUdTdT-3′; p65 siRNA, 5′-GCCCUAUCCCUUUACGU-CAdTdT-3′. siControl Non-Targeting siRNA#1 (Dharmacon) or siRNA against lamin a/c were used as controls.

Cell death ELISA. Apoptosis was quantified using the Cell Death Detection ELISA (Roche). Cells were washed in PBS and lysed in 500 μL incubation buffer at room temperature for 30 min. Clarified lysates (100 μL aliquots) containing 2.5×10^4 cell equivalents were used in the ELISA assay.

Results and Discussion

TMS1 is a proapoptotic protein that is subject to epigenetic silencing in a significant proportion of breast and other cancers (16–20). We have previously shown that in normal breast tissue, TMS1 is selectively expressed in the ductal and lobular epithelium, whereas its expression is absent from the underlying myoepithelial and stromal cell compartments (18). We extended these findings to examine the expression of TMS1 in DCIS lesions and invasive breast carcinomas. In DCIS lesions occurring adjacent to areas of invasive ductal carcinoma, we found that although the ductal epithelial cells in direct contact with the stroma retained TMS1 expression, a subset of the DCIS lesions examined showed reduced TMS1 expression in the majority of the epithelial cells that had filled the breast duct (Fig. 1*A, i–iii*). Furthermore, it seemed that the cells closest to the basement membrane retained TMS1 expression, whereas those in the center of the duct lacked TMS1 expression. This pattern was noted in both solid-form (Fig. 1*A, i* and *ii*) and

cribriform (Fig. 1*A, iii*) DCIS. This did not seem to be a reflection of a generalized loss of epithelial cell characteristics, as the central cells that selectively lose TMS1 expression retain E-cadherin expression (Supplementary Fig. S1; Fig. 1*B*). In addition, although the majority of invasive breast carcinomas examined showed varying degrees of TMS1 expression (Fig. 1*A, iv* and *v*), ~16% (3 of 19) of invasive ductal carcinomas had completely lost TMS1 expression (Fig. 1*A, vi*). Note that even in invasive breast carcinomas that lose TMS1 expression (Fig. 1*A, vi, ca*), infiltrating "normal" inflammatory cells retain TMS1 expression (Fig. 1*A, iv* and *vi, inf*). These data suggest that TMS1 expression is selectively lost in the aberrant epithelial cells filling the breast duct in some DCIS lesions and invasive breast carcinomas. Considering the limited data set, we cannot determine the relationship between TMS1 expression in DCIS and invasive carcinomas (i.e., whether loss of TMS1 in certain DCIS lesions ultimately gives rise to invasive ductal carcinomas lacking TMS1 expression). Nevertheless, the data are suggestive that loss of TMS1 accompanies the transition from DCIS to invasive carcinoma during the progression of breast cancer.

Considering the proapoptotic nature of TMS1 (13, 14) and the unusual expression pattern observed in DCIS lesions, we hypothesized that down-regulation of TMS1 might play a role in the resistance of breast epithelial cells to anoikis. To test this idea, we examined the effect of detachment on TMS1 expression in the nontransformed breast epithelial cell line MCF10A. Forced suspension of MCF10A cells led to a significant induction of

TMS1 expression (Fig. 2). This up-regulation occurred with similar kinetics to that of Bim, a proapoptotic BH3-only protein known to be up-regulated during anoikis (Fig. 2). Previous work from our laboratory has shown that TMS1 is up-regulated in breast epithelial cells in response to TNFα stimulation in an NF-κB and c-Jun-NH$_2$-kinase (JNK)-dependent manner (13). In contrast, neither expression of a dominant-negative IκB, siRNA-mediated knock-down of NF-κB, nor chemical inhibition of JNK had any effect on suspension-induced up-regulation of TMS1 (data not shown; Fig. 2C and D), indicating that up-regulation of TMS1 occurs independently of these pathways.

We next determined the effect of TMS1 silencing on the apoptotic response to detachment in MCF10A cells. Cells transfected with control siRNA or siRNA directed against TMS1 were seeded onto poly-HEMA–coated tissue culture plates and analyzed over 48 hours. The forced suspension of control MCF10A cells induced a time-dependent cleavage of procaspase-8 and the downstream caspase target PARP (Fig. 3A), indicating that the cells are undergoing apoptosis. Strikingly, knockdown of TMS1 led to a significant delay in the apoptotic events associated with anoikis. Neither cleavage of procaspase-8 nor PARP cleavage were observed in TMS1 knockdown cells until 48 hours postdetach-

ment (Fig. 3A). Consistent with the observed delay in PARP cleavage, knockdown of TMS1 in MCF10A cells conferred a ∼2-fold protection from cell death after 24 hours in suspension (Fig. 3B). This degree of protection is similar to previous reports describing the effect of Bim knockdown on detachment-induced apoptosis in breast epithelial cells (3). In this regard, we also examined the effect of TMS1 on the regulation of Bim. Induction of Bim was evident by 8 hours postdetachment in control cells (Fig. 3A). Strikingly, both the magnitude and timing of Bim protein accumulation were significantly inhibited in TMS1 knockdown cells (Fig. 3A). The effect of TMS1 knockdown was specific, as a similar delay in apoptotic events (caspase-8 cleavage, PARP cleavage) and inhibition of Bim up-regulation were observed when MCF10A cells were transfected with a second independent siRNA targeting TMS1 (Supplementary Fig. S2) but not when transfected with siRNAs targeting lamin a/c or caspase-1 (data not shown). Taken together, these data illustrate that caspase-8 cleavage, PARP cleavage, and Bim up-regulation are severely inhibited in cells lacking TMS1 expression, and that loss of TMS1 confers resistance to anoikis.

Bim is regulated by both transcriptional and posttranslational mechanisms. In the latter case, integrin-mediated survival signaling through the mitogen-activated protein kinase (MAPK) pathway leads to ERK-mediated phosphorylation of serine 69 on Bim$_{EL}$, the largest isoform of Bim (22). This phosphorylation stimulates Bim$_{EL}$ ubiquitylation and subsequent degradation by the proteasome (22). The cessation of ERK signaling after detachment from the ECM induces both an increase in Bim transcription (3), and the dephosphorylation of Bim$_{EL}$, allowing for stabilization and accumulation of Bim protein (22). To examine in more detail the effect of TMS1 down-regulation on detachment-induced up-regulation of Bim, we performed extensive time course experiments. Suspension of MCF10A cells caused a time-dependent increase in TMS1 protein expression, which preceded that of Bim$_{EL}$ (Fig. 4A). Again, knockdown of TMS1 severely inhibited the induction of Bim$_{EL}$ expression (Fig. 4A). The effect of TMS1 loss on Bim expression occurred primarily at the level of protein accumulation, as there was little effect of TMS1 knockdown on the detachment-induced up-regulation of Bim mRNA (Fig. 4A). We further examined the effect of TMS1 knockdown on ERK activation. Interestingly, even under attached, unstimulated conditions, there were increased levels of phospho-ERK in cells lacking TMS1 relative to controls (e.g., 48-hour monolayer cultures; time, 0 hour; Fig. 4B). Detachment and a shift to fresh serum-containing medium led to a stimulation of ERK phosphorylation that was both greater in magnitude and persisted longer in TMS1 knockdown cells compared with control cells (Fig. 4B). These data indicate that there is a pool of ERK that is constitutively active in the absence of TMS1 and that the delay in detachment-induced Bim up-regulation occurring in the absence of TMS1 may be due to misregulation of ERK signaling. Taken together, these data suggest that TMS1 acts upstream of Bim and that the resistance to anoikis observed in TMS1 knockdown cells may be, in part, due to the inhibition of Bim induction.

To determine whether the inhibition of detachment-induced Bim up-regulation in TMS1 knockdown cells is due directly to TMS1 loss or rather is a consequence of the observed delay in anoikis, we compared the effects of TMS1 knockdown on the induction of anoikis in the presence of the pan-caspase inhibitor z-VAD-FMK. As shown above, knockdown of TMS1 inhibited both the up-regulation of Bim$_{EL}$ protein and apoptosis after 24 hours in
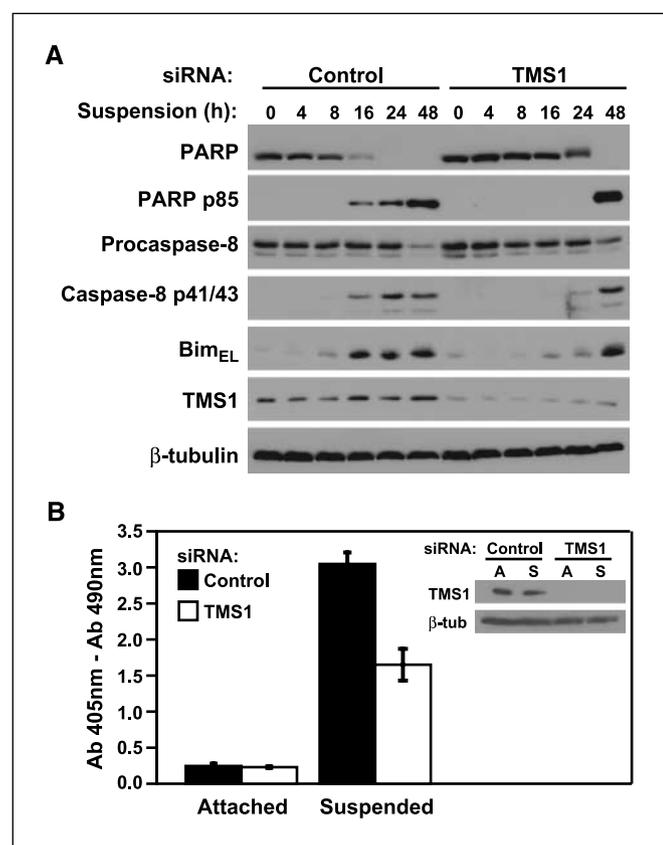

Figure 3. Knockdown of TMS1 expression inhibits anoikis. *A,* MCF10A cells were transfected with 200 nmol/L of scrambled siRNA (*control*) or siRNA#1 targeting TMS1 (*TMS1*). After 36 h, cells were plated on poly-HEMA–coated dishes, and protein was isolated at timed intervals and subjected to western analysis with the indicated antibodies. *B,* MCF10A cells were transfected as in *A.* After 36 h, cells were plated on poly-HEMA–coated dishes. The following day, the cells were harvested and analyzed for apoptosis using the DNA fragmentation cell death detection ELISA. A portion of the cell sample was retained, harvested for protein, and subjected to western blot analysis for TMS1 and β-tubulin (*inset*).
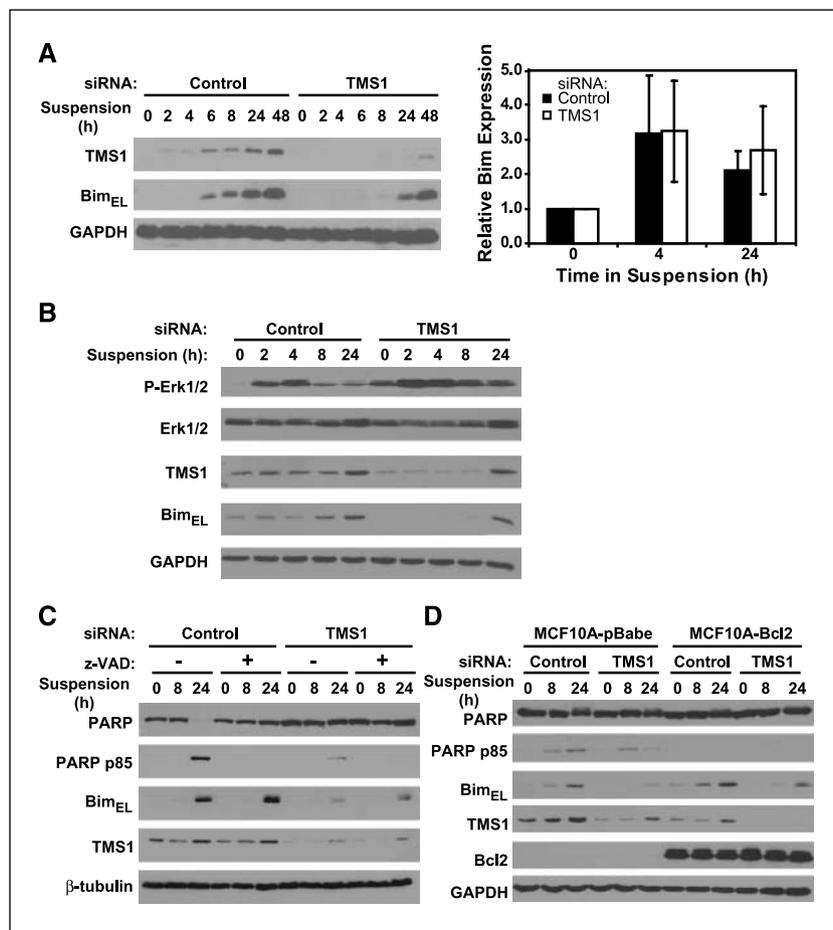
Figure 4. Impact of TMS1 knockdown on ERK signaling and Bim up-regulation during anoikis. *A,* detachment-induced up-regulation of Bim requires TMS1. MCF10A cells were transfected with 50 nmol/L of scrambled siRNA (*control*) or siRNA#1 targeting TMS1 (*TMS1*). *Left,* after 48 h, cells were suspended in growth medium containing 0.5% methylcellulose and plated onto poly-HEMA–coated plates. *Left,* lysates were collected at the indicated time points and subjected to Western blot analysis using the indicated antibodies. *Right,* Bim mRNA expression levels were quantified by real-time PCR analysis. Relative starting quantities were determined by comparison to MCF7 cDNA standard curve included in each run. Shown is the increase in Bim mRNA relative to time zero after normalization to an 18s rRNA control. *Columns,* mean of three independent experiments assayed in triplicate; *bars,* SD. *B,* TMS1 loss promotes persistent ERK activation. MCF10A cells were transfected with 50 nmol/L scrambled siRNA or siRNA#1 targeting TMS1. After 48 h, cells were suspended in growth medium containing 0.5% methylcellulose and plated onto poly-HEMA–coated plates. Lysates were collected at the indicated time points and subjected to western blot analysis using the indicated antibodies. *C* and *D,* the requirement for TMS1 in detachment-induced Bim accumulation is apoptosis-independent. *C,* MCF10A cells were transfected with 200 nmol/L of scrambled siRNA or TMS1#1 siRNA. After 48 h, the cells were plated onto poly-HEMA coated plates in the absence or presence of 250 nmol/L z-VAD-FMK. Protein was harvested at the indicated time points and analyzed by western blot analysis with the indicated antibodies. *D,* MCF10A-pBABE and MCF10A-Bcl-2 cells were transfected with 200 nmol/L scrambled or TMS1#1 siRNA. After 48 h, cells were suspended in growth medium containing 0.5% methylcellulose and plated onto poly-HEMA–coated plates. Lysates were collected at the indicated time points and subjected to Western blot analysis with the indicated antibodies.

suspension (Fig. 4*C*). Treatment with z-VAD-FMK had no effect on detachment-induced increase in Bim_{EL} or TMS1 expression in control cells, nor did it affect the delayed kinetics of Bim accumulation in TMS1 knockdown cells (Fig. 4*C*). It did, however, prevent detachment-induced apoptosis, as indicated by the reduced cleavage of PARP (Fig. 4*C*). We also examined the effect of detachment on TMS1 in MCF10A cells constitutively over-expressing Bcl2 (21). As expected, overexpression of Bcl2 blocked detachment-induced apoptosis (as indicated by the reduction in PARP cleavage) but again had no effect on detachment induced up-regulation of TMS1 or Bim, nor on the delayed up-regulation of Bim and inhibition of PARP cleavage afforded by TMS1 knockdown (Fig. 4*D*). Taken together, these data indicate that the detachment-induced increase in TMS1 protein expression is caspase-independent and not a downstream consequence of anoikis, as has been observed for Bim_{EL} induction (22). These data further indicate that the effect of TMS1 loss on detachment-induced up-regulation of

Bim_{EL} protein expression is direct, and not due to an overall inhibition of anoikis.

In summary, we find that TMS1 plays an important role in breast epithelial cell anoikis. We provide evidence that loss of TMS1 expression promotes resistance to anoikis, which may be mediated at least in part by the inhibition of detachment-induced up-regulation of Bim_{EL}. Previous work has shown that anoikis in breast epithelial cells is dependent on Bim, in that factors that inhibit Bim up-regulation, such as siRNA against Bim itself, or constitutive activation of the MAPK/ERK pathway, also prevent detachment-induced cell death (3, 22). Our data indicate that apoptosis induced by detachment of MCF10A cells is similarly dependent on the induction of TMS1, and furthermore, that TMS1 is necessary for detachment-induced accumulation of Bim_{EL}. TMS1 seems to play a role in the suppression of ERK signaling, as its down-regulation leads to ERK activation. These data suggest that TMS1 acts upstream of Bim, and may act as a link between loss of integrin

signaling and the onset of anoikis, such that in the absence of TMS1, survival signaling through the MAPK/ERK pathway persists even in the absence of matrix attachments.

In previous work, we and others have shown that TMS1 overexpression or forced oligomerization induces apoptosis, and that TMS1-induced apoptosis is dependent on caspase-8 (11–13). TMS1 induces the cleavage of procaspase-8 independent of death receptor–ligand interactions, and is necessary for TNFα−induced activation of caspase-8 and apoptosis in some cell types (13).[5] Others have shown that TMS1 directly interacts with caspase-8 in cotransfection experiments (12). Anoikis may be another context in which TMS1 regulates caspase-8 activity. First described by Helfer et al. (7), several recent studies have shown a novel, nonapoptotic role for caspase-8 in cell adhesion and migration (8–10). Collectively, these studies have shown that caspase-8 is required for adhesion to the ECM and src-dependent activation of the MAPK/ERK pathway. This scaffolding function is independent of caspase-8 catalytic activity, and seems to be mediated by an adhesion-stimulated recruitment of procaspase-8 and src to a complex at the lamella and src-mediated phosphorylation of procaspase-8 (8–10, 23). Our data suggest that TMS1 is necessary for the efficient cleavage of procapase-8 at the onset of anoikis, which would both limit the pool of procaspase-8 available for adhesion and promote downstream apoptotic signaling.

In conclusion, we report a novel role for TMS1 in anoikis. TMS1 is up-regulated after detachment of breast epithelial cells, and

siRNA-mediated knockdown of TMS1 causes these cells to be resistant to anoikis. This resistance correlates with a delay in Bim protein up-regulation and procaspase-8 cleavage, providing two mechanisms by which TMS1 may contribute to anoikis. Significantly, our results demonstrating the deleterious effects of TMS1 loss on sensitivity to anoikis are supported by *in vivo* data from DCIS lesions, where we see that aberrant epithelial cells persisting in the lumen of the breast duct in DCIS lesions no longer express TMS1. Together, our data illustrate that the epigenetic silencing of TMS1 observed in breast cancer and other tumor types might contribute to the progression of carcinomas by allowing epithelial cells to bypass anoikis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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[5] M.J. Parsons and P.M. Vertino, unpublished observations.

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