Epithelial-to-Mesenchymal Transition Induced by TGF-β1 Is Mediated by Blimp-1–Dependent Repression of BMP-5

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Abstract

Induction of epithelial-to-mesenchymal transition (EMT) by TGF-β1 requires Ras signaling. We recently identified the transcriptional repressor Blimp-1 (PRDM1) as a downstream effector of the NF-κB, RelB/Bcl-2/Ras-driven pathway that promotes breast cancer cell migration. As the RelB/Blimp-1 pathway similarly required Ras signaling activation, we tested whether Blimp-1 plays a role in TGF-β1–mediated EMT. Here, TGF-β1 treatment of untransformed NMuMG mammary epithelial and MDA-MB-231 breast cancer cells was shown to induce Blimp-1 expression, which promoted an EMT signature and cell migration. TGFβ1 and BLIMP1 RNA levels were correlated in patient breast tumors. BLIMP1 gene transcription was activated by TGF-β1 via a c-Raf (RAF1) to AP-1 pathway. Blimp-1 induced expression of the EMT master regulator Snail (SNAI1) via repressing BMP-5, which inhibited Snail expression upon TGF-β1 treatment. Interestingly, a similar cascade was observed during postnatal mouse mammary gland development. RelB expression was detected early in pregnancy followed progressively by Blimp-1 and then Snail; whereas, BMP-5 levels were high in nulliparous and regressing glands. Finally, lower BMP5 RNA levels were detected in patient breast tumors versus normal tissues, and correlated with cancer recurrence. Thus, the Ras effector Blimp-1 plays an essential role in TGF-β1–induced EMT via repression of BMP-5 in breast cancer.

Cancer Res; 72(23); 1–11. ©2012 AACR.

Introduction

Aberrant constitutive expression of NF-κB subunits, reported in more than 90% of breast cancers (1, 2) and multiple other malignancies, plays a pivotal role in tumorigenesis (3, 4). Notably, the RelB NF-κB subunit is more highly expressed in more aggressive estrogen receptor (ER)α-negative breast tumors versus ERα-positive ones, and promotes their invasive phenotype (5). Similarly, nuclear RelB expression in prostate cancer correlates directly with Gleason score (6) and plays a radioprotective role in aggressive prostate cancer cells (7). In breast cancer cells, RelB enhances migration and invasion via induction of the BCL2 gene (5). The resulting Bcl-2 protein recruits Ras to the mitochondria, where it is activated (8).

The zinc-finger B-lymphocyte–induced maturation protein (Blimp-1) was identified as a pivotal downstream mediator of the migratory phenotype regulated by RelB and Ras (8). Blimp-1, which was not previously known to be expressed in breast cancer, reduced ERTα expression by directly repressing ERTα (ESR1) gene transcription (8). Consistently, higher BLIMP1 RNA expression was detected in ERTα negative breast tumors. Blimp-1 was originally identified as a silencer of IFNB gene transcription (9), and subsequently as a master regulator essential for the differentiation of B and T lymphocytes (10, 11). Blimp-1 also plays crucial roles in early embryonic development, including the specification and migration of primordial germ cells (12, 13). Consistently, we have shown that Blimp-1 promotes breast cancer cell migration (8).

TGF-β, which suppresses growth of normal cells, functions as a tumor promoter in the presence of active Ras signaling. TGF-β1 induces epithelial-to-mesenchymal transition (EMT) and promotes the invasive phenotype of breast cancer cells that are signaling via a Ras to Erk cascade (14). TGF-β1 treatment of the ERTα-negative untransformed NMuMG mouse mammary epithelial line (15, 16) or of MDA-MB-231 human breast cancer cells promotes EMT as judged by enhanced cell migration and wound closure in vitro (17), and metastasis to bone (18) and lung (19) in mouse models. As Blimp-1 is induced downstream of Ras signaling, we hypothesized a role for this master regulator in TGF-β1–mediated EMT. Here, we elucidate a new EMT pathway mediated by Blimp-1 that leads to Snail induction via the repression of bone morphogenetic protein 5 (BMP-5) expression, which has been previously implicated in

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certain features of TGF-β–induced EMT in renal epithelial cells (20). Consistently, lower BMP5 RNA levels are detected in breast tumors versus normal tissues, and correlate with disease recurrence in patients, suggesting the clinical relevance of this pathway.

Materials and Methods

Cell culture and treatment conditions
ERα-negative MDA-MB-231, NMuMG and Hs578T and ERα-positive MCF-7 and ZR-75 cells were purchased from the American Type Culture Collection (ATCC), grown in medium recommended by ATCC and vials frozen immediately. Fresh vials were used approximately every 6 weeks. Cells were confirmed to be free of mycoplasma contamination using PCR (VenorGeM Mycoplasma Detection Kit, Sigma). The identity of the MDA-MB-231 line was authenticated using short tandem repeat analysis (Genetica DNA Laboratories), which has shown 100% identity with the MDA-MB-231 cell line of ATCC. Hs578T pRELβ-shRNA (RelB shRNA) or pRELβ-sense (Control) cell lines were established as described (5) and grown in the presence of 1 μg/mL puromycin (Sigma). NMuMG and MDA-MB-231 cells were treated with 2 and 5 ng/mL TGF-β1, respectively, or with 100 ng/mL BMP-5 (R&D Systems). For both reagents, the equivalent volume of vehicle, 4 mmol/L HCl containing 1 mg/mL BSA in PBS, was used as control. Plasmids, transfection analysis and wound-healing assays are described in the Supplementary Materials.

RNA and immunoblot analyses
RNA isolation, RT-PCR, and real-time quantitative Q-PCR analyses were conducted as detailed in Supplementary Data. Whole-cell extracts (WCE) and nuclear extracts (NE) were prepared and subjected to immunoblotting using the antibodies described in the Supplementary Materials. Protein extracts were prepared from the 4th mammary gland of FVB/N mice following removal of the lymph node as previously described (21), see Supplementary Materials.

ChIP assay
The chromatin immunoprecipitation (ChIP) assay for AP-1 binding was conducted using Hs578T cells as previously described (8), see Supplementary Materials. The Blimp-1 ChIP assay for the BMP5 promoter was conducted using a pcDNA4/Blimp1-V5-tag expression vector and the EZ-ChIP Kit (Millipore Corporation; Supplementary Materials).

Gene expression and statistical analysis
Individual cancer datasets were downloaded from Oncomine (Compendia Bioscience), as previously described (5); see Supplementary Data. The prognostic value of BMP5 in recurrence of breast cancer was assessed with Kaplan–Meier Plotter (www.kmplot.com), which used gene expression microarray data and survival information of 2,324 patients with breast cancer downloaded from GEO (Affymetrix HGU133A and HGU133+2). To compare the different conditions used in wound-healing assays, the Student t-test was conducted and a P value less than 0.05 was considered statistically significant.

Results

Blimp-1 is required for TGF-β1–induced EMT
Incubation of MDA-MB-231 or NMuMG cells with TGF-β1 resulted in robust increases in BLIMP1 RNA and Blimp-1 protein levels (Fig. 1A). Using 2 different Blimp-1 siRNAs to knockdown Blimp-1 levels in MDA-MB-231 cells, the normally observed induction of fibronectin caused by TGF-β1 treatment was attenuated (17) and the decrease in basal E-cadherin levels was prevented (Fig. 1B). MDA-MB-231 cells consist of 2 subpopulations with different levels of E-cadherin, 1 negative and 1 positive (22); the mesenchymal marker N-cadherin is not detectable (23). No significant increase in the high basal levels of vimentin was noted. TGF-β1 treatment of NMuMG cells has been shown to lead to EMT, as judged by the upregulation of mesenchymal markers fibronectin, N-cadherin, and vimentin (15). Knockdown of Blimp-1 overrode the characteristic upregulation of all 3 mesenchymal makers by TGF-β1 (Fig. 1C). Notably, ectopic expression of Blimp-1 largely rescued the effects of Blimp-1 knockdown in these cells, confirming the specificity of the Blimp-1 siRNA (Fig. 1D). These results were recapitulated in another ERα-negative breast cancer line Hs578T using both knockdown and dominant negative strategies (Supplementary Fig. S1).

Another measure of EMT is increased cell migration (16). TGF-β1 treatment led to more rapid wound closure by both MDA-MB-231 and NMuMG cells. Blimp-1 knockdown reduced the basal level of wound closure and prevented its acceleration by TGF-β1 in MDA-MB-231 (Fig. 1E and F and Supplementary Fig. S2) and NMuMG cells (Fig. 1G). Furthermore, ectopic Blimp-1 expression in NMuMG cells partially rescued the decreased migration seen upon Blimp-1 knockdown (Fig. 1G). Consistently, the acquisition of a fibroblastoid phenotype by NMuMG cells upon TGF-β1 treatment was prevented by knockdown of Blimp-1 (Fig. 1H). Thus, TGF-β1 treatment induces Blimp-1 expression, which is essential for the acquisition of a mesenchymal phenotype.

Increased TGF-β1 production has been observed in breast carcinomas compared with normal tissues (24), and associated with disease progression in human breast cancer (25). Using the bc-GenExMiner database of 2,920 patients with breast cancer, a significant correlation was observed between TGFB1 and BLIMP1 RNA levels (r = 0.35, P < 0.0001; Fig. 1I), which was also observed when one stratified by ERα status (Supplementary Fig. S3), thus extending the clinical relevance of these findings to human disease.

Blimp-1 expression in breast cancer cells is mediated via a c-Raf to AP-1 pathway
To address the mechanism of Blimp-1 activation, we focused on Ras signaling, as it is implicated downstream of RelB signaling (8) and also required for the transforming activity of TGF-β1 (14, 26). As c-Raf is an effector of Ras-mediated migration (27), its involvement in Blimp-1 induction was tested. Expression of a constitutively active form of c-Raf (Raf CA) into ERα-positive ZR-75 and MCF-7 breast cancer cells, which contain low levels of Blimp-1, led to increased Blimp-1 levels (Fig. 2A). Conversely, a dominant negative variant of c-Raf (Raf DN) prevented Blimp-1 induction by ectopic
expression of RelB and its binding partner p52 (Fig. 2A). Consistent with a c-Raf to Erk pathway downstream of RelB and Bcl-2, we confirmed the ability of RelB and Bcl-2 to activate Erk1/2 (Supplementary Fig. S4A and S4B), and showed that their effects on Blimp-1 induction could be abrogated with the MEK inhibitor PD98059 (Supplementary Fig. S4C). Thus, c-Raf and Erk1/2 are important downstream effectors of the Ras pathway leading to induction of Blimp-1 expression in breast cancer cells.

Dimeric AP-1 factors are important mediators of transformation by the Ras/c-Raf/Erk1/2 pathway and have been implicated in TGF-β1–mediated transformation (28). AP-1 elements have been implicated in the transcriptional control of the BLIMP1 gene although different subunits were identified, suggesting cell-type specificity (29). Following ectopic expression of RelB/p52 or Bcl-2 in MCF-7 cells, activation of c-Jun, c-Fos, Fra-1, and Fra-2 AP-1 subunits was observed, as judged by an increase in their levels and/or relative phosphorylation status (Fig. 2B).

**Figure 1.** Blimp-1 is induced by TGF-β1 and required for TGF-β1–induced EMT. A, MDA-MB-231 and NMuMG cells were treated with TGF-β1. RNAs were subjected to RT-PCR analysis, and NEs or WCEs to immunoblotting. B, MDA-MB-231 cells were transiently transfected with 2 siRNAs specific for Blimp-1 or with Control (siCtrl) siRNA. After 24 hours, cells were incubated with TGF-β1 or vehicle (--) and WCEs analyzed. FN, fibronectin. C, NMuMG cells were transfected with siCtrl or siBlimp1-1 and processed as in B. D, NMuMG were transfected with Blimp-1 expressing vector or empty vector (EV). After 24 hours, cells were transfected with siRNAs and processed as in C. E and F, MDA-MB-231 cells were transfected with siCtrl or siBlimp1-1 and subjected to a wound-healing assay with TGF-β1 or vehicle. E, cultures were photographed at ×10 magnification right after the scratch and 20 hours later. Bar, 200 μm. F, the percentage of wound closure (mean ± SD) from 3 independent experiments is shown. *, P = 1.3 × 10⁻⁶; ††, P = 2.3 × 10⁻³; ‡‡, P = 1.5 × 10⁻⁴. G, NMuMG cells were transfected as in D and subjected to a wound-healing assay with TGF-β1 or vehicle. The percentage of wound closure (mean ± SD) from 3 independent experiments is shown. *, P < 0.05; ††, P < 0.005. H, NMuMG cells were transfected with siControl or siBlimp1-1. After 24 hours, cells were incubated with TGF-β1 and photographed at ×20 magnification. Bar, 100 μm. I, the Pearson’s pairwise correlation plot depicts the expression of TGFβ1 and BLIMP1 from datasets (bc-GenExMiner Database), showing a positive correlation in human breast tumors.
Consistently, Blimp-1 expression was induced. Conversely, knockdown of RelB in Hs578T cells led to decreased levels of these same AP-1 subunits as well as of endogenous Blimp-1 expression (Fig. 2C). Cotransfection of vectors expressing c-Jun with c-Fos, Fra-1, or Fra-2 induced BLIMP1 promoter activity (Supplementary Fig. S4D). Expression of these AP-1 subunits increased endogenous levels of Blimp-1 protein (Fig. 2D). Complexes of c-Jun/Fra-2 resulted in the largest induction in increased endogenous levels of Blimp-1 protein (Fig. 2D). Knockdown of c-Jun/c-Fos or c-Jun/Fra-1 also led to decreased levels (reduction of C24 70% with this combination of siRNAs; shown). ChIP assays found endogenous c-Jun, c-Fos, Fra-1, and Fra-2 protein bound to the AP-1 site in the BLIMP1 promoter in Hs578T cells (Fig. 3B), suggesting that they all contribute to induction of BLIMP1 transcription. Thus, activation of AP-1 signaling potently induces Blimp-1 expression in breast cancer cells.

**TGF-β1 induces Blimp-1 via a c-Raf to AP-1 pathway**

We next addressed whether increased AP-1 activity by TGF-β1 was responsible for Blimp-1 induction. TGF-β1 treatment increased nuclear levels of c-Jun and Fra-2 in both NMuMG and MDA-MB-231 cells (Fig. 3C). In NMuMG cells, c-Fos and Fra-1 were downregulated, whereas they were modestly increased in MDA-MB-231 cells. The role of c-Jun/Fra-2 activation in TGF-β1-mediated induction of Blimp-1 was tested. Knockdown of both c-Jun and Fra-2 greatly reduced the induction of Blimp-1 in MDA-MB-231 cells treated by TGF-β1 (Fig. 3D), which is consistent with the partial rescue of E-cadherin expression and the decrease in fibronectin induction seen upon TGF-β1 treatment (Fig. 3E). The remaining Blimp-1 expression may be because of the increase in c-Fos and Fra-1 seen in these cells upon TGF-β1 treatment (Fig. 3C). Finally, c-Raf knockdown reduced basal Blimp-1 expression and virtually completely prevented its induction by TGF-β1, which led to the rescue of E-cadherin expression and the attenuation of fibronectin induction by TGF-β1 (Fig. 3F), confirming the importance of the Ras/c-Raf signaling axis. Together, we conclude that TGF-β1 activates AP-1, thereby inducing Blimp-1 expression, which promotes EMT.

**Blimp-1 is required for the induction of Snail by TGF-β1**

Blimp-1 was shown to promote EMT through the direct repression of ERF-positive breast cancer cells (8). However, as NMuMG and MDA-MB-231 cells are ERα negative and its reexpression was not observed upon Blimp-1 knockdown (data not shown), we sought to identify the TGF-β1–induced EMT mediator downstream of Blimp-1. Snail and Slug are 2 key transcription factors that regulate the TGF-β-induced EMT molecular program during tumor progression (30). Blimp-1 knockdown greatly reduced the induction of Snail/SNAIL RNA and Snail protein resulting from TGF-β1 treatment (Fig. 4A), whereas the induction of Slug was unaffected (data not shown). Moreover, ectopic Blimp-1 expression was sufficient to increase levels of Snail in NMuMG and multiple breast cancer lines (Fig. 4B). Thus, Blimp-1 promotes the induction of Snail expression and its knockdown appears to profoundly decrease Snail protein levels following treatment with TGF-β1.

**Blimp-1 repression of the BMP5 gene relieves inhibition of Snail expression by BMP-5 and promotes EMT by TGF-β1**

We next sought to elucidate the mechanism whereby Blimp-1, which is a zinc finger protein only known to repress transcription, induces Snail expression. As Blimp-1 did not appear to affect the canonical TGF-β1 pathway mediated by Smad signaling (see Discussion), we hypothesized that Blimp-1
reduced the expression of an inhibitor of Snail expression. Microarray analysis was conducted to identify genes controlled by Blimp-1 in the MDA-MB-231 line. Cells were reverse transfected with the 2 Blimp-1 siRNAs used above for 48 hours. The quality of RNA from 2 independent experiments was confirmed using an Agilent RNA 6000 Pico kit and effective Blimp-1 knockdown using Western blot analysis (data not shown). The RNA was analyzed using Affymetrix 1.0 ST arrays, representing 28,869 human genes. Sixty-eight genes were significantly upregulated with Blimp-1 siRNAs, which represent potential direct Blimp-1 targets. BMP5 was found upregulated 2.75-fold ($P = 5 \times 10^{-4}$) with the 2 Blimp-1 siRNAs compared with Control siRNA, which was confirmed by RT-PCR (Fig. 4C). Interestingly, both TGF-β1 and BMP-5 belong to the TGF-β superfamily and their signaling pathways display complex and frequently antagonistic crosstalk during organogenesis and tissue homeostasis (31). To confirm the effects of Blimp-1 on BMP5 expression, Blimp-1 was ectopically expressed in NMuMG cells and led to a profound repression of BMP5 RNA (Fig. 4C). Importantly, TGF-β1 treatment resulted in almost complete loss of BMP5 expression; whereas, BMP5 levels were substantially maintained upon knockdown of Blimp-1 in NMuMG (Fig. 4D) and MDA-MB-231 cells (Fig. 4D and data not shown). Using Transfac analysis, 4 putative Blimp-1 binding sites were identified in the BMP5 promoter (Fig. 4E). As there are currently no commercially available Blimp-1 antibodies for ChIP analysis, V5-tagged Blimp-1 protein was ectopically expressed in MDA-MB-231 cells. Blimp-1 binding to the region-containing sites 1 and 2 was shown using 2 different V5 antibodies (Fig. 4E). Together these findings identify BMP5 as a direct target of Blimp-1 repression.

To assess the role of BMP-5 in Snail induction by TGF-β1, NMuMG and MDA-MB-231 cells were treated with TGF-β1 or BMP-5 alone, or the 2 in combination. Notably, addition of recombinant BMP-5 significantly attenuated Snail induction by TGF-β1 and reduced the induction of fibronectin in both lines (Fig. 5A). Moreover, the loss of E-cadherin expression on the surface of NMuMG cells in response to TGF-β1 was largely overridden by BMP-5 (Fig. 5B) and the fibroblastoid phenotype was also substantially reversed (Fig. 5C). Finally, the acceleration of wound closure seen with TGF-β1 treatment was abrogated with addition of BMP-5 (Figs. 5D and E). Together,
the data indicate that repression of BMP-5 levels by TGF-β1 contributes to the induction of EMT.

The RelB/Blimp-1/Snail pathway is active during mammary gland development

Previously, we detected RelB expression in the developing mouse mammary gland and found this subunit involved in ductal branching during pregnancy (21). As pathways used in malignant cells are often derived from normal development, we examined the expression of RelB and Blimp-1 in the mouse mammary gland. For each time point during pregnancy, nuclear and WCEs were prepared from 3 individual female mice. As seen previously, expression of RelB was detected early during pregnancy and increased between days 10.5 and 18.5 (Fig. 6A). During lactation and regression, RelB levels continued to decline. Blimp-1 expression followed RelB, with amounts increasing during late pregnancy and peaking during lactation (Fig. 6A), as judged by quantification of the 3 samples per time point (Fig. 6B). In comparison, Snail expression was quite low during pregnancy and increased following Blimp-1 induction. Expression of Blimp-1 and Snail dropped precipitously during regression (Fig. 6). Thus, the relative patterns of Blimp-1 and Snail were consistent with signaling downstream of RelB. Notably, we could detect the precursor and mature forms of BMP-5 in nulliparous mice and during regression. Mature BMP-5 expression decreased progressively during pregnancy until it disappeared at the latest time point of pregnancy (P18.5) and during lactation, which is consistent with our findings identifying BMP-5 as a target of Blimp-1 repression and involved in Snail repression,
suggesting a similar cascade occurs during adult mouse mammary gland development.

Breast cancer and poor prognosis are associated with low BMP5 expression

To assess the role of BMP-5 in breast cancer, we used the Oncomine database to evaluate their levels of BMP5 expression. The TCGA_Breast study was selected initially, as this dataset includes a substantial number of normal breast tissues (n = 25), as well as a large number of invasive breast carcinoma samples (n = 346; Fig. 7A). BMP5 RNA was significantly lower in overall invasive tumors versus normal breast tissues (see legend for individual P values). Data from other studies that include fewer normal tissue samples showed similar results (Supplementary Fig. S5A and S5B). Overall, when all the studies containing informative BMP5 expression levels were combined, BMP5 was identified as one of the most downregulated genes in breast cancer compared with normal tissue (Fig. 7B). Furthermore, numerous BMPs were found to be aberrantly downregulated in breast cancer, whereas TGFBI was upregulated when compared with normal tissues (Supplemental Fig. S5C).

Discussion

These studies elucidate a new pathway of transformation by TGF-β1 via induction of Snail. Blimp-1 was induced by TGF-β1 via a c-Raf/AP-1 pathway, and directly inhibited the BMP5 gene (see scheme in Fig. 7E). The resulting decrease in BMP-5 levels led to a derepression of Snail expression. Thus Blimp-1 and BMP-5 are crucial intermediates between the Ras/c-Raf/AP-1 signaling axes and the induction of...
Snail and EMT by TGF-β1 in breast epithelial and cancer cells.

The transcriptional master regulator Snail is induced in virtually all EMT processes and notably by TGF-β1 in a broad spectrum of cell types (30). Snail expression has been correlated with mesenchymal phenotype and metastasis of breast cancer (32). Abnormal production of TGF-β1 in breast cancer is associated with disease progression (25). Interrogation of microarray datasets has shown a significant correlation between TGBF1 and BLIMP1 RNA levels in 2,920 patients with breast cancer, extending our findings to human disease. The induction of Snail by Blimp-1 is mediated via release of repression by BMP-5, and consistently Blimp-1 is shown here to directly bind to and repress the BMP5 gene. To our knowledge, this is the first study showing the direct binding of a transcription factor to the mammalian BMP5 promoter leading to its transcriptional regulation. Previous studies of the BMP5 promoter identified putative regulatory elements (i.e., GATA-1, engrailed and long-range cis regulatory elements), but binding studies or more fine-tuned mapping was not conducted (33, 34). In untransformed renal epithelial cells, BMP-5 expression was decreased by TGF-β1 and its addition attenuated TGF-β-induced EMT, as judged by levels of smooth muscle cell actin and ZO-1 (20); although the target(s) of BMP-5 was not elucidated in these cells, TGF-β1 and BMP signaling pathways display complex (positive and negative) cross-talk during organogenesis and homeostasis of many tissues (31). In mammary cells, BMP-5 and TGF-β1 had antagonistic activities on Snail expression. Blimp-1 tilted the balance toward TGF-β1-induced EMT by repressing BMP-5 expression, which led to the derepression of Snail.

Bmp5 was identified as the gene mutated in the short-ear mouse model, which displays various skeletal defects (35). Interestingly, these mice also have a 2-fold increase in skin tumor susceptibility (36). Decreased BMP-5 expression was observed in a number of primary tumors or cancer cell lines. In pancreatic cancers, which are typified by active Ras signaling, low expression of BMP5 RNA was detected in 16 tumor cell lines compared with 4 normal samples (37). BMP-5 expression was also significantly lower in adenocarcinomas and tumor cell lines compared with normal adrenal glands (38), and in malignant schwannoma versus benign lesions (39). We observed that BMP5 RNA is one of the most underexpressed transcripts in breast tumors compared with normal tissue, and its decreased expression correlated with disease recurrence in patients with breast cancer. Reduced BMP5 levels also correlated with more invasive tumors in these patients. Previously, Blimp-1 was found more highly expressed in ERα-negative versus ERα-positive breast tumors (3). Consistently, the prognostic value of BMP-5 was restricted to ERα-negative breast tumors, validating the biologic relevance of this inhibitory pathway. Interestingly, the Blimp-1/BMP-5/Snail axis appeared active in vivo during adult mammary gland development. In contrast to BMP-5, Blimp-1 and Snail were not expressed in nulliparous or regressing mammary glands, and only detected during pregnancy and lactation. This suggests that after pregnancy, Blimp-1 expression might need to be shut down in mammary tissue to reactivate BMP-5, decreasing Snail levels and allowing the gland to go back to a resting stage.

AP-1 complexes drive the oncogenic capacity of TGF-β at the transcriptional level (40). Here, AP-1 dimers of c-Jun and Fra-2 were identified as critical downstream targets required for Blimp-1 induction in response to TGF-β1. Transcriptional activation of other TGF-β1-induced genes involved in tumor progression, such as Collagen, MMP2, Laminin α3a, and the autoinduction of TGF-β1 itself is similarly mediated by the direct promoter binding of AP-1 complexes (40). Interestingly, overexpression of c-Jun in MCF-7 cells strongly promoted their migratory and invasive properties and allowed for tumor formation independent of estrogen (41). Fra-1 and Fra-2 were implicated in the malignant behavior of breast cancer cells and the progression of mammary carcinoma (8, 42).

This study identifies Blimp-1 as a critical node in the cooperation between the Ras pathway and TGF-β1 signaling in favor of Snail expression and induction of EMT. Blimp-1 did not appear to affect the canonical TGF-β1 pathway.
mediated by Smad signaling as its knockdown did not affect either levels of Smad2/3 and Smad4 in the nucleus, or RNA levels of Smad6 and Smad7 (data not shown). The antagonistic effects of BMP-5 on TGF-β1–induced EMT provide a rationale for the prognostic value of BMP-5 observed in patients with invasive breast cancer. Interestingly, addition of BMP-5 did not recapitulate all the EMT marker modulations seen with Blimp-1 knockdown upon TGF-β1, suggesting that Blimp-1 regulates genes other than BMP5 and SNAIL to promote certain features of TGF-β1–induced EMT in ERα negative cells. Studies are in progress to identify these additional target genes and pathways.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: M. Romagnoli, D.C. Seldin, G.E. Sonenshein Development of methodology: M. Romagnoli, Z. Yu Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Romagnoli, K. Belgaise, Z. Yu, X. Wang, E. Landesman-Bollag, D.C. Seldin, D. Chalbos, S. Barrille–Nion, M.L. Seldin
Grant Support
These studies were supported by NIH grants P01 ES11624 (D.C. Selden and G. E. Sonenshein) and R01 CA129129 (G.E. Sonenshein), and by the DOD postdoctoral fellowship award W81XWH-10-1-0003 (M. Romagnoli).

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Received June 7, 2012; revised September 7, 2012; accepted September 25, 2012; published OnlineFirst October 10, 2012.

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Acknowledgments
The authors thank Pat Hogan for assistance with mammary gland preparations and Drs. Nura Sánchez-Morgan and Albert Tai for help with the immunofluorescence microscopy.

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Cancer Res  Published OnlineFirst October 10, 2012.

Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-12-2270

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2012/10/10/0008-5472.CAN-12-2270.DC1

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