Krüppel-like Factor 5 Transcription Factor Promotes Microsomal Prostaglandin E2 Synthase 1 Gene Transcription in Breast Cancer*

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Background: The mechanism by which the KLF5 transcription factor promotes breast cancer is not entirely understood.

Results: KLF5 promotes breast cell proliferation partially through inducing mPGES1 gene expression.

Conclusion: mPGES1 is a direct transcriptional target gene of KLF5.

Significance: We discovered a new functional mechanism for KLF5 and a regulation mechanism for mPGES1.

The KLF5 (Krüppel-like factor 5) transcription factor is specifically expressed in a subset of estrogen receptor α-negative breast cancers. Although KLF5 promotes breast cancer cell cycle progression, survival, and tumorigenesis, the mechanism by which KLF5 promotes breast cancer is still not entirely understood. Here, we demonstrate that mPGES1, encoding microsomal prostaglandin E2 synthase 1 (mPGES1), is a KLF5 direct downstream target gene. KLF5 overexpression or knockdown positively altered the levels of mPGES1 mRNA and protein in multiple breast cell lines. 12-O-Tetradecanoylphorbol-13-acetate induced the expression of both KLF5 and mPGES1 in dosage- and time-dependent manners. The induction of KLF5 was essential for 12-O-tetradecanoylphorbol-13-acetate to induce mPGES1 expression. Additionally, KLF5 bound to the mPGES1 gene proximal promoter and activated its transcription. Both KLF5 and mPGES1 promoted prostaglandin E2 production; regulated p21, p27, and Survivin downstream gene expression; and likewise stimulated cell proliferation. Overexpression of mPGES1 partially rescued the KLF5 knockdown-induced downstream gene expression changes and growth arrest in MCF10A cells. Finally, we demonstrate that the expression of mPGES1 was positively correlated with the estrogen receptor α/progesterone receptor/HER2 triple-negative status. These findings suggest that mPGES1 is a target gene of KLF5, making it a new biomarker and a potential therapeutic target for triple-negative breast cancers.

KLF5 (Krüppel-like factor 5)/IKLF/BTEB2 belongs to the human Sp1/KLF family of transcription factors containing three conserved C2H2-type zinc fingers in the C-terminal domain (1) and is highly expressed in estrogen receptor α (ERα)-negative breast cancers (2). KLF5 promotes breast cell proliferation, survival, and tumorigenesis, partially by inducing FGF-BP1 (FGF-binding protein 1) gene expression (3), activating ERK signaling, and stabilizing the MKP-1 phosphatase (4). However, the mechanism by which KLF5 promotes breast cancer development is not completely understood.

As a transcription factor, KLF5 promotes cell proliferation and survival by regulating a number of downstream target genes, e.g. CCND1 (5), p21 (6), FGF-BP1 (3), Survivin (7), and so on. KLF5 binds to the promoters of these genes through GC-rich DNA sequences using its three zinc finger domains. In our previous study of microarray analysis using TSU-Pr1 clones stably expressing KLF5, we identified a number of genes, including mPGES1 as potential KLF5-regulated genes (8). Of these, mPGES1 is of particular interest; it plays a critical role in mediating prostaglandin E2 (PGE2) synthesis and cell proliferation.

Accumulated evidence suggests that mPGES1 is a promising therapeutic target in cancer treatments (9). The expression of mPGES1 is induced by various proinflammatory stimuli, and mPGES1 acts downstream of COX-2 (cyclooxygenase-2) for PGE2 production (10). PGE2 is a bioactive lipid that can regulate a wide range of biological effects associated with inflammation and cancer. PGE2 activates a series of proliferative signals by binding to a family of E-prostanoid receptors; PGE2 has long been implicated in promoting tumor cell growth and invasion via angiogenesis (11). PGE2 is produced through three sequential enzyme reactions: (a) release of arachidonic acid from

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† The abbreviations used are: ERα, estrogen receptor α; PGE2, prostaglandin E2; PGH2, prostaglandin H2; TPA, 12-O-tetradecanoylphorbol-13-acetate; IHC, immunohistochemistry; PR, progesterone receptor; qRT-PCR, quantitative RT-PCR.
mPGES1 Is a KLF5 Target Gene

membrane glycerophospholipids by phospholipase A₂, (b) conversion of arachidonic acid to the unstable intermediate prostanoid prostaglandin H₂ (PGH₂) by cyclooxygenases, and (c) isomerization of PGH₂ to PGE₂ by terminal PGE₂ synthase (12). Intriguingly, the expression of mPGES1 is elevated in a number of human cancers, including breast cancer (13). Knockdown of mPGES1 in both the DU145 prostate cancer cell line and the A549 non-small cell lung cancer cell line inhibits xenograft tumor growth in nude mice (14). mPges1 knockout mice likewise exhibit a significant reduction in both the number and size of intestinal tumors induced by an Apc mutant (15). Accordingly, mPGES1 is an attractive novel drug target for cancer treatments.

The human mPGES1 gene promoter contains two GC boxes (16). Previous studies showed that inflammatory factors, such as TNFα (17), IL-1β (17–19), LPS (20), and 12-O-tetradecanoylphoribol-13-acetate (TPA) (18), induce mPGES1 expression. Several transcription factors, such as Sp1/3 (21), EGR1 (10, 22), CREB (cAMP-responsive element-binding protein) (20), C/EBPβ (CCAAT/enhancer-binding protein β) (19), and NF-κB (18, 23), have also been shown to regulate mPGES1 gene transcription. However, the transcriptional regulatory mechanism of mPGES1 in breast cancer has not been explored to date.

In this study, we hypothesized that mPGES1 is a KLF5 direct target gene that partially mediates KLF5 function in breast cancer. Our analysis showed that KLF5 manipulation regulates mPGES1 mRNA and protein expression in multiple breast cell lines. Luciferase reporter and ChIP experiments suggested that KLF5 directly binds to the mPGES1 proximal promoter, activating its transcription. Functionally, both KLF5 and mPGES1 similarly promote PGE₂ synthesis and breast cell proliferation. Finally, an immunohistochemistry (IHC) study in primary breast tumors demonstrated that the expression of mPGES1 is positively associated with ERα/progesterone receptor (PR)/HER2-negative status. These results provide the first evidence for the expression and functional coupling between KLF5 and mPGES1 in breast cancer cells.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection—The immortalized breast cell line MCF10A and breast cancer cell lines MCF7, BT20, Hs578T, and HCC1937 (American Type Culture Collection) were cultured according to the manufacturer’s protocols. All plasmids and siRNAs were transfected into different cell lines using Lipofectamine 2000 (Invitrogen) following the manufacturer’s instructions. The final siRNA concentration was 10 nm. The target sequences of KLF5 siRNA are as follows: KLF5 siRNA-#5, 5’-CGAUUCAGCGGAGAGCU-3’ and KLF5 siRNA-#1, 5’-AAGCUGAAGAGCU-3’. The target sequences of mPGES1 siRNA are as follows: mPGES1 siRNA-#1, 5’-CGCGUGCUAGCUAGAUA-3’ (reagent s18305) and mPGES1 siRNA-#2, 5’-ACCCUUUCUGGCU-3’ (reagent s18306). These siRNA were purchased from Invitrogen.

Antibodies and Western Blotting—Western blotting and anti-KLF5 antibody were described in our previous study (24). Anti-mPGES1 antibody was purchased from Cayman Chemical (catalog no. 10004350) and diluted 1000 times for Western blotting. Anti-p21Waf1/Cip1 antibody (12D1) was obtained from Cell Signaling (catalog no. 2947S), anti-p27Kip1 antibody was from BD Biosciences (catalog no. 610241), and anti-Survivin antibody was from Santa Cruz Biotechnology. The secondary antibody was diluted 10,000 times for all primary antibodies.

Semiquantitative RT-PCR and qRT-PCR—RT-PCR was used to measure the mRNA expression of KLF5 and mPGES1. The forward primer sequence for mPGES1 was 5’-ACCGCT-GCTGGTCTACGAAG-3’, and the backward primer sequence was 5’-AGGGCCACACCCACCATCTTGC-3’. The primer sequences for KLF5 and β-actin were described in our previous study (25).

SYBR Select Master Mix (catalog no. 4472908, ABI) was used for qRT-PCR in an ABI-7900 HT machine. The primers were same as for RT-PCR. qPCR on cDNA products was performed using the following parameters: pre-denaturation at 95 °C for 2 min and at 95 °C for 15 s and annealing at 60 °C for 1 min, running 40 cycles. The mRNA levels of KLF5 and mPGES1 were analyzed using the ΔΔCT method. GAPDH was used as the loading control.

Dual-Luciferase Assays—The mPGES1 proximal promoters (−789, −209, and −131 from the ATG codon) were amplified using normal human DNA as templates. PCR products were cloned into pGL3-Basic (Promega) and confirmed by DNA sequencing. MCF7 cells were seeded in 24-well plates at 1 × 10⁵ cells/well. The following day, the cells were transfected with the mPGES1 promoter reporter constructs (0.6 μg/well) and a pRL-β-actin internal control (5 ng/well) in triplicate. At 24 h after transfection, the cells were infected with a GFP control adenovirus and a KLF5 adenovirus (8) for 4 h. After transfection, the cells were infected with a GFP control adenovirus and a KLF5 adenovirus (8) for 4 h (~50% cells were infected under a fluorescence microscope). Luciferase activities were measured 24 h later using the Dual-Luciferase reporter assay system (Promega).

ChIP Assays—ChIP assay was performed using SW527 cells following a protocol provided by Abcam (Cambridge, MA). The diluted DNA-protein complex (25 μg of protein) was incubated overnight at 4 °C with different antibodies (rabbit anti-KLF5, rabbit IgG, and anti-H3K4me4, Abcam) in the presence of herring sperm DNA and protein A/G beads. PCR was performed using primers for the mPGES1 promoter region: 5’-GGACACCCCGAGCTCTGCTTC-3’ (forward) and 5’-CTCTGGCCAGCGCAGCTCAACTGTG-3’ (reverse).

IHC Staining—In total, 111 tumor samples (87 invasive ductal carcinomas and 24 invasive lobular carcinomas) were collected from the First and Third Affiliated Hospitals of the Kunming Medical University; their clinical and pathological parameters are listed in Table 1. The ages of the patients are from 29 to 82 years, with a median age of 48. The IHC antibodies used were anti-ERα (RMA-0501, Maixin), anti-PR (RMA-0502, Maixin), anti-HER2 (RMA-0555, Maixin), and anti-mPGES1. Tissue sections (4 μm thick) were first deparaffinized in xylene and rehydrated in graded ethanol. Following this process, antigen retrieval was completed by immersing slides in 10 mM citrate buffer (pH 6.0) at 120 °C for 8 min using an autoclave. Endogenous peroxidase was blocked with 45 ml of methyl alcohol and 5 ml of 30% hydrogen peroxide. After three washes with PBS, PBS with 2% BSA was used to block the nonspecific reaction for 1 h at room temperature. After three washes with
PBS, slides were incubated overnight at 4 °C with 1:100 diluted primary antibodies in 4% goat serum in 1/H11003 PBS with 0.2% Triton X-100. The slides were washed three times with PBS and subsequently incubated with biotinylated goat anti-rabbit secondary antibody at a dilution of 1:200 for 20 min, followed by three washes with PBS. We used 3,3'/H11032-diaminobenzidine as the chromogen to detect the signal. Slides were counterstained with hematoxylin for 3 min at room temperature, washed with running water, stained with 10–20 drops of ammonium hydroxide, washed with running water, cleaned with xylene, and mounted in Permount. The stained slides were scored independently by a pathologist and a technician. The study was approved by an institutional ethical committee.

Retrovirus Construction—pBABE-puro-mPGES1 was constructed by inserting the mPGES1 coding region into the pBABE-puro retroviral vector. The MCF10A and MCF7 cell populations expressing mPGES1 or the empty vector were generated according to a previously published protocol (26).

mPGES1 Enzyme Activity Measurement—The mPGES1 enzyme activity was measured by converting PGH2 to PGE2. The PGE2 concentration was measured following a previously published method (17). MCF7 or MCF10A cells were cultured in 24-well plates and then transfected with luciferase, KLF5, or mPGES1 siRNA. After 48 h, the cells were washed with 500 μl of PBS, and the medium was changed to 250 μl of fresh medium. Plates were then placed on ice, and 10 μM PGH2 (catalog no. 17020, Cayman Chemicals) was added. After 10 min, reactions were stopped by the addition of 25 μl of 400 mM FeCl2 and 4 mM citric acid. The same volume of acetone in which PGH2 was dissolved was also added for 10 min as a control. The concentration of PGE2 was measured by enzyme-linked immunosassay (catalog no. 514010, Cayman Chemical).

Cell Viability Assays—MCF10A and MCF7 cell proliferation and viability were measured by sulforhodamine B assay, as described previously (27).

Statistical Analysis—All data were analyzed using SPSS 13.0 (SPSS Inc., Chicago, IL). The luciferase and cell viability assays were conducted in triplicate and repeated at least three times. Where appropriate, data were pooled to generate means ± S.D. and analyzed by t test. Pearson’s chi-squared test was used to examine the correlation between mPGES1 expression and other clinicopathological parameters in primary tumors. For all tests, p < 0.05 was considered significant.

RESULTS

KLF5 Positively Regulates mPGES1 Expression in Breast Cancer at Both the mRNA and Protein Levels—In our previous study (3), KLF5 was found to be highly expressed in the immortalized breast epithelial cell line MCF10A and several ERα-negative breast cancer cell lines, including BT20 and MDA-MB-468, but expressed at low levels in MCF7 and Hs578T cells. When endogenous KLF5 was knocked down in MCF10A and BT20 cells by KLF5 siRNA (8), the mPGES1 mRNA levels were down-regulated in both cell lines as determined by Western blotting. Luciferase siRNA was used as the negative control. KLF5 knockdown by siRNA reduced the mPGES1 protein level in MCF10A cells as determined by Western blotting. Luciferase siRNA was used as the negative control, and β-actin was used as the loading control. In addition, KLF5 overexpression by an adenovirus in both MCF7 and Hs578T cells (two breast cancer cell lines with low expression of KLF5) dramatically increased the mPGES1 mRNA levels.
compared with the GFP control as determined by RT-PCR (Fig. 1B).

To test whether endogenous KLF5 ultimately regulates mPGES1 protein expression, we knocked down KLF5 in MCF10A cells and found that KLF5 depletion dramatically decreased the mPGES1 protein level in these cells. Consistent with this result, KLF5 overexpression increased the mPGES1 protein level in MCF7 cells (Fig. 1C). Additionally, both KLF5 and mPGES1 proteins were found to be coexpressed in three ERα-negative breast cell lines (MCF10A, BT20, and MDA-MB-468) by Western blotting (Fig. 1D). In contrast, both KLF5 and mPGES1 proteins were not detectable in two ERα-positive breast cancer cell lines (MCF7 and T47D) (Fig. 1D).

KLF5 Is Essential for Phorbol Ester (TPA) to Induce mPGES1 Expression—To further test whether KLF5 induces mPGES1 expression, we treated the ERα-positive and KLF5-negative breast cancer cell line MCF7 with TPA, a drug that induces KLF5 mRNA expression (3), thereby stimulating the mPGES1 promoter (10) and increasing PGE₂ production (28). The dosage and time course experiments indicated that both KLF5 and mPGES1 mRNA levels were up-regulated by TPA (Fig. 2, A and B). We further demonstrated that TPA failed to induce mPGES1 mRNA expression when KLF5 induction was blocked by KLF5 siRNA (Fig. 2C), suggesting that KLF5 is necessary for mPGES1 mRNA induction by TPA and that mPGES1 could be a KLF5 downstream target gene.

KLF5 Promotes mPGES1 Gene Transcription by Directly Binding to the mPGES1 Promoter—The human mPGES1 gene is located at chromosome 9q34.3, spanning ~15 kb divided into three exons. The human mPGES1 gene proximal promoter contains two CACCC and two GC boxes (10). To test whether KLF5 transcription factor activates the mPGES1 promoter, we generated mPGES1 gene promoter-luciferase reporter plasmids based on pGL3-Basic. KLF5 was overexpressed upon adenovirus infection. pGL3-Basic was used as the negative control. The β-actin promoter-driven Renilla luciferase gene (pRL-β-actin) was used as the internal control. **, p < 0.01 (t test). E, KLF5 specifically binds to the mPGES1 promoter in vivo as determined by ChIP assays. The input DNA and water were used as positive and negative controls. Anti-H3K4me antibody was used as a positive control for ChIP because trimethylated Lys-4 of histone H3 is associated with active gene transcription (56). The pre-bleed sera from the same rabbit used for generating anti-KLF5 antibody and rabbit IgG (RigG) were used as negative controls.
in which both KLF5 and mPGES1 are highly expressed (data not shown), and found that anti-KLF5 antibody, but not control IgG, specifically immunoprecipitated the mPGES1 gene promoter (Fig. 2E).

Both KLF5 and mPGES1 Promote Breast Cell Proliferation and PGE\(_2\) Production—KLF5 has been well established to promote breast cell proliferation (3). However, it is unclear if mPGES1 has a similar function, so we knocked down both KLF5 and mPGES1 using two different siRNAs in MCF10A cells and measured protein expression and cell viability. As expected, depletion of either KLF5 or mPGES1 significantly decreased the expression of target proteins (Fig. 3A) and cell viability in MCF10A cells (Fig. 3B) compared with luciferase siRNA. Likewise, depletion of either KLF5 or mPGES1 significantly decreased HCC1937 cell viability (Fig. 4, D and E).

Because mPGES1 is predominately responsible for the production of PGE\(_2\) from PGH\(_2\), we sought to measure PGE\(_2\) production after manipulation of KLF5 and mPGES1. As expected, depletion of either KLF5 or mPGES1 significantly decreased PGE\(_2\) production from PGH\(_2\) in MCF10A cells during the course of a 6-day treatment, especially on the day 4 (Fig. 3C).

In our previous study (3), KLF5 overexpression in MCF7 cells promoted cell proliferation. Subsequently, we were curious about whether mPGES1 overexpression also promotes cell proliferation. Here, we found that stable overexpression of mPGES1 in MCF7 cells (Fig. 3D) significantly promoted cell growth during a time course experiment (Fig. 3E). KLF5 or mPGES1 overexpression in MCF7 cells also significantly increased PGE\(_2\) production from PGH\(_2\) (Fig. 3F).

mPGES1 Overexpression Partially Rescues KLF5 Depletion-induced Gene Expression Changes and Growth Arrest—KLF5 has previously been shown to inhibit the expression of the cell cycle-dependent kinase inhibitors p21 (6) and p27 (8) and to increase the expression of Survivin (7). mPGES1 has also been reported to inhibit p21 and p27 expression in hepatocellular carcinoma cells (29). As shown in Fig. 4A, knockdown of either KLF5 or mPGES1 by siRNA in MCF10A cells elevated p21 and p27 protein levels while simultaneously decreasing Survivin protein levels. Similar results were observed in HCC1937 cells (Fig. 4D). To test whether KLF5 regulates the expression of p21, p27, and Survivin partially through mPGES1, we performed a rescue experiment in MCF10A cells and noted that mPGES1 overexpression partially rescued the KLF5 depletion-induced p21 and p27 increase and Survivin decrease (Fig. 4B). Consistently, KLF5 depletion decreased MCF10A cell viability to 62.9% compared with the luciferase control siRNA. Forced overexpression of mPGES1 partially, although significantly, elevated the cell viability percentage to 75.8% (Fig. 4C). These results support our hypothesis that mPGES1 partially mediates the pro-proliferative function of KLF5 in breast cells.

mPGES1 Protein Expression Is Associated with Triple-negative Breast Tumors—We performed IHC staining for mPGES1, ER\(_\alpha\), PR, and HER2 in 111 primary breast tumors obtained from Chinese patients. ER\(_\alpha\), PR, and HER2 were positive in 56, 55, and 27% of the breast tumors, respectively (Table 1). IHC staining revealed that the expression of mPGES1 was detected in 41% of the tumors examined (Table 1). mPGES1 expression was not correlated with ER\(_\alpha\), PR, or HER2 status. However, the expression of mPGES1 positively correlated with ER/PR/HER2 triple-negative breast tumors (\(p = 0.032\), Pearson’s chi-squared test) (Fig. 5 and Table 1).

DISCUSSION

KLF5 has been suggested as an oncogene in several carcinomas, including colon (30), intestinal (31), esophageal (32), bladder (8), and breast (3). Higher KLF5 mRNA and protein expression is associated with a short survival time in breast cancer patients (33, 34). Our previous studies demonstrated that KLF5 promotes MCF7 and HCC1937 breast cancer cell growth in vitro and in vivo (3, 35). In this study, we showed several lines of evidence indicating that mPGES1 is a KLF5 direct target gene that partially mediates the pro-proliferative function of KLF5 in breast cancer cells. First, in multiple breast cell lines, KLF5 tightly controlled mPGES1 mRNA and protein expression, and both KLF5 and mPGES1 were correlativey expressed (Fig. 1). Without KLF5 induction, TPA failed to induce mPGES1 mRNA expression (Fig. 2). Second, KLF5 bound to the mPGES1 promoter in vitro and activated the mPGES1 gene promoter in vitro (Fig. 2). Additionally, KLF5 and mPGES1 regulated in parallel p21, p27, and Survivin gene expression; PGE\(_2\) production; and cell proliferation (Figs. 3 and 4). Moreover, mPGES1 overexpression partially reversed the KLF5 depletion-induced p21, p27, and Survivin gene expression changes and growth arrest (Fig. 4). Aside from these results, both KLF5 and mPGES1 have previously been reported to promote cell proliferation, cell survival, tumor growth, and angiogenesis (36, 37). Taken collectively, these results led us to conclude that KLF5 promotes breast cell proliferation, at least partially, by inducing mPGES1 gene transcription.

Here, we demonstrated that KLF5 is a critical transcription factor positively regulating mPGES1 transcription through binding to the mPGES1 proximal promoter. KLF5 expression can be induced by a variety of oncogenic and proinflammatory factors, such as LPS (38), TNF\(_\alpha\) (39), TPA (3, 40), and so on. These proinflammatory stimuli also induce mPGES1 (10, 17, 41). We also showed that KLF5 is essential for the induction of mPGES1 by TPA (Fig. 2). These findings imply that both KLF5 and mPGES1 may function in the same signaling pathways. The mPGES1 promoter contains two proximal GC boxes (10), to which Sp1, Sp3, KLF11, and EGR1 transcription factors can bind and regulate gene transcription (10, 22). As a member of the Sp/KLF family, KLF5 also binds to GC boxes through its three zinc finger domains. Interestingly, EGR1 was previously suggested to induce KLF5 expression (42, 43). Two other mPGES1 transcription factors, C/EBP\(\beta\) (19) and NF-\(\kappa\)B (18, 23), were shown to be KLF5 cofactors (40, 44). However, how these transcription factors coordinately regulate mPGES1 gene transcription needs further investigation.

PGE\(_2\) is the most common prostanoid, possessing a variety of bioactivities implicated in several inflammatory pathologies, including cancer. The PGE synthases convert PGH\(_2\) to PGE\(_2\) and include three different enzymes: mPGES1, mPGES2, and cytosolic PGES (45). Only mPGES1 is induced by proinflammatory signals, coupling with COX-2 to produce PGE\(_2\) in several cancers (46). COX-2 overexpression was previously reported to correlate with invasive breast cancer (47). Forced overexpres-
sion of COX-2 likewise induces mammary tumorigenesis in multiparous mice through a PGE2-EP2 pathway (48), whereas inhibition of COX-2 significantly suppresses mammary tumorigenesis in rats treated with 7,12-dimethylbenzanthracene and N-methyl-N-nitrosourea (49, 50). However, the expression of mPGES1 may be independent of COX-2 (13, 51). Therefore, mPGES1 is a promising therapeutic target, and several mPGES1 inhibitors have already been developed (52–54). Our

**FIGURE 3.** Both KLF5 and mPGES1 promote breast cell proliferation and PGE2 production. A, knockdown of endogenous KLF5 and mPGES1 protein expression by two different siRNAs (KLF5si and mPGES1si) in MCF10A cells was detected by Western blotting. Luciferase siRNA (Lucsi) was used as the negative control. Mock, no siRNA. B, knockdown of endogenous KLF5 or mPGES1 by two different siRNAs significantly decreases MCF10A cell viability in a 6-day time course experiment. *, p < 0.05; **, p < 0.01 (t test). C, knockdown of endogenous KLF5 or mPGES1 by siRNA significantly decreases PGE2 production in MCF10A cells. *, p < 0.05; **, p < 0.01 (t test). D, transient overexpression of KLF5 or stable overexpression of mPGES1 protein in MCF7 cells was detected by Western blotting. GFP and pBABE were used as negative controls. E, stable overexpression of mPGES1 in MCF7 cells increases cell viability in a 6-day time course experiment. *, p < 0.05; **, p < 0.01 (t test). F, overexpression of either KLF5 or mPGES1 increases PGE2 production in MCF7 cells. *, p < 0.05 (t test).
results are consistent with these earlier findings, suggesting that depletion of mPGES1 significantly inhibits breast cell growth.

A previous study showed the mPGES1 expression was detected in 79% of breast tumors, but its expression did not correlate with any pathological biomarkers (13). In this study, we found that mPGES1 protein expression was detected in only 41% of tumors and did not correlate with the status of ERα, PR, and HER2. However, mPGES1 expression significantly and positively correlated with the ERα/PR/HER2 triple-negative status. We do not know if mPGES1 expression is associated with patient survival or not because the clinical follow-up for the tumor samples has not been long enough. KLF5 mRNA expression is positively associated with short survival, HER2,
and Ki67 and is negatively correlated with the age of the patients at diagnosis (33). An IHC study using rabbit anti-KLF5 polyclonal antibody (sc-22797, Santa Cruz Biotechnology) in 60 breast cancer patients showed that the expression of KLF5 was increased in breast tumors and that positive KLF5 staining was associated with high tumor grades (55). Another IHC study using mouse anti-KLF5 monoclonal antibody (Santa Cruz Biotechnology) in 113 breast cancer patients suggested significant correlations between KLF5 immunoreactivity and the androgen receptor, increased risks of recurrence, lymph node metastasis, and disease-free survival. We did not examine KLF5 protein expression in this study because several anti-KLF5 antibodies showed high background (data not shown). According to our cell line study (3), KLF5 appears to be highly expressed in basal-like triple-negative breast cancers. Nevertheless, mPGES1 may be a prognostic biomarker for triple-negative breast cancers.

In summary, we have demonstrated that KLF5 tightly controls mPGES1 gene transcription by binding to the mPGES1 gene promoter in breast cells. mPGES1 partially mediates the proliferative function of KLF5, and the expression of mPGES1 positively correlates with triple-negative breast cancers. These findings may provide a rationale for developing mPGES1 as a breast cancer diagnosis biomarker and therapeutic target.

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