Psoriasin Interacts with Jab1 and Influences Breast Cancer Progression

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ABSTRACT

Psoriasin (S100A7) is expressed at low levels in normal breast epithelial cells but is highly expressed in preinvasive ductal carcinoma in situ. Persistent psoriasin expression occurs in some invasive carcinomas and is associated with poor prognostic factors. Whereas there is evidence that secreted psoriasin can act as a chemotactic factor for CD-4-positive lymphocytes in psoriatic skin lesions, an intracellular biological function is unknown. We have found that psoriasin physically interacts with Jab1 (c-jun activation-domain binding protein 1) in the yeast two-hybrid assay and confirmed this by coimmunoprecipitation assay in breast cancer cells. Psoriasin-transfected breast cancer cells showed increased nuclear Jab1 and demonstrated several features consistent with an alteration in Jab1 activity including an increase in activator protein-1 (AP-1) activity, increased expression of AP-1 and HIF-1-dependent genes, and reduced expression of the cell-cycle inhibitor p27Kip1. Psoriasin overexpression was also associated with alteration of cellular functions that are associated with increased malignancy, including increased growth, decreased adhesion, and increased invasiveness in vitro, as well as increased tumorigenicity in vivo in nude mice. We conclude that intracellular psoriasin influences breast cancer progression and that this may occur through stimulation of Jab1 activity.

INTRODUCTION

We have identified psoriasin (S100A7) previously as a differentially expressed gene between DCIS and invasive carcinoma. The expression of psoriasin is low in normal breast and benign pathologies (1), but psoriasin is among the most highly expressed genes in high grade DCIS (2, 3). Whereas expression is often reduced in invasive carcinoma, persistent high expression is associated with markers of poor prognosis (4). This profile of gene expression raises the possibility that psoriasin may be functionally involved in invasion and early tumor progression (5). Psoriasin is a small calcium-binding protein belonging to the S100 gene family (6, 7), among which several other members have been associated with breast tumor progression (8, 9). Most interest has been focused on S100A4 (10), which was also initially identified as a differentially expressed gene between non-metastatic and metastatic rodent mammary tumor cell lines (11). In later studies by several groups, S100A4 has been shown to directly influence the invasive and metastatic phenotype in breast cancer cell lines (12–14) and tumors (15), and expression is also associated with poor prognostic factors and patient survival in human breast tumors (16).

Psoriasin was originally described as highly expressed within psoriatic skin lesions (17) and found to be a secreted protein (18), but has since been observed to be present in the cytoplasm and nucleus of both abnormally differentiated keratinocytes (19) and breast carcinoma cells (2, 4). Whereas there is evidence that secreted psoriasin can act as a chemotactic factor for CD-4-positive lymphocytes in psoriatic skin lesions (18), a function for intracellular psoriasin also appears likely but has yet to be established.

We sought to identify proteins that might interact with psoriasin in breast epithelia by using the yeast two-hybrid system (20). Jab1 (21) was found to specifically interact with psoriasin in the yeast system, and this interaction was confirmed by biochemical assay in breast cancer cells. Jab1 is a component of a multimeric protein complex (22, 23), the CSN/COP9 signalosome, which is involved in signal transduction and protein degradation via the Ub-26S proteasome (24, 25). The effect of overexpression of psoriasin on Jab1 distribution and function in a breast cancer cell line was studied. Psoriasin overexpression resulted in redistribution of Jab1 to the nucleus and multiple functional changes that can be attributed to activation of Jab1, as well as enhanced tumorigenesis and metastasis in an in vivo assay. These data support our hypothesis that psoriasin enhances early tumor progression and the process of invasion in breast cancer cells in part by interacting with Jab1 and positively enhancing its activity.

MATERIALS AND METHODS

Yeast Two-Hybrid System. For yeast two-hybrid studies, the coding region of human psoriasis protein was fused in-frame with the GAL4 DNA-binding domain of the pGBT9 vector (Clontech). The resulting bait plasmid (pGBT9-psor) was used to screen a normal human mammary epithelial cDNA library (Clontech) by the yeast two-hybrid method as we have described previously (26). Clones were isolated that could grow on Trp" Leu" His medium, did not autoactivate the β-galactosidase reporter gene, and demonstrated specificity for their interaction with psoriasin. This was done by testing the interaction of psoriasin with specific “prey” constructs not identified in the screen. Jab1 was analyzed in a similar fashion. The NH2-terminal “bait” psoriasin plasmid used to define the region of psoriasin involved in Jab1 binding encoded amino acids 1–52 (pGBT9-N-term-psor), and the COOH-terminal bait psoriasin plasmid encoded amino acids 43–101 (pGBT9-C-term-psor).

Cell Culture, Transfections, and Antibodies. The human breast carcinoma cell lines MDA-MB-231 and MDA-MB-468 were cultured in DMEM supplemented with 10% FBS under standard conditions (4). The former cell line is negative for psoriasin, whereas the latter expresses psoriasin mRNA and protein (confirmed by RT-PCR and Western blot; data not shown). The full psoriasin protein coding sequence was cloned into pcDNA3.1 (Invitrogen) and transfected into MDA-MB-231 cells using Superfect (Qiagen) followed by G418 selection. Resistant colonies were isolated and expanded. Psoriasin protein expression was determined by Western blot using a rabbit antisera against the COOH terminus of psoriasin. The specificity of the antibody was established by comparison with a similar antibody generated previously against the same epitope (4), and by immunohistochemistry and Western blot, using transfected breast cancer cell lines and tumors as described previously (4). Three MDA-MB-231 clones were found to express psoriasin.
(designated as clone 231-LP1) exhibiting low psoriasin expression, and clones 231-HP1 and 231-HP2 both exhibiting similar high levels of psoriasin expression. Wild-type MDA-MB-231 and clone 231-neo (generated by transfection with the empty vector) do not express psoriasin. Jab1 and p27kip1 antibodies were obtained from Santa Cruz Biotechnology, Inc. Hypoxic stimulation of cells was performed in a Forma Scientific Model 1025 Anaerobic System containing an atmosphere of 0.7% O2, 5% CO2, and 5% H2 at 37°C for 24 h as we have described previously (27).

**Immunoprecipitation and Western Blot.** Human breast cancer cell lines expressing psoriasin (231-HP2 and MDA-MB-468) were lysed on ice in 25 mM HEPES (pH 7.7), 0.4 mM NaCl, 1.5 mM MgCl2, 2 mM EDTA, 1% Triton X-100, 0.5 mM DTT, and protease inhibitor mixture (Roche). Complexes were immunoprecipitated by Jab1 antibody/protein G-Sepharose (Pierce) at 4°C for 2 h. Binding and washes were performed in the same buffer, except the NaCl concentration was diluted 4-fold (28). Coimmunoprecipitated psoriasin protein was detected by immunoblotting using the psoriasin-specific antibody. Total protein lysates were extracted from the cell-line pellets in SDS-Isolation Buffer (50 mM Tris (pH 6.8), 20 mM EDTA, 5% SDS, 5 mM β-glycerophosphate, and a mixture of protease inhibitors (Roche)). Protein concentrations were determined using the Micro-BCA protein assay kit (Pierce). Protein lysates were run on a mixture of protease inhibitors (Roche). Protein lysates were run on a 16.5% SDS-PAGE mini gel using Tricine SDS-PAGE to separate the proteins, and then transferred to 0.2 μm nitrocellulose (Bio-Rad). After blocking in 10% skimmed milk powder in Tris-buffered saline-0.05% Tween, blots were incubated with primary antibodies (~15 μg/ml in Tris-buffered saline-0.05% Tween) followed by incubation with appropriate secondary antibodies and visualization by incubation with Supersignal (Pierce) as per the manufacturer’s instructions and exposure on X-ray films.

**Reporter Gene Assay and Transcription of AP-1-dependent Genes.** MDA-MB-231 parental cells and clones stably transfected with psoriasin were transfected with an AP-1-driven luciferase reporter gene (Stratagene) and a β-galactosidase expression vector, in triplicate experiments using Effectene (Qiagen). Luciferase was measured in cell lysates (Promega) 18 h after transfection and standardized to β-galactosidase activity (Promega). Total RNA from the MDA-MB-231 clones was isolated using TRIZol (Sigma) and reverse transcribed in duplicate from triplicate samples as described (4). Specific primers for VEGF, MMP13, and GAPDH were used for PCR as follows: VEGF-UPPER (sense) CGC AGA GT AAA TTA TGC TCC and VEGF-LOWER (antisense) AAG AAA AAT AAA ATG GCG AAT CC; MMP13-UPPER (sense) ATG CGG GGT TCC TGA T and MMP13-LOWER (antisense) CGC AGC AAG AAA ACG ACA; and GAPDH-UPPER (sense) ACC CAC TCC ACC TTG G and GAPDH-LOWER (antisense) CTC TTG TGC TCT TGC TTG TGT. Reactions were stopped during the log-linear stage of PCR amplification and samples electrophoresed through an agarose gel that was poststained with ethidium bromide for band visualization. Image capture and analysis was done using an LCD camera and MCID software (Imaging Research, St. Catharine’s, Ontario, Canada).

**Immunohistochemistry.** Cultured cells were grown on microscope slides for 24 h, and then fixed and processed as described previously (4). Immunohistochemical staining for psoriasin was performed essentially as described previously, using an automated tissue immunostainer (Ventana Medical Systems, Phoenix, AZ), and 3,3’-diaminobenzidine immunohistochemistry kit and bulk reagents supplied by manufacturer. Briefly, the staining protocol was set to “Extended Cell Conditioning” procedure, followed by 12 h incubation with primary antibody (concentration 1:3000) and 32-min incubation with secondary antibody. Positive staining was assessed by light microscopy.

**Adhesion, Growth, and Invasion Assays.** MDA-MB-231 clones were trypsinized from flasks that were 60–70% confluent. Cells (10,000) were plated in triplicate on three different days in 96-well plates having fibronectin, collagen I, or uncoated plastic surfaces (Becton Dickinson). After 1 h at 37°C, nonadherent cells were gently washed away with PBS. Adherent cells were stained with crystal violet, and their relative abundance determined by spectrophotometric absorbance. For growth assay, 1000 cells/well were plated in plastic 96-well plates in triplicate on three different days and allowed to grow for 18, 24, 48, and 72 h. Cells were stained with crystal violet and their relative abundance determined by spectrophotometric absorbance. Invasion assays were performed in triplicate on a Matrigel-coated modified Boyden-invasion chamber (24-well plate inserts with 8-μm pores; Becton Dickinson). FBS DMEM (10%) was used as a chemoattractant in the lower chamber. Cells (350,000) were added to the upper chamber, and allowed 12 h to degrade the Matrigel and invade through the porous membrane. Cells that invaded and were adhering to the bottom of the membrane were stained with crystal violet. Invaded cells were visualized by light microscopy and enumerated by counting the number of cells per high power field in five random fields. In Vivo Mouse Studies. Breast cancer cells (four experimental groups comprising MDA-MB-231 parental cells, 231-neo control, 231-LP1, and 231-HP1) were grown in culture and then suspended in 0.2 ml of PBS at a concentration of 5 × 10⁶ cells before injection into mammary fat pads of female nude mice according to a protocol approved by the University of Manitoba Animal Care Committee. Each experimental group included 5 animals, and two injections were sited bilaterally in each animal to achieve a total of 10 possible tumor sites per group. Tumor diameters were measured by calipers at weekly intervals, and the tumor volume was calculated from the formula: volume = 4/3 × (0.5 × smaller diameter)² × 0.5 × larger diameter). The experiment was continued for up to 8 weeks at which time all of the animals were euthanized, and all of the injection sites, tumors, and multiple organ tissues (abdominal lymph nodes, lungs, liver, and spleen) were examined grossly for the presence of tumor. Representative tissue blocks from all of the primary injection sites and all of the organ sites suspicious for metastatic tumor were subsequently processed by 10% formalin fixation, paraffin embedding, and preparation of H&E-stained sections for light microscopic examination.

**RESULTS**

**Identification and Confirmation of Psoriasin Interacting Proteins.** We used full-length psoriasin fused to the GAL4 DNA-binding domain as bait in a yeast two-hybrid assay (20) and screened 1.74 × 10⁶ clones from a normal human breast cDNA library. Among 4 true positive clones (26), 1 (Fig. 1a) contained almost the full protein sequence (amino acids 42–335) for Jab1. As shown in Fig. 1a, controls including unrelated bait (Rad18) and prey (Mad2) constructs, and empty bait and prey vectors did not show any activation of reporter genes. We noted that a Jab1-binding motif common to several Jab1 interacting proteins described recently is also contained within psoriasin (Fig. 1b), so we tested whether this region was necessary for the psoriasin-Jab1 interaction. As shown (Fig. 1a), only the COOH-terminal portion of psoriasin that contains this motif interacted with Jab1. To additionally confirm the psoriasin-Jab1 interaction in breast cancer cells, psoriasin was stably transfected into MDA-MB-231 cells, and coimmunoprecipitation experiments performed using Jab1 and psoriasin antibodies. Psoriasin-Jab1 protein complexes were detected in both psoriasin-transfected MDA-MB-231 cells (231-HP2) and the breast cell line MDA-MB-468 (which exhibits endogenous psoriasin expression) when Jab1 antibody was used for immunoprecipitation (Fig. 1c). However no psoriasin-containing complex was detected in control lanes in the absence of Jab1 antibody or protein G beads. Psoriasin-specific antibody immunoprecipitated psoriasin from cell lysates but was unable to coimmunoprecipitate Jab1 (data not shown), presumably because of the proximity and partial overlap of the epitope recognized by the antibody (amino acids 88–101 of psoriasin) and the proposed Jab1-binding domain (amino acids 57–89 of psoriasin). **Psoriasin and Jab1 Cellular Localization.** We localized psoriasin and Jab1 in the MDA-MB-231 clones by immunohistochemistry. Jab1, like psoriasin, has been found previously to be both nuclear and cytoplasmic in cell types other than breast. In MDA-MB-231 cells and all 4 of the transfected cell lines (231-neo, 231-LP1, 231-HP1, and 231-HP2) Jab1 is expressed at comparable levels in the cytoplasmic compartment (Fig. 2, right panel). However, in all three of the psoriasin-expressing clones, 231-LP1, 231-HP1, and 231-HP2 (Fig. 2, left panels), there is a relative increase in Jab1 within the nucleus. However, the total amount of Jab1 protein as detected by Western blot is similar in all of the cell clones and does not change in the presence of psoriasin (Fig. 3a). Psoriasin can also be detected by immunoprecipitation of medium conditioned by 231-HP2 and MDA-MB-468.
cells (data not shown), suggesting that psoriasin is also secreted by breast cancer cells in culture.

Psoriasin Overexpression Influences Several Jab1-related Functions. Jab1 influences a number of cellular proteins. Among these, Jab1 effects the level of the negative cell cycle-regulating protein p27Kip1 by promoting the export of p27Kip1 from the nucleus to the cytoplasm and the subsequent degradation by the Ub-28S proteasome (29). Therefore, we first examined p27Kip1 expression in our MDA-MB-231 clones and found that psoriasin-overexpressing clones showed a consistent reduction in levels of p27Kip1 relative to wild-type and control cells (Fig. 3a).

To determine whether psoriasin influences other Jab1 functions in breast cancer cells we examined AP-1-dependent transcription in the MDA-MB-231 clones using an AP-1-driven luciferase reporter (Fig. 3b). AP-1 activity was increased in all 3 of the psoriasin-transfected clones in close proportion to the level of psoriasin expression (Fig. 3a). In the high psoriasin-expressing clones (231-HP1 and 231-HP2) there was a 6.5-fold increase in luciferase activity (P < 0.0001). These psoriasin-expressing cells showed no difference in total Jab1 protein detected by Western blot, compared with non-psoriasin-expressing controls (Fig. 3a). However, the effect on AP-1 activity is consistent with the redistribution and relative increase in nuclear Jab1 protein detected by immunohistochemistry (Fig. 2) and the findings of others (21). Expression of endogenous AP-1-dependent genes was next examined by RT-PCR (Fig. 3c). Psoriasin expression is also associated with an increase in mRNA levels of the endogenous AP-1-regulated genes VEGF (30) and MMP13 (31), and this increase is proportional to the levels of psoriasin in the MDA-MB-231 control and transfected cells.

Jab1 also interacts with HIF-1 (32) and enhances its activity. Expression of HIF-1 and the HIF-1-regulated gene CAIX (27) was examined by Western blot. Under hypoxic conditions (0.7% O2), psoriasin-expressing clones showed a marked and higher induction of HIF-1 compared with control cells (Fig. 3d, top panel) and a parallel increase in CAIX protein (Fig. 3d, middle panel). However, it was noted that CAIX expression was also increased in psoriasin-expressing 231-HP1 and 231-HP2 cells under normoxic conditions. The latter observation is consistent with the recent finding that CAIX can also be regulated by AP-1 (33) and indicates that a component of the CAIX induction seen under hypoxic conditions might be attributable to AP-1, given the involvement of AP-1 as well as HIF-1 in the cellular hypoxic response (34, 35).

Psoriasin Overexpression Influences Breast Tumor Progression in Vitro. We next looked for a relationship between psoriasin expression and biological end points relevant to tumor progression in breast cancer cells. The effect of psoriasin on growth of MDA-MB-231 cells was examined and found to be associated with a modest but significant increase in growth rate (Fig. 4a) of up to 1.3 fold (P = 0.0009). The influence of psoriasin on cellular adhesion, an important parameter of invasion, was measured in an in vitro assay. We observed a consistent reduction in cell-substrate adhesion (Fig. 4b) in psoriasin-expressing clones plated on plastic (0.42-fold reduction; P < 0.0001), collagen I (0.20-fold reduction; P < 0.0001), and fibronectin (0.18-fold reduction; P < 0.0001). The influence of psoriasin on invasion was then assessed in a modified Boyden chamber assay. There was a 1.4-fold increase in invasiveness in the high psoriasin-expressing clones (P < 0.0001) after 12 h (Fig. 4c), at which time there was no significant difference in growth (data not shown).

Psoriasin Overexpression Influences Breast Tumor Progression in Vivo. To determine whether psoriasin expression can also influence invasion and metastasis in vivo, psoriasin-overexpressing cells (231-LP1 and 231-HP1) and control cells (parental 231 and 231-neo) were injected into the mammary fat pad of nude mice, and the generation of tumors and metastasis was assessed (Fig. 5). Control cell lines (231 and 231-neo) generated tumors in 2 of 10 and 3 of 10 sites, respectively, after 8 weeks. These tumors were first noted between 2 and 3 weeks after injection, and increased slowly in size (Fig. 5a). Both psoriasin-expressing cell lines (LP1 and HP1) generated grossly detectable tumors in 7 of 10 and 6 of 10 sites. These tumors were also first noted between 2 and 4 weeks after injection but
increased rapidly in size (Fig. 5, b and c). By week 8 there was no difference in incidence or mean tumor size between parental 231 cells and 231-neo controls, or between the two psoriasin-expressing clones (Fig. 5e). However, both psoriasin-expressing clones were significantly different from both parental and neo-transfected control cells (P = 0.017 and P = 0.024, Mann Whitney; Fig. 5f). Overall mean tumor sizes (mm³) for each experimental group were: MDA-231 = 2711, 231-neo = 548, LP1 = 336223, and HP1 = 370270. When control groups and psoriasin transfecant groups were combined, the mean tumor sizes (mm³) were also significantly different: MDA-213 + 231-neo = 4020 and LP1 + HP1 = 352236 combined (P = 0.0016, Mann Whitney test). Microscopic examination of primary injection sites identified one additional microscopic tumor in the LP1 cell line group. The primary tumors derived from both control and psoriasin-expressing cells showed similar histological appearances. Expression of psoriasin was confirmed in representative tumors derived from psoriasin-transfected cell clones by immunohistochemistry (data not shown) and by Western blot (Fig. 5d). Psoriasin expression was only detected in tumors from psoriasin-transfected cells (although only a very weak signal was detected in the LP1 cell line). p27 expression was reduced in both psoriasin-transfected cell clone tumors. Grossly evident metastasis was identified and confirmed by microscopy in abdominal lymph nodes distant from the primary injection sites in 2 of 10 mice injected with psoriasin-overexpressing cells (both in the HP1 cell line group) compared with 0 of 10 mice in the control experimental groups.

**DISCUSSION**

The transition from normal epithelium through DCIS to invasive breast cancer is likely to involve many complex processes that are influenced by dynamic changes in gene expression (36). Perhaps the most critical of these processes is the acquisition of the invasive phenotype (37) that occurs with the transition from DCIS to invasive disease, because this event transforms an otherwise local disease into one that is capable of distant spread to threaten the host. It is likely that some of those genes that show alterations in expression between preinvasive and invasive components of breast tissues may be relevant to the process of invasion and offer markers of risk of early tumor progression (36). In this study we demonstrate that the psoriasin gene,
which is highly expressed in DCIS and associated with poor prognostic factors when expressed in invasive disease, can enhance growth, adhesion, and invasiveness of a breast cell line in in vitro assays and tumorigenicity in nude mice in vivo. Furthermore, we describe a potential mechanism for these effects through a direct interaction between psoriasin and the multifunctional intracellular protein Jab1 (21).

Jab1 was originally identified in mammalian cells as a factor influencing c-jun transcription of AP-1-regulated genes (21). It soon became clear that Jab1 was also a component (CSN5) of a multimeric protein complex (22, 23). The CSN/COP9 signalosome had been studied previously in other systems and shown to be involved in protein degradation via the Ub-26S proteasome (24, 25). Jab1 has since been shown to be involved in a diversity of interactions with components of cell signaling pathways in vitro, yeast, and human cell line model systems. These interactions appear to result in either translocation of Jab1 from cytoplasm to nucleus (integrin LFA-1 [38], erbB-2 [39] signaling), enhanced activity of transcription factors (including c-jun/AP-1 [21], HIF-1 [32], steroid receptors and cofactors [40, 41]) or the promotion of degradation of the interacting protein (including Smad4 [42], p53 [43], HIF-1 [32], MIF1 [28], and p27Kip1 [29, 43]), often but not always associated with translocation from nucleus to cytoplasm. However, the physiological relevance of some of these interactions, and specifically in the context of breast epithelial cells, is mostly unknown.

In ovarian tumors, increased nuclear Jab1 is associated with progression and poor outcome (44), and altered Jab1 has also been implicated in renal cancer (45). A direct role for Jab1 in breast cancer has not been identified previously; however, several proteins including p53 and erbB-2, which are known to interact with or to influence Jab1, are altered at an early stage within high-risk DCIS (46–49) and may exert some of their effects through Jab1. The interaction between psoriasin and Jab1 also has the potential to directly facilitate several aspects of early tumor progression. We have shown here that overexpression of psoriasin is associated with translocation of Jab1 to the nucleus, alterations in expression of several Jab1 "downstream" genes, and increased proliferation, altered response to hypoxia, and promotion of invasion. Increased proliferation may be specifically attributable to increased AP-1 activity and down-regulation of the cell cycle inhibitor p27Kip1 in this model. Alteration of Jab1 might also lead to increased activation of estrogen receptor and progesterone receptor, and up-regulation of cyclin D1 and alteration of transforming growth factor β signaling in other cell models (39, 50, 51), but these aspects of Jab1 function remain to be examined in the context of breast cancer. Increased capacity to survive hypoxic stress may occur through augmented HIF-1 activity and hypoxic response. Increased invasiveness may result from activation of AP-1 and HIF-1-dependent genes (52, 53), such as matrix metalloproteinases and VEGF, which are already implicated as critical factors in breast tumor progression (37, 54).

The estrogen receptor-negative MDA-MB-231 breast cell line was selected to reflect the context of psoriasin expression that we and others have observed previously in breast tumors in vivo (2, 4). The modest although significant increase in proliferation and invasiveness seen in our in vitro assays may reflect the fact that this cell line is already a highly proliferative and invasive cell in in vitro assay. More
striking increases in growth and invasiveness were observed in vivo in the nude mice experiments, where metastasis was also associated with psoriasin-expressing tumors. This difference is consistent with the anticipated effects of enhanced metalloproteinase and VEGF expression on extracellular matrix and angiogenesis, spheres of influence that are not adequately replicated in in vitro assays, and has been observed by others studying the effects of overexpression of VEGF in breast cell lines (55). Nevertheless, additional detailed studies will be necessary to confirm the direct relationship and functional role of these specific factors in the enhanced growth and invasiveness seen in this model in vivo.

Alteration of Jab1 activity in tumors could be attributable in part to alterations in either the cytoplasmic-nuclear distribution (Refs. 38, 45, 56; as appears to be the case for the effect of psoriasin), the ratio of free Jab1:COP9-associated Jab1 (56), competition between different interacting proteins (42), or direct elevation of Jab1 expression and activation. The relevance of these potential mechanisms of action to breast cancer remains to be resolved, both for psoriasin and several other Jab1-interacting proteins. Nevertheless, it has been demonstrated that the many important activities of Jab1 can be influenced by competition between different interacting proteins (42). For example, p53 can compete with and down-regulate Jab1 activation of c-jun (57), and inhibition of Jab1 causes reciprocal up-regulation of p53 (42) and down-regulation of c-jun in HeLa cells (57). It is also interesting to note that the chemokine MIF can exert the opposite effect on Jab1 to psoriasin (28) with respect to modulation of AP-1 activity and p27Kip1 expression. This raises the question of whether these different chemokine molecules might compete to modulate Jab1 activity.

Whereas our data support the involvement of Jab1 in mediating many of the biological actions of psoriasin, additional experiments will be needed to confirm that a direct interaction occurs between the putative Jab1 binding motif (29) on psoriasin and the Jab1 protein, and that direct alterations of Jab1 indeed exert effects on these specific target genes and pathways. It is also possible that some of the functions of psoriasin are mediated through other pathways (58). For example, it has been shown that other secreted S100 proteins (S100B and S100A12) can bind to and stimulate the receptor for advanced glycation end products, leading to activation of intracellular signaling pathways including up-regulation of ras, mitogen-activating protein kinase and nuclear factor κB in immune cells (59, 60). Expression of receptor for advanced glycation end products is also associated with invasion in gastric carcinoma (61) and is functionally involved in metastasis (62). Unlike some other S100s with chemokine activities such as S100A9 and S100A12, which are expressed by both epithelial and stromal inflammatory cells (63), expression of S100A7 (psoriasin) is restricted to epithelium, at least in skin and breast. However,
psoriasin is also secreted and could potentially interact with cell surface receptors on immune or epithelial cells.

In summary, we have shown that psoriasin can contribute to breast tumor progression and that its action may be mediated, at least in part, through Jab1. Although other important cellular proteins also influence and may compete for Jab1, psoriasin is one of the most abundant proteins in high-risk DCIS (2) and is likely to exert an important effect on Jab1 activity in breast tumor cells at an early stage of tumor progression. Thus, therapies aimed at modulating the effect of psoriasin may have important potential in the treatment of early breast cancer.

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11. Psoriasin ALTERS JAB1 FUNCTION IN BREAST CANCER CELLS


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