Persistent activation of STAT3 by PIM2-driven positive feedback loop for epithelial-mesenchymal transition in breast cancer

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Metastasis of breast cancer is promoted by epithelial–mesenchymal transition (EMT). Emerging evidence suggests that STAT3 is a critical signaling node in EMT and is constitutively activated in many carcinomas, including breast cancer. However, its signaling mechanisms underlying persistent activation of STAT3 associated with EMT remain obscure. Here, we report that PIM2 promotes activation of STAT3 through induction of cytokines. Activation of STAT3 caused an increase in PIM2 expression, implicating a positive feedback loop between PIM2 and STAT3. In agreement, targeting of either PIM2, STAT3 or PIM2-dependent cytokines suppressed EMT-associated migratory and invasive properties through inhibition of ZEB1. Taken together, our findings identify the signaling mechanisms underlying the persistent activation of STAT3 and the oncogenic role of PIM2 in EMT in breast cancer.

Despite the fact that surgical resection and adjuvant therapy can cure well-confined primary tumors, breast cancer mortality rates remain high owing to clinical relapse associated with metastasis to distant organs. Because primary tumors in most cases are not the main cause of fatality, the efficacy of breast cancer treatment is largely dependent on our capacity to interfere in the process of metastasis.[1]

PIM kinase is a family of proto-oncogenes that contains three members, PIM1, PIM2 and PIM3, constituting a group of closely related serine/threonine kinases.[2] PIM kinases have unique structural properties, and are characterized by a constitutive serine/threonine kinase activity that does not depend on post-translational modifications for activation. PIM kinases play an important role in cellular proliferation, survival and differentiation.[3,4] Because PIM1 and PIM2 are highly expressed in hematopoietic tissues, their oncogenic roles are relatively well-known in hematopoietic cancers. However, their oncogenic mechanisms in breast cancer remain obscure.

In the current study, we report that PIM2 contributes to the persistent activation of STAT3 in breast cancer cells through an autocrine positive feedback loop. In previous studies, STAT family proteins have been identified as regulators of EMT, a key mechanism of metastasis in breast cancer.[5–9] STAT proteins are latent transcription factors located in cytoplasm in an inactive state.[10] Upon phosphorylation, activated STAT proteins translocate to the nucleus where they initiate transcription of its target genes.[11,12] In normal cells, STAT3 tyrosine phosphorylation is transient. In contrast, STAT3 has been found to be constitutively phosphorylated to high levels in more than 50% of breast cancer cell lines and in more than 30% of breast adenocarcinomas and is associated with a poor prognostic indicator.[13,14] However, the molecular mechanisms underlying persistent activation of STAT3 in breast cancer remain obscure. In this study, we show that constitutive activation of STAT3 is achieved by a PIM2-driven positive feedback loop, finally leading to EMT in breast cancer cells. Collectively, our findings implicate the importance of PIM2 for persistent activation of STAT3 leading to EMT in breast cancer.

Materials and Methods

Chemical reagents and antibodies. Polyclonal antibodies to PIM1, vimentin (VIM), SLUG, TWIST and STAT3 were purchased from Santa Cruz (Santa Cruz, CA, USA). Polyclonal antibodies to PIM2, SNAIL, AKT, phospho-AKT(S473), phospho-AKT(T308), phospho-STAT3(S727), phospho-STAT3 (Y705), STAT5 and phospho-STAT5(Y694) were purchased from Cell Signaling Technology (Beverly, MA, USA). Polyclonal antibody to N-cadherin was purchased from BD (Beverly, MA, USA). Polyclonal antibody to ZEB1, monoclonal antibody to β-actin and DAPI were purchased from Sigma (St Louis, MO, USA). Anti-rabbit IgG alexa 488 and anti-mouse IgG alexa Fluor 488 were purchased from Invitrogen (Carlsbad, CA, USA). Neutralizing antibodies against IL-1α and IL-8 were purchased from Abcam (Cambridge, MA, USA) and the corresponding control anti-mouse IgG and anti-rabbit IgG were purchased from Santa Cruz.

Cell culture. Human breast cancer cell lines BT549 and MDA-MB231 were established from the American Type Culture Collection (Manassas, VA, USA). MDA-MB231 cells were grown in DMEM and BT549 in RPMI supplemented with 10% FBS, penicillin (100 units/mL) and streptomycin (100 μg/mL). The DMEM, RPMI, FBS, penicillin, streptomycin and trypsin were purchased from Gibco (Seoul, Korea).

Transfection. Cancer cells were transfected with siRNA by using Lipofectamine2000 (Invitrogen), according to the manufacturer's instructions. All siRNA targeting PIM1, PIM2, STAT3, STAT5, IL-1α and IL-8 and a negative scrambled control siRNA were purchased from Genolution Pharmaceuticals (Seoul, Korea). At 48 h after transfection, cells were harvested for western blot or mRNA expression analysis.

Transduction. Retroviral vectors pMSCV and pMSCV-PIM2 were kindly provided from Hee-Yong Chung (Department of Medicine, Hanyang University). For retroviral production, H29D cells were cultured in DMEM supplemented with 10% FBS, 2 mmol/L GlutaMAX (Invitrogen), 50 U/mL penicillin/streptomycin, 1 μg/mL tetracyclin, 2 μg/mL puromycin, and 0.3 mg/mL G418 sulfate (Calbiochem, San Diego, CA, USA) and transfected with MSCV or MSCV-PIM2 using the Lipofectamine 2000 reagent. After 48 h, the viral supernatant was harvested and the viral supernatant was stored at −80°C. Cells were transduced overnight in the viral supernatant supplemented with 4 μg/mL Polybrene (Sigma, St. Louis, MO, USA).

Invasion and migration assays. For the invasion assays, cells were loaded in the upper well of the Transwell chamber (8-μm pore size [Corning Glass, Seoul, Korea]) that was pre-coated with 10 mg/mL growth factor-reduced Matrigel (BD Biosciences, Seoul, Korea). After 48 h, non-invaded cells on the upper surface of the filter were removed with a cotton swab, and migrated cells on the lower surface of the filter were fixed and stained with a Diff-Quick kit (Fisher, Pittsburgh, PA, USA) and photographed. Invasiveness was determined by counting cells in five microscopic fields per well, and the extent of invasion was expressed as an average number of cells per microscopic field. Cells were imaged by phase contrast microscopy (Leica Microsystems, Bannockburn, IL, USA). For the Migration assay, we used the Transwell chambers with inserts that contained the same type of membrane but without the matrigel coating.

Western blot analysis. Cell lysates were prepared by extracting proteins with lysis buffer (40 mM Tris-HCl [pH 8.0], 120 mM NaCl, 0.1% Nonidet-P40) supplemented with protease inhibitors. Proteins were separated by SDS-PAGE, and transferred to a nitrocellulose membrane (Amersham, Arlington Heights, IL, USA). The membrane was blocked with 5% nonfat dry milk in Tris-buffered saline, and incubated with primary antibodies overnight at 4°C. The blots were developed with a peroxidase-conjugated secondary antibody, and proteins were visualized by enhanced chemiluminescence procedures (Amersham, Arlington Heights, IL, USA), following the manufacturer's protocol.

Reverse transcription PCR. RNA was isolated using Trizol (Invitrogen). RT-PCR were performed using Super-Script III (Invitrogen) according to the manufacturer's instructions. Quantitative RT-PCR (qRT-PCR) was performed in Rotor Gene Q (Qiagen, Seoul, Korea).

Immunocytochemistry. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100 in PBS. Following cell fixation, cells were incubated with the appropriate primary antibodies in a solution of PBS with 1% BSA and 0.1% Triton X-100 at 4°C overnight. Staining was visualized using anti-rabbit or anti-mouse IgG Alexa Flour 488 (Molecular Probes). Nuclei were counterstained using DAPI. Stained cells were visualized with an Olympus IX71 fluorescence microscope (Olympus, Seoul, Korea).

Statistical analysis. All experimental data are reported as means and the error bars represent the experimental standard errors. Statistical analysis was performed using the non-parametric Student *t*-test in the GraphPad Prism statistical program.

Results

PIM2 promotes the migratory and invasive properties of breast cancer cells through epithelial–mesenchymal transition. To examine the role of PIM kinases in the malignancy of breast cancer cells, we examined the effect of PIM kinase knockdown on migration and invasion of basal type MDA-MB231 and BT549 breast cancer cells. Of note, siRNA-mediated downregulation of either PIM1 or PIM2 greatly inhibited the migratory and invasive properties of breast cancer cells without causing cell death (Fig. 1a–c). Because the effect was more pronounced by PIM2 knockdown, we further investigated the functional role of PIM2 on the metastatic ability of breast cancer cells.

Because the acquisition of migratory and invasive properties is often associated with EMT, we next examined whether PIM2 regulates the EMT in breast cancer cells. Importantly, we found that EMT marker proteins VIM and N-cadherin were decreased in MDA-MB231 and BT549 breast cancer cells, respectively, after silencing PIM2 with siRNA (Fig. 1d). These findings were also confirmed by immunocytochemical staining (Fig. 1e). Furthermore, as expression of mesenchymal marker proteins is known to be regulated by master EMT transcription factors such as SNAIL, SLUG, TWIST and ZEB1, we examined whether their expression levels can be regulated by PIM2. As expected, downregulation of PIM2 caused a decrease in ZEB1 in both cancer cell lines, while it had no effect on other transcription factors (Fig. 1f). Consistent with these results, immunocytochemistry and qRT-PCR analysis also revealed that downregulation of PIM2 decreases ZEB1 in breast cancer cells (Fig. 1g,h). Taken together, these results suggest that PIM2 regulates the migratory and invasive properties of basal-type breast cancer cells through ZEB1-driven EMT.

PIM2 enhances mesenchymal phenotypes through the activation of STAT3 signaling. To further investigate the mechanism
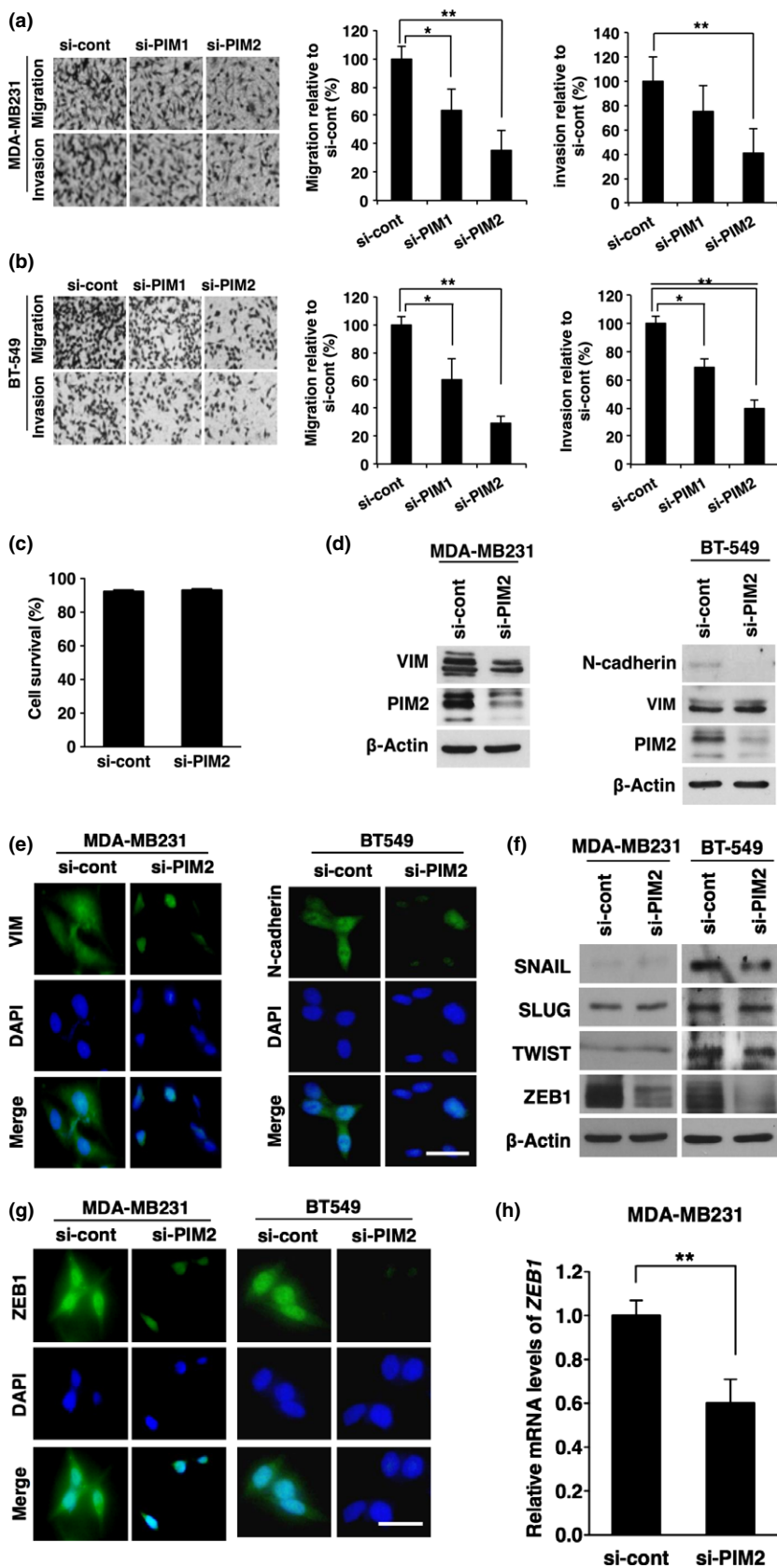
Fig. 1. PIM2 promotes epithelial–mesenchymal transition (EMT) in breast cancer cells. (a, b) Migration and invasion assay in MDA-MB231 (a) and BT549 (b) breast cancer cells treated with siRNA against PIM1, PIM2 or scrambled control siRNA. (c) FACS analysis for the effect of PIM2 on cell survival of breast cancer cells. (d) Western blot analysis for EMT markers, vimentin (VIM) and N-cadherin in breast cancer cells that were treated with siRNA against PIM2. (e) Immunocytochemistry for EMT markers, VIM and N-cadherin in breast cancer cells treated with siRNA against PIM2. (f, g) Western blot analysis for EMT transcription factors SNAIL, SLUG, TWIST and ZEB1 (f), and immunocytochemistry for EMT transcription factor ZEB1 (g) in breast cancer cells treated with siRNA against PIM2. (h) Quantitative RT-PCR for ZEB1 transcript levels in MDA-MB231 breast cancer cells after treatment with siRNA against PIM2. β-actin was used as a loading control. Error bars represent mean ± SD of triplicate samples. Scale bar, 10 μm. ∗$P < 0.05$; ∗∗$P < 0.01$.

of PIM2-mediated invasiveness of breast cancer, we sought to determine its kinase activity in well-known signaling regulators of EMT such as STATs and AKT.[15–17] We observed that

phosphorylation of STAT3 was reduced specifically on Ser727 residue, while phosphorylation of AKT was not altered in response to downregulation of PIM2 (Fig. 2a). By contrast,
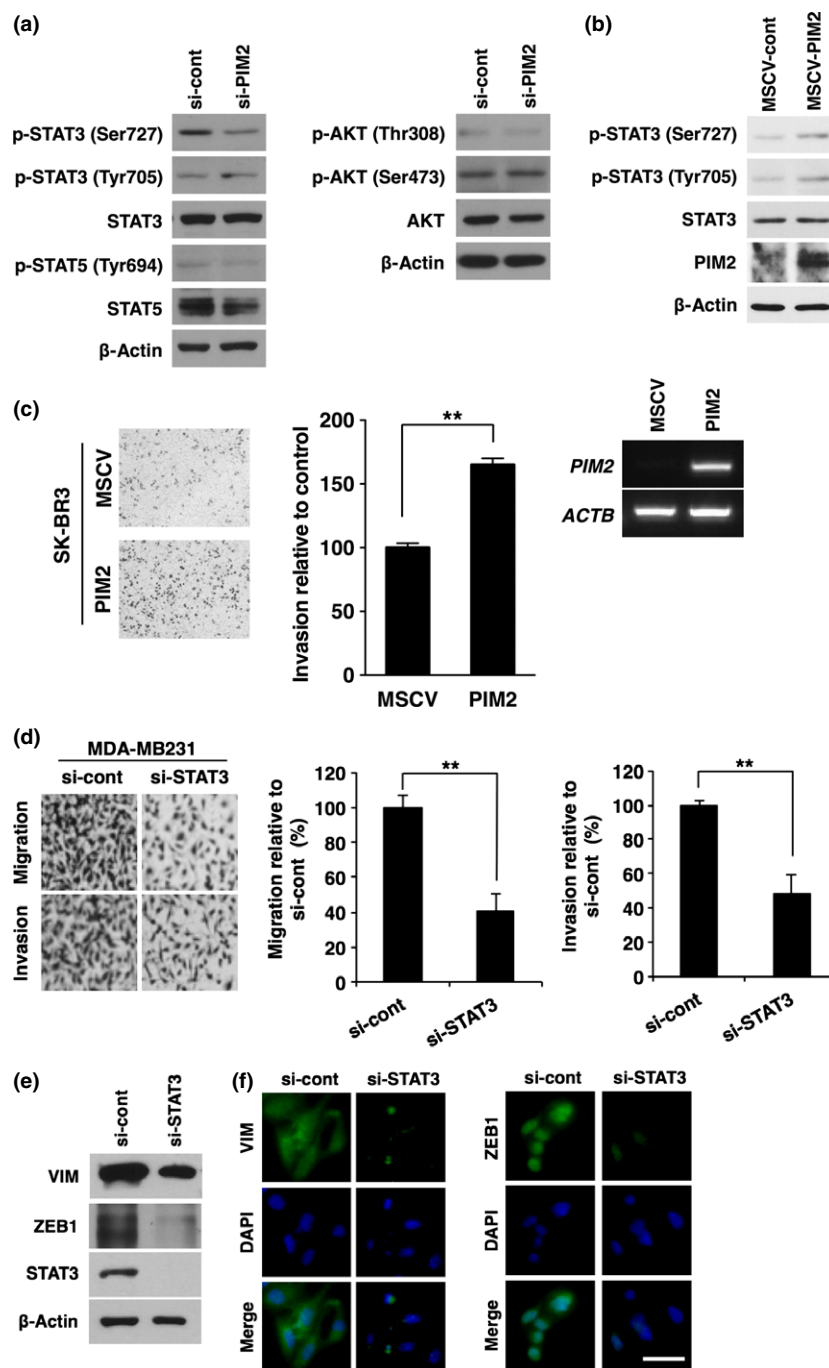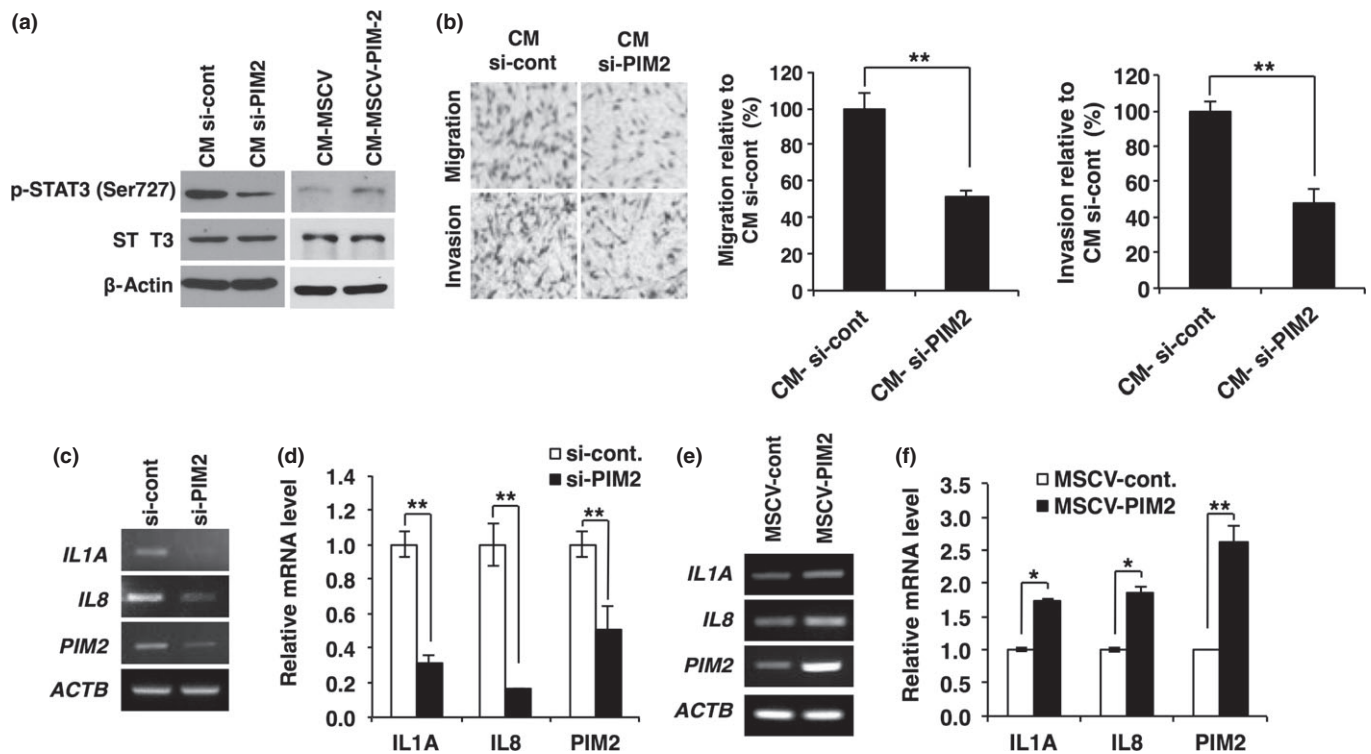
Fig. 2. PIM2 contributes to epithelial–mesenchymal transition (EMT) through activation of STAT3. (a) Western blot analysis for phosphorylation status of STATs and AKT after treatment of MDA-MB231 breast cancer cells with siRNA targeting PIM2. (b) Western blot analysis for phosphorylation status of STAT3 in MDA-MB231 breast cancer cells after transduction with PIM2. (c) Invasion assay in SKBR3 breast cancer cells after transduction with PIM2. (d) Migration and invasion assay in MDA-MB231 breast cancer cells after treatment with siRNA against STAT3. (e, f) Western blot analysis (e) and immunocytochemistry (f) for vimentin (VIM) and ZEB1 in MDA-MB231 breast cancer cells after treatment with siRNA against STAT3. β-actin was used as a loading control. MSCV, control vector. Scale bar, 10 μm. Error bars represent mean ± SD of triplicate samples. ∗∗*P* < 0.01.

overexpression of PIM2 enhanced the phosphorylation of STAT3 at Ser727 residue (Fig. 2b). In parallel, overexpression of PIM2 enhanced the invasiveness of relatively non-invasive luminal-type SKBR3 breast cancer cells (Fig. 2c). Next, we asked whether PIM2 promotes mesenchymal phenotypes of breast cancer cells through STAT3 signaling. To this end, invasion and migration assays were performed after treatment with siRNA targeting STAT3. As expected, we observed that downregulation of STAT3 decreases the migration and invasion of breast cancer cells (Fig. 2d). In agreement with these results, downregulation of STAT3 decreased expression of EMT marker VIM and its regulator ZEB1, similar to the effect of PIM2 downregulation (Fig. 2e). Consistently, immunocyto-

chemical staining of cancer cells transfected with siRNA against STAT3 showed lower expression levels of VIM and ZEB1, compared with cells transfected with scrambled control siRNA (Fig. 2f). Collectively, these data show that PIM2 promotes mesenchymal phenotypes of breast cancer cells through activation of STAT3 signaling.

PIM2 promotes activation of STAT3 through secretion of IL-1α and IL-8. We next examined how PIM2 promotes STAT3 activation in breast cancer cells. Because PIM2 is a serine-threonine protein kinase, we checked whether PIM2 directly binds to and activates STAT3. However, immunoprecipitation and PIM2 kinase assay showed that PIM2 does not interact with STAT3 and phosphorylates it directly (Suppl. Fig. S1a and

Fig. 3. PIM2 promotes epithelial–mesenchymal transition (EMT) through induction of IL-1α and IL-8 cytokines. (a) Western blot analysis for phosphorylation status of STAT3 in MDA-MB231 cancer cells after treatment with conditioned media (CM) harvested from MDA-MB231 cancer cells transfected with siRNA against PIM2 or transduced with PIM2. MSCV, control vector. (b) Migration and invasion assay in MDA-MB231 cancer cells after treatment with CM harvested from cells transfected with siRNA against PIM2. (c, d) Semi-quantitative RT-PCR (c) and quantitative RT-PCR (qRT PCR) (d) for mRNA levels of *IL1A* and *IL8* in cancer cells after treatment with siRNA against PIM2. (e, f) Semi-quantitative RT-PCR (e) and qRT PCR (f) for mRNA levels of *IL1A* and *IL8* in cancer cells after transduction with PIM2. β-actin was used as a loading control. Scale bar, 10 μm. Error bars represent mean ± SD of triplicate samples. *$P < 0.05$; **$P < 0.01$.

data not shown). Because STAT3 is known to be activated by many cytokines, we hypothesized the possibility that PIM2 induces secretion of cytokines, which stimulates the activation of STAT3 in breast cancer cells. To examine the possibility, we analyzed the phosphorylation status of STAT3 in MDA-MB231 breast cancer cells after treatment with conditioned media (CM) from MDA-MB231 breast cancer cells that were transfected with siRNA targeting PIM2 or scrambled control siRNA. Of note, phosphorylation of STAT3 was decreased in the breast cancer cells by treatment with CM from PIM2-knockdown breast cancer cells, compared to control (Fig. 3a). To further validate this, we also analyzed the phosphorylation status of STAT3 in breast cancer cells after treatment with CM from PIM2-overexpressing MDA-MB231 cells. Of note, the phosphorylation of STAT3 was increased in cells treated with CM from PIM2-overexpressing breast cancer cells compared to control, indicating that PIM2-dependent cytokine secretion is responsible for STAT3 activation (Fig. 3a). In agreement with this data, treatment with CM from PIM2-knockdown cells reduced the ability of MDA-MB231 cells to migrate and invade through Transwells as compared to control (Fig. 3b).

Because these results indicated that PIM2 promotes the phosphorylation of STAT3 through secretion of soluble factors, we proceeded to determine the PIM2-dependent secretion factors. To this end, we analyzed whether down-regulation of PIM2 alters the expression levels of interleukin family. Importantly, we found that downregulation of PIM2 causes a decrease in interleukin-1α (IL-1α) and IL-8 (also known as

CXCL8, CXC chemokine ligand 8) in breast cancer cells (Fig. 3c,d and Suppl. Fig. S1b). To further confirm that IL-1α and IL-8 are regulated by PIM2, we analyzed whether PIM2 overexpression increases the expression levels of the cytokines. As expected, PIM2 overexpression increased the expression levels of two cytokines, IL-1α and IL-8 (Fig. 3e,f).

To examine whether PIM2 contributes to the invasiveness of breast cancer cells through secretion of cytokines, we next analyzed the effect of IL-1α and IL-8 on the migratory and invasive properties of breast cancer cells. Importantly, downregulation of either IL-1α or IL-8 caused a decrease in the migratory and invasive properties of breast cancer cells (Fig. 4a). Consistent with these results, treatment with a neutralizing antibody against either IL-1α or IL-8 also inhibited migration and invasion of MDA-MB231 breast cancer cells (Fig. 4b). In parallel, both siRNA-mediated downregulation and treatment with a neutralizing antibody against IL-1α or IL-8 led to a decrease in the mesenchymal marker VIM and EMT regulator ZEB1 in breast cancer cells (Fig. 4c,d).

In accordance with these data, we next examined whether STAT3 can be activated by these PIM2-dependent cytokines. Importantly, siRNA-mediated downregulation of IL-1α or IL-8 effectively decreased the phosphorylation of STAT3 at Ser727 (Fig. 4e). To further confirm that PIM2-dependent activation of STAT3 is mediated by these cytokines, we examined whether downregulation of IL-1α and IL-8 could block the effect of PIM2 on phosphorylation of STAT3. To this end, these cytokines were down-regulated by treatment with siRNA against them in PIM2-overexpressing breast cancer cells and
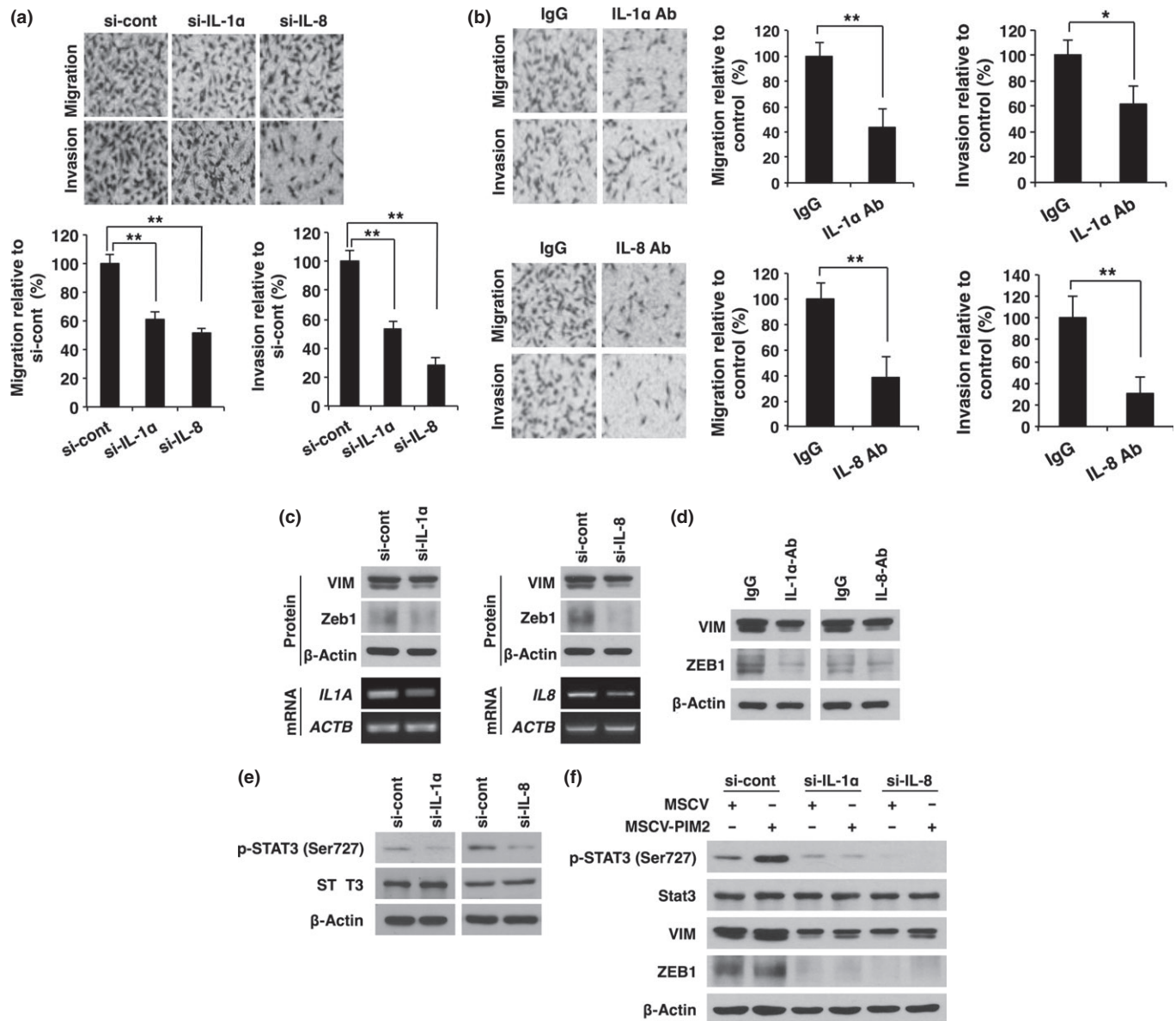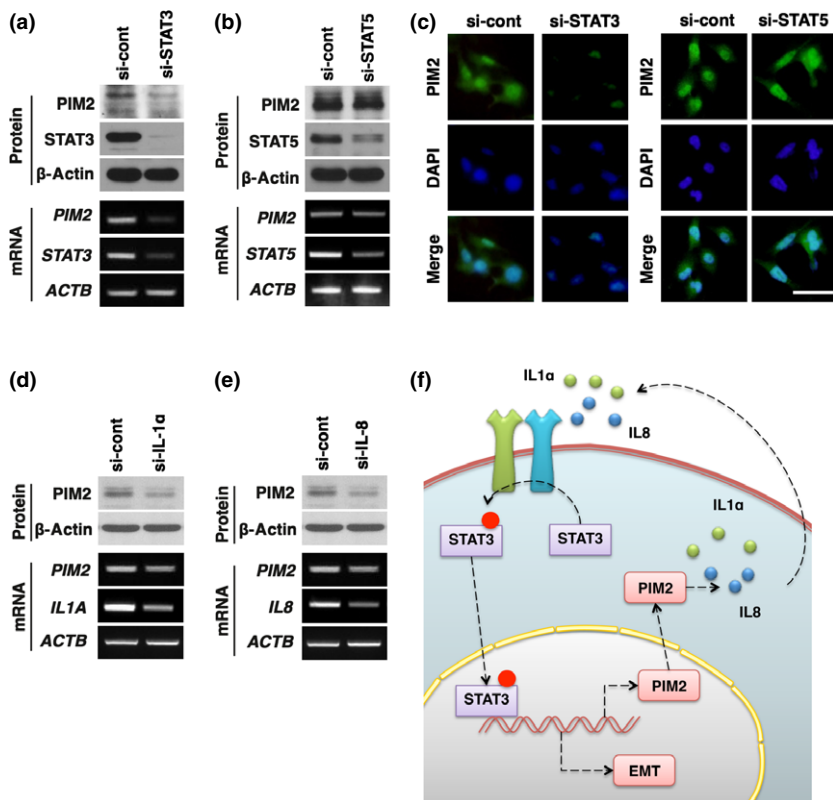
Fig. 4. PIM2 activates STAT3 signaling through induction of IL-1α and IL-8. (a, b) Migration and invasion assay after treatment of MDA-MB231 breast cancer cells with siRNA (a) or neutralizing antibodies (b) against IL-1α or IL-8. (c, d) Western blot analysis for vimentin (VIM) and ZEB1 in MDA-MB231 breast cancer cells after treatment with siRNA (c) or neutralizing antibodies (d) against IL-1α or IL-8. (e) Western blot analysis for phosphorylation status of STAT3 in MDA-MB231 cancer cells after treatment with siRNA against IL-1α or IL-8. (f) Western blot analysis for phosphorylation status of STAT3 and protein levels of VIM and ZEB1 in MDA-MB231 cancer cells that were transduced with PIM2 prior to transfection with siRNA against IL-1α or IL-8. β-actin was used as a loading control. Error bars represent mean ± SD of triplicate samples. *$P < 0.05$; **$P < 0.01$.

followed to examine the phosphorylation status of STAT3. Consistent with the above data, PIM2 overexpression caused an increase in the phosphorylation of STAT3 along with the levels of VIM and ZEB1; however, knockdown of IL-1α or IL-8 abolished the effect of PIM2 on activation of STAT3 as well as expression of VIM and ZEB1 in PIM2 overexpressing breast cancer cells (Fig. 4f). These results demonstrate that PIM2 promotes activation of STAT3 through the induction of IL-1α and IL-8, thereby enhancing the mesenchymal phenotypes of breast cancer cells.

Activation of STAT3 is maintained by PIM2-dependent positive feedback loop. Because STATs have been known to act as a transcription factor for PIM2 in various cell types,[18] we next

examined whether STAT3 could regulate the expression of PIM2 in breast cancer cells as well. We found that siRNA-mediated downregulation of STAT3 decreased the expression of PIM2 at both protein and mRNA levels, while downregulation of STAT5 had no effect (Fig. 5a,b). Consistently, immunocytochemical analysis also confirmed that downregulation of STAT3 decreases the expression level of PIM2 (Fig. 5c), suggesting that expression of PIM2 is regulated by STAT3 in breast cancer cells as well. Because STAT3 is activated by PIM2-induced IL-1α and IL-8 in the above, these results indicate that PIM2 and STAT3 could be regulated reciprocally by a positive feedback loop. To test this possibility, we examined whether expression levels of PIM2 can be increased by these

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Fig. 5. PIM2-dependent cytokine mediates positive feedback loop between PIM2 and STAT3. (a, b) Western blot and semi-quantitative RT-PCR for PIM2 after treatment of MDA-MB231 breast cancer cells with siRNA against STAT3 (a) or STAT5 (b). (c) Immunocytochemistry for PIM2 after treatment of MDA-MB231 breast cancer cells with siRNA against STAT3 or STAT5. (d, e) Western blot and semi-quantitative RT-PCR for PIM2 after treatment of MDA-MB231 breast cancer cells with siRNA against IL-1α (d) and IL-8 (e). (f) Schematic model illustrating persistent activation of STAT3 by PIM2-dependent positive feedback loop for epithelial–mesenchymal transition (EMT). β-actin was used as a loading control.

cytokines through an autocrine loop. Importantly, treatment with siRNA targeting either IL-1α or IL-8 decreased the expression of PIM2 both at protein and mRNA levels (Fig. 5d, e). Taken together, our findings suggest that STAT3 regulates the expression of PIM2 on a transcriptional level, which, in turn, promotes activation of STAT3 through the secretion of cytokines such as IL-1α and IL-8, thereby promoting ZEB1-driven mesenchymal phenotypes in breast cancer cells (Fig. 5f).

Discussion

Metastasis to distant organs is a major reason behind cancer-associated deaths, and EMT is considered a key mechanism in the procedure. As a signaling component of EMT, several lines of strong evidence suggest that STAT proteins (in particular STAT3) are a critical regulator for EMT in breast cancer.[7–9] Under physiologic conditions, STATs are temporally phosphorylated on tyrosine residues by receptor and nonreceptor tyrosine kinases. However, in pathological conditions such as cancer, STATs are constitutively phosphorylated and contribute to the pathology.[19–22] In particular, STAT3 was found to be constitutively phosphorylated to high levels in more than 50% of breast cancer cell lines and in more than 30% of breast adenocarcinomas, and is associated with a poor prognostic indicator.[13,14] However, the molecular mechanisms underlying the constitutive activation of STAT3 in breast cancer remain unknown.

In the current study, we found that PIM2 contributes to persistent activation of STAT3, thereby promoting the invasiveness of breast cancer cells. Knockdown of PIM2 decreased the phosphorylation of STAT3, whereas its overexpression increased the phosphorylation. Although PIM2 belongs to the serine-threonine kinase family, our study suggests that PIM2

activates STAT3 indirectly through induction of cytokines such as IL-1α and IL-8. Targeting of IL-1α or IL-8 effectively inhibited phosphorylation of STAT3, decreased EMT and suppressed the invasiveness of breast cancer cells. In previous studies, IL-1α and IL-8 were found to be highly expressed in breast cancer compared with normal breast tissue, and associated with poor prognosis.[23–26] Pantschenko et al.[23] suggest that the IL-1 family of cytokines are present within human breast cancer and trigger other protumorigenic cytokines in tumor microenvironment, such as the angiogenic factor and growth factor including IL-8. In addition, they demonstrate that expression levels of some members of the IL-1/IL-8 network of cytokines are correlated with prognostic indicators such as estrogen and progesterone receptors. Moreover, IL-8 has been recognized as an important regulator of breast cancer stem cell activity through its receptors, CXCR1 and CXCR2.[27] In this aspect, targeting CXCR1/2 signaling has already proved its efficacy in preclinical studies, although drug development is still in the early stages.[28] In addition to these previous observations, our study suggests that IL-1α and IL-8 mediate a positive feedback loop between PIM2 and STAT3, thereby allowing constitutive activation of STAT3 in breast cancer cells. Indeed, PIM2-activated STAT3, in turn, caused an increase in the expression of PIM2. Thus, our findings explain how the expression levels of IL-1α and IL-8 and activation of STAT3 are sustained at higher levels in breast cancer compared to normal counterpart tissues. In addition, although we showed that IL-1α and IL-8 are secreted by cancer cells, these cytokines are also known to be produced by macrophages, epithelial cells and endothelial cells.[29,30] Presumably, in the in vivo situation, those stromal cells in tumor microenvironment might also contribute to elevation of the cytokine levels and strengthen the positive feedback loop between PIM2 and STAT3 in a paracrine manner.

We also found that downregulation of PIM2 suppresses the invasiveness of breast cancer cells along with mesenchymal cell markers such as VIM and N-cadherin. Because ZEB1 was specifically decreased by knockdown of PIM2, our findings suggest that PIM2 promotes EMT through ZEB1 induction. Supporting this notion, targeting of STAT3 also decreased the EMT accompanying the downregulation of ZEB1, as PIM2 does. Collectively, our findings suggest that ZEB1 is a critical regulator for PIM2/STAT3-driven EMT in breast cancer cells; however, the possibility is not completely excluded that STAT3 also promotes invasiveness of breast cancer cells through other EMT transcription factors such as SNAIL, SLUG and TWIST. Further study on the role and regulation of ZEB1 might clarify its importance in breast cancer metastasis.

In summary, our study suggests that PIM2 is a critical regulator in the maintenance of higher levels of IL-1α, IL-8 and persistent activation of STAT3, and thereby contributes to breast cancer progression through EMT. Considering that IL-1α, IL-8 and STAT3 are evaluated as novel therapeutic targets, our finding that PIM2 regulates EMT and signaling components, IL-1**a**, IL-8 and STAT3 via a positive feedback loop implicates PIM2 as a novel therapeutic target for breast cancer as well.

Disclosure Statement

The authors declare no conflict of interest.

Abbreviations

EMT	epithelial-mesenchymal transition
siRNA	small interfering RNA
STAT	signal transducer and activator of transcription

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Supporting Information

Additional supporting information may be found in the online version of this article:

Fig. S1. PIM2-dependent secretion of cytokines promotes activation of STAT3 in breast cancer cells.