12-O-Tetradecanoylphorbol-13-acetate Promotes Breast Cancer Cell Motility by Increasing S100A14 Level in a Kruppel-like Transcription Factor 4 (KLF4)-dependent Manner*

Received for publication, November 13, 2013, and in revised form, February 14, 2014. Published, JBC Papers in Press, February 14, 2014, DOI 10.1074/jbc.M113.534271

Huan He 1, Sheng Li 1, Hongyan Chen, Lin Li, Chengshan Xu, Fang Ding, Yun Zhan, Jianlin Ma, Shuguang Zhang, Yaoting Shi, Chunfeng Qu, and Zhihua Liu* 2

From the State Key Laboratory of Molecular Oncology, Cancer Institute and Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021, China

Abstract

Background: The transcriptional regulation of S100A14 and its underlying mechanism have not been fully elucidated.

Results: The activation of S100A14 promoter by KLF4 mediates its up-regulation upon TPA stimulation.

Conclusion: S100A14 partly mediates TPA-induced cell motility in a KLF4-dependent manner.

Significance: KLF4 plays a significant and previously unrecognized role in regulation of S100A14.

The S100 protein family represents the largest subgroup of calcium binding EF-hand type proteins. These proteins have been reported to be involved in a wide range of biological functions that are related to normal cell development and tumorigenesis. S100A14 is a recently identified member of the S100 protein family and differentially expressed in a number of different human malignancies. However, the transcriptional regulation of S100A14 and its role in breast cancer needs to be further investigated. Here, we determined that 12-O-tetradecanoylphorbol-13-acetate (TPA) up-regulated the expression of KLF4 and facilitated its binding directly to two conserved GC-rich DNA segments within the S100A14 promoter, which is essential for the transactivation of KLF4 induced S100A14 expression. Furthermore, stable silencing of KLF4 significantly suppressed breast cancer cell migration induced by TPA. Collectively, these results offer insights into the fact that TPA provokes cell motility through regulating the expression and function of S100A14 in a KLF4-dependent manner.

The family of the S100 proteins includes more than 20 calcium-binding proteins belonging to the EF-hand superfamily, which manipulates their influence on multitude vital cellular processes through interacting with specific target proteins and modulating their subcellular translocalization (1–3). The expression of an individual S100 protein displays specific patterns of cellular and developmental distribution, and changes in S100 protein expression are usually depending on the state of cellular proliferation, differentiation, or transformation and precisely regulated under a variety of conditions (4). For instance, in vitro studies of S100A8 and S100A9 with myeloid and endothelial cells revealed strong induction of transcription by numerous proinflammatory cytokines, such as TNF-α and IL-1 (5, 6), whereas in keratinocytes, enhanced basal and TPA-induced S100A8 and S100A9 mRNA levels in the skin of fos-deficient animals suggested that the transcription of both genes is negatively controlled by the subunit of AP-1 (7, 8). Recent study indicates that the extent of DNA methylation in the S100 gene regulatory regions is also decisive in the regulation of S100 gene expression both in development and during malignant transformation (9).

S100A14 is a member of the family involved in several functional and pathological processes and is predicted to be under tight transcription and post-translational regulation (10, 11). Loss of S100A14 expression has been illustrated in tumors of the colon, rectum, kidney, oral, and esophageal squamous cell carcinoma and small intestinal adenocarcinoma, whereas up-regulation of S100A14 has been documented in a variety of tumors, including ovarian, breast, lung, and uterine tumors (11–17). Recent studies substantiate that S100A14 induces cell cycle arrest, apoptosis, or metastasis in oral and esophageal squamous cell carcinomas and regulates these processes in a p53-dependent or receptor for advanced glycation end products (RAGE)-dependent manner (12, 18–20). In addition, S100A14 is up-regulated in basal type breast cancer and significantly associated with patient outcome (15). These results suggest that the expression and functional role of S100A14 in malignant tumors is organ-specific. However, the context-specific transcriptional regulation of S100A14 varying in different tumors has not been fully elucidated. In the present study, we sought to dissect the molecular mechanism by which the expression of S100A14 might be regulated in the breast cancer. Moreover, we evaluated the role of a context-related transcriptional factor KLF4 in the transcriptional regulation of S100A14.
KLF4 is a zinc finger protein of the Kruppel-like factor family, which are zinc finger transcription factors defined by containing a composition of three Cys2/His2 zinc fingers. It is involved in the regulation of cell survival, proliferation, differentiation, development, and inflammation and plays significant roles in stem cell function (21–26). KLF4 regulates responsive promoter via either binding to its canonical GC box (27), as well as to the CACCC DNA motif (28), or functioning as a modulator of chromatin acetylation (29). KLF4 has been reported to have a dual function as a tumor suppressor or an oncogene in a context-specific manner and can cooperate with or compete with other factors to either activate or repress various target promoters. Recent researches demonstrated that the “epidermal differentiation complex” protein expression, which includes the S100A family, is regulated in a gene- and tissue-specific manner by KLF4 (30). Furthermore, S100 was found to be upregulated in KLF4-overexpressing neuroblastoma cells (24). Although the transcriptional regulation between S100 proteins and KLF4 is of particular interest, the mechanism by which KLF4 activates the expression of S100A14 remains to be further deciphered.

In this study, we confirmed that TPA-induced cell type-specific expression of S100A14 in a time- and dose-dependent manner. Computer-aided transcription factor-binding site analysis identified two consensus CACCC motifs in the promoter of S100A14. Further analysis by ChIP and luciferase reporter assay indicates that KLF4 can specifically bind to the CACCC motif in the promoter region of S100A14, which is required for KLF4 to transactivate S100A14. Stable silencing of KLF4 or klf4−/− MEF cells substantially attenuated the effect of TPA on the modulation of S100A14. Knockdown of KLF4 expression impaired TPA-mediated migration. Analyzing gene expression profiling and clinical outcome data from published studies revealed that the expression level of S100A14 in human breast cancer is inversely correlated with metastasis-free survival, and it is significantly associated with KLF4 in both breast cancer cell lines and clinical breast cancer samples.

**EXPERIMENTAL PROCEDURES**

**Reagents, Cell Culture, Transfection, RNA Interference, and Mice**—TPA was purchased from Cell Signaling Technology (Danvers, MA). Staurosporine was purchased from Jiamay Biotech (Beijing, China). MCF7, HCT116, HEK293T, mouse embryonic fibroblast (MEF) cells generated from wild-type or klf4−/− homozygous mice (21) and MDA-MB-231 cells were maintained in DMEM and L15 supplemented with 10% fetal bovine serum and antibiotics. To delete exons 2 and 3 of KLF4 in embryonic stem cells, we crossed floxed klf4 (21) obtained from the Missouri Mutant Mouse Regional Resource Center (Columbia, MO) to CMV-cre (Nanjing Biomedical Research Institute of Nanjing University, Nanjing, China) mice. Transfection and establishment of stable cell lines were performed as described previously (22).

**Plasmid Construction and Site-directed Mutagenesis**—DNA fragments of the KLF4 and S100A14 cDNA coding regions were cloned into the mammalian expression vectors pcDNA3.1 and pcDEF. The wild-type promoter region construct of S100A14 (−511 to +6 bp), designed as P1, was described previously (12).

**Three point mutations were introduced into each target site by mutagenesis PCR. The resulting construct was verified by direct sequencing.**

**RNA Isolation and PCR Analysis**—RNA purification and real time RT-PCR were performed as described previously (22). The primers used are listed in Table 1.

**Chromatin Immunoprecipitation Assay**—ChIP was performed as described previously (22). The antibody used was anti-KLF4 from Santa Cruz Biotechnology (sc-20691; Santa Cruz, CA).

**Western Blot Analysis**—Western blots were performed as described previously (22). Antibodies used were anti-KLF4 (sc-20691; Santa Cruz Biotechnology) and anti-S100A14 (gifts of Dr. Iver Petersen, University Hospital Charité, Berlin, Germany, and Dr. Youyong Lü, Beijing Cancer Hospital and Institute, Beijing, China).

**Luciferase Assay**—The luciferase assay was performed as described previously (22).

**Wound Healing Assay**—Cells were seeded in the chambers of the culture dish for 24 h, then a yellow pipette tip was used to make a straight scratch, and fresh culture medium was added to start the migration process. Pictures were acquired at 0 and 24 h.

**Migration Assays**—Cell motility ability was analyzed using real time cell analysis (RTCA). Briefly, cells were starved in serum-free medium for 24 h and added to the top chamber of RTCA CIM-16 plates (xCELLigence Roche, Penzberg, Germany) at the desired density in serum-free medium. Full growth medium was used as a chemoattractant in the lower chamber. Migration is monitored in a time-resolved manner using the RTCA device. Cell motility ability analyzed by 24-well Boyden chambers was described as previously (31).

**Statistical Analysis**—We statistically evaluated experimental results using two-independent sample t test, one-way analysis of variance test, and Pearson correlation analysis. The Kaplan-Meier method was used to calculate the survival rates and was evaluated by the log rank test. All other data were expressed as the means ± S.D. A value of P less than 0.05 was considered to be statistically significant.

**RESULTS**

**TPA Indirectly Up-regulates Levels of S100A14 mRNA and Protein Expression**—Previous studies indicated that S100 proteins are commonly up-regulated in a TPA-induced carcino-

**TABLE 1**

<table>
<thead>
<tr>
<th>RT-qPCR primers</th>
<th>Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human KLF4 mRNA F</td>
<td>CGAGGACCAAGCAAGGAGAA</td>
</tr>
<tr>
<td>KLF4 mRNA R</td>
<td>TCCGGAGCTCTGTCATTCC</td>
</tr>
<tr>
<td>S100A14 mRNA F</td>
<td>ACCCTCCACAGGATCTGCA</td>
</tr>
<tr>
<td>S100A14 mRNA R</td>
<td>CGAGGAGCTCTGACGAGGC</td>
</tr>
<tr>
<td>GAPDH F</td>
<td>GTCGAGATCCGAGGATGTTG</td>
</tr>
<tr>
<td>GAPDH R</td>
<td>AAGAGCAGCGCAGGTGAGCC</td>
</tr>
<tr>
<td>Mouse KLF4 mRNA F</td>
<td>CAGGCTGAAGATTGCTGCC</td>
</tr>
<tr>
<td>KLF4 mRNA R</td>
<td>AGGGCAAGAGGGAGAAGAG</td>
</tr>
<tr>
<td>S100A14 mRNA F</td>
<td>GAGGUUCAATGAGGCTTCC</td>
</tr>
<tr>
<td>S100A14 mRNA R</td>
<td>AGGGCAAGAGGGAGAAGAG</td>
</tr>
<tr>
<td>GAPDH F</td>
<td>CTAGGAGGAGGTAGGCTCC</td>
</tr>
<tr>
<td>GAPDH R</td>
<td>CTAGGAGGAGGTAGGCTCC</td>
</tr>
</tbody>
</table>
To examine whether TPA influences S100A14 activity, we first analyzed the expression of S100A14 in MCF7 cells treated with TPA. RT-qPCR and Western blot results clearly showed that TPA induced the expression of S100A14 (Fig. 1, A and B). We subsequently examined the expression of S100A14 by different dosage of TPA treatments ranging from 5 to 100 ng/ml, and a dose-dependent stimulation of S100A14 expression was observed (Fig. 1C). To explore whether the increase of S100A14 mRNA levels triggered by TPA treatment was linked to post-transcriptional regulation, we then measured the half-life of S100A14 mRNA by incubating cells with actinomycin D to block de novo gene transcription. Quantitative RT-PCR analysis revealed that the mRNA stability of S100A14 was not influenced by TPA treatment (data not shown). To determine whether it is a direct link between TPA and S100A14 expression, we performed experiments using cycloheximide to block de novo protein translation to study the expression of S100A14 in MCF7 cells. As shown in Fig. 1D, TPA-induced S100A14 expression was influenced by cycloheximide treatment, suggesting that S100A14 is indirectly induced by TPA, and this regulation requires de novo protein synthesis. These results indicate that TPA can indirectly induce S100A14 expression by a transcriptional mechanism.

**TPA Regulates S100A14 Required KLF4 Activation**—Previous studies showed that TPA directly induces KLF4 expression in a PKCδ-dependent manner in keratinocytes (32, 33), to clarify this we tested KLF4 expressions in MCF7 cells treated with or without TPA. RT-qPCR and Western blot results clearly showed that TPA-induced up-regulation of KLF4 was dose- and time-dependent (Fig. 2A), suggesting that KLF4 may involve in TPA-induced S100A14 up-regulation. Additionally, the induction of KLF4 and S100A14 by TPA was blocked by cotreatment with the PKC antagonist staurosporine (Fig. 2B), suggesting that this induction is PKC-dependent. To examine the interplay between KLF4 and S100A14, we performed experiments using HA-KLF4 or KLF4 siRNA to study S100A14 in MCF7 cells. As shown in Fig. 2C, the expression of S100A14 was up-regulated with the overexpression of KLF4 and down-regulated in the absence of KLF4. The expression of S100A14 induced by TPA and KLF4 had also been detected in other breast cancer cells such as T47D and SKBR3 and colorectal cancer cells HCT116 (data not shown). Furthermore, we measured the mRNA level of S100A14 in klf4-null or wild-type MEFs. In line with the KLF4 knockdown results, S100A14 significantly decreased in klf4-null MEFs (Fig. 2D). These results indicated that KLF4 may involve in TPA-induced S100A14 up-regulation.

**Up-regulating S100A14 upon TPA Activation Is Mediated through KLF4 Binding Sites**—KLF4 regulates responsive promoter via binding to its canonical GC and CACCC boxes...
located in the promoter regions in the target genes (27, 28). To further determine the direct regulation of S100A14 by KLF4, we searched for putative KLF4-binding sites on the human S100A14 promoter. Remarkably, we identified three potential KLF4-binding sites at /H11002 to /H11002 bp, /H11002 to /H11002 bp, and /H11002 to /H11002 bp upstream of the S100A14 ATG codon, respectively. Moreover, the last two of the putative CACCC sites were highly conserved in the human and mouse DNA (Fig. 3A), suggesting that S100A14 mRNA might be a direct target of KLF4.

To confirm whether the putative KLF4-binding sites were involved in transactivation, we next used S100A14 gene promoter reporter constructs containing either three wild-type putative KLF4-binding sites (P1) or mutations of each site. These constructs were cotransfected with or without FLAG-KLF4, and then luciferase activity was determined. As shown in Fig. 3B, a 2-fold increase of P1 and M1 (−293 to −289 bp site mutation) cotransfected with FLAG-KLF4 was observed, indicating that KLF4 activates S100A14 activity and the first putative binding site has no activity. In contrast, no significant activation of M2 (−124 to −120 bp site mutation), M3 (−75 to −71 bp site mutation) or M4 (both −124 to −120 bp and −75 to −71 bp site mutation) was observed, suggesting that KLF4 activates S100A14 through the last two conserved KLF4-binding sites.

Stable Silencing of KLF4 or S100A14 Attenuates the Effect of TPA on the Modulation of S100A14—To determine whether the TPA-induced S100A14 increase was KLF4-dependent, we measured the expression of KLF4 and S100A14 in MCF7 cells stably silenced with two KLF4 short hairpin RNAs or control short hairpin RNA. As shown in Fig. 4 (A and B), shRNA markedly inhibited KLF4 expression following TPA treatment. The
The response of S100A14 to TPA was determined by real-time RT-PCR and Western blotting, comparing KLF4-silencing cells to control-silencing cells. As expected, TPA treatment resulted in an up-regulation of S100A14 and KLF4 in control-silencing cells (Fig. 4, A and B). Ablation of KLF4 expression significantly compromised the effect of TPA on the expression of S100A14 (Fig. 4, A and C), suggesting that TPA-induced S100A14 increase was KLF4-dependent. In additional experiments, similar results were observed in S100A14-silencing cells (Fig. 4D). Taken together, these results indicated that elimination of KLF4 or S100A14 attenuated the effect of TPA on the modulation of S100A14.

**TPA Treatment or Ectopic Expression of KLF4 or S100A14 Promotes Breast Cancer Cell Motility**—To study the role of TPA in cell motility, we first assessed the metastasis capacity of MCF7 breast cancer cells after TPA treatment in real-time and in end-point migration assays using the RTCA and wound-healing assay, respectively. Consistent with early observations, TPA treatment strongly promoted the migration of the MCF7 cells in both assays (Fig. 5, A, upper panel, and B, left panel), without affecting the viability of the cells (Fig. 5C). To determine the role of KLF4 and S100A14 in cell motility, we assessed migration capacity of MDA-MB-231 breast cancer cells ectopic expression of KLF4 or S100A14. Similarly, overexpression of KLF4 (data not shown) or S100A14 also promoted migration of MDA-MB-231 cells in both assays (Fig. 5, A, lower panel, and B, right panel). Taken together, these results indicated that TPA treatment or ectopic expression of KLF4 or S100A14 impinged on cell migration in breast cancer cells.

**Ablation of KLF4 or S100A14 Expression Reduces TPA-mediated Migration in Breast Cancer Cells**—To assess whether targeting of KLF4 could contribute to TPA-induced phenotype,
we next investigated whether individual ablation of KLF4 or S100A14 would inhibit the effects of TPA-induced cell migration. Indeed, individual silencing of KLF4 or S100A14 resulted in a decrease in migration of the MCF7 cells, not only in the basal condition, but also when cells were treated with TPA as measured by RTCA, Transwell (Figs. 6A and 7A), and wound healing assay (Figs. 6B and 7B), without affecting the viability of the cells (Fig. 6C). As shown in Fig. 6B, TPA treatment promoted cell migration by >30% in the control silencing cells, as opposed to 10% in the KLF4 silencing cells (Fig. 6A). The difference is statistically significant (p < 0.001). In additional experiments, similar results were observed in S100A14 silencing cells (Fig. 7B). When taken together with the KLF4 and S100A14 silencing data, our results showed the important role of the KLF4-S100A14 signaling pathway in mediating the effect of TPA on breast cancer cell migration.

**KLF4 and S100A14 Are Correlated in Breast Cancer Cell Lines and Patients, and Their Expression Levels Are of Prognostic Value for Breast Carcinoma Patient Survival**—To evaluate the up-regulation of KLF4 on S100A14 expression in breast cancer, we examined the mRNA levels of KLF4 and S100A14 in breast cancer cell lines and patients. Pairwise correlation analysis showed a statistically significant positive correlation between KLF4 and S100A14 in two independent data sets (Fig. 8, A and B). To investigate the role of KLF4/S100A14 in breast cancer progression, we first analyzed gene expression profiling and clinical outcome data from published studies for correlations between KLF4/S100A14 expression levels and metastasis-free survival (Fig. 8C). Patients were divided into two equal groups, high and low, based on the median S100A14 expression level. Kaplan-Meier survival analyses in combined four data sets demonstrated that the expression of S100A14 exquisitely and inversely correlated with metastasis-free survival. Interestingly, further stratification of patient groups based on positive KLF4/S100A14 expression improves the predictive capability of S100A14. It strikingly supports a potential role for KLF4/S100A14 in breast cancer metastasis and its predictive capability for prognosis.

**DISCUSSION**

The effort of reducing morbidity and mortality of cancer putatively depends on validating novel biomarkers that favor in the early detection and the accurate prediction of tumor behavior. The S100 family is one group of proteins that has a potentially important role in a clinical setting in the diagnosis and therapeutic monitoring of multiple tumor types (34). Cumulative literatures have documented that 21 of the human S100 genes (S100A1–S100A18, trichohyalin, filaggrin, and repetin)
clustered at the chromosome region 1q21, frequently deleted, translocated, or duplicated in epithelial tumors and tumors of soft tissues (1, 3, 8, 35). The expression of S100 proteins is tissue- and cell-specific and is altered in many tumors, often in association with tumor progression. S100 proteins are commonly up-regulated in tumors. However, S100A2, S100A9, S100A11, and S100A14 have been substantiated as tumor suppressors in some cancers but as oncogenes in others (34). This indicates that the functions of the S100 family are complex and variable. Therefore, the precise expressional regulation of S100 proteins in tumor progression has not been thoroughly examined to date. Here, we have further delineated a previously unrecognized mechanism by which TPA can modulate the expression of S100A14 in a KLF4-dependent manner. Previous studies indicated that in TPA-induced multistage skin carcinogenesis model, S100A8 and S100A9 were massively and coordinately up-regulated in the infiltrating inflammatory cells as well as differentiating epithelia keratinocytes (7). In line with this, we have shown that TPA substantially invigorated S100A14 mRNA and protein levels in a time-dependent (Fig. 1, A) and dose-dependent (Fig. 1C) manner. Previous studies showed the rapid and tight association of cytosolic calcium, phospholipid-dependent protein kinase activity with the plasma membrane was an early event in mediating some of the effects of TPA (36). TPA enhanced the release of death receptor ligands and the activation of the extrinsic apoptotic cascade, which resulted in the apoptosis of prostate cancer cells via allostERIC activation of PKC (37, 38). It has also been proposed that PKCa, a known upstream element of the MAPK signaling pathway, was involved in the TPA-induced activation and release of the calcium-binding proteins S100A8 and S100A9 (7, 39). Here, we have shown that TPA-induced S100A14 expression was ablated in the presence of staurosporine, a PKC antagonist, suggesting the PKC-dependent manner of TPA-induced S100A14 expression (Fig. 2B). Furthermore, increased gene transcription rather than suppressed mRNA turnover is involved in the increase of S100A14 in response to TPA treatment because our data show that the mRNA stability of S100A14 was not influenced upon TPA application by incubating cells with actinomycin D (data not shown). However, we found that this regulation is indirect, because TPA induced the expression of S100A14, influenced by coincubation with cycloheximide, implicating that there is de novo protein synthesis required (Fig. 1D). Next, we explore which transcription factor is involved in regulating S100A14 expression in a PKC-dependent manner. With computer-aided transcription factor-binding site analysis, we identified three CACCC motifs located at −511 to +6 bp upstream of the S100A14 ATG codon, and the last two of them are highly
Conserved among different species (Fig. 3A). The CACCC motif is one of the most common regulatory elements widely distributed in promoters, enhancers, and was generally regarded as the transcription factor SP1/KLF family functional binding elements (40, 41). Luciferase assay unequivocally demonstrated that KLF4 transactivates S100A14 by binding the two proximal CACCC motifs (Fig. 3B); ChIP assay also confirmed the specificity of the KLF4-binding sites (Fig. 3C). A significant positive correlation between KLF4 and S100A14 expression analyzed by gene expression profiling data from published studies also confirmed the observations (Fig. 8, A and B). Accumulating evidence showed that KLF4 is one of the downstream targets of the
PKC-MAP kinase-signaling pathway in many tissues. PKC increased KLF4 expression and promoted its interaction with the GC-rich DNA elements in the proximal p21Cip1, which activate p21Cip1 gene expression leading to the inhibition of keratinocyte proliferation (32). In addition, in differentiating keratinocytes, PKC drives involucrin gene transcription through promoting KLF4 expression (33). On the other hand, PKC phosphorylated KLF4 at Thr401, which mediated Sp1 dissociating from KLF4-Sp1 complex on TGF-β/1 activating and suppressed the expression of target gene (42). These results implicated that the mechanism and function of KLF4 are regulated by PKC in a complex and variable manner. Here, we confirmed that the PKC specific activator TPA increased both KLF4 mRNA and protein level and enhanced KLF4 binding to the CACCC motif in the S100A14 proximal promoter (Figs. 2A and 3C). In addition, KLF4 deletion blunted TPA-induced expression of S100A14 (Fig. 4, A and C), which evidently shows that KLF4 is involved in the increase of S100A14 by TPA.

TPA has been reported to reduce the lung metastasis and growth of melanoma cells through suppressing MMP-9 expression via the inhibition of NF-κB-ERK1/2 or AP-1/FAK signaling pathway (43, 44). Conversely, TPA mediated the motility of lung cancer cells through activating Rac signaling (45). Therefore, TPA modulated the motility of cancer cells through diverse signaling pathway and in a context-specific manner. Here, we have illustrated that the enhanced expression of KLF4 (data not shown) and S100A14 plays an important role in TPA-induced migration of breast cancer cells (Fig. 5, A and B), and knockdown of KLF4 or S100A14 attenuated the motility of cells mediated by TPA (Figs. 6 and 7). Therefore, TPA-induced cell motility was conferred at least partially by KLF4-S100A14 signaling pathway. However, deletion of KLF4 can neither completely abolish the expression of S100A14 nor antagonize the effect of TPA-induced breast cancer cell migration, which recalls the existence of other transcriptional factors regulating S100A14 expression and other downstream targets participating in TPA-mediated alteration of cell motility.

Recent publications revealed that mesenchymal to epithelial transition initiates and is required for reprogramming, cancer stem cell formation, and metastatic colonization (46–48). TPA

**FIGURE 8.** The expression of KLF4 and S100A14 are correlated in breast cancer cell lines and tissues and display prognostic value for breast carcinoma patients. A and B, a statistically significant positive correlation between KLF4 and S100A14 mRNA was observed by Pearson’s method in breast cancer cell lines and patients in two independent published data sets (GSE14020 (S1)). C, Kaplan-Meier analysis for metastasis-free survival of 630 patients with breast cancer in GEO databases (GSE12276, GSE2034, GSE2603, and GSE5327). Patients were divided into two groups based on the median value for each marker. The log rank test p values are shown.
KLF4 and S100A14 are involved in breast cancer cell biology.

anticancer strategies and provide considerable insight into results in the elevation of cell motility in breast cancer. Additionally, consensus CACCC motifs within the S100A14 promoter, KLF4 expression and increasing its binding directly to the two TPA modulates the expression level of S100A14 via enhancing aggressive behavior of breast cancer.

Many of the S100 protein family have been reported to be associated with metastases through interactions with matrix metalloproteinases or by acting as chemoattractants (34). S100A14 is known to promote esophageal squamous cancer cell motility and invasiveness by regulating the expression and function of MMP2 in a p53-dependent manner (18). S100A14 has also been documented to be regulated by TIMP-1, which promotes tumorigenesis of breast cancer cells (49). Moreover, S100A14 is known to be up-regulated in basal type breast cancer and significantly associated with patient outcome (15). These findings indicate that S100A14 plays an important role in cancer metastasis and may serve as a predictor of survival and prognosis in breast cancer. Here, by using the existing GEO database, we show that patients with higher expression of S100A14 exhibit a more aggressive behavior (Fig. 8C). It strikingly supports a potential role for S100A14 in breast cancer metastasis and its predictive capability for prognosis. In addition, Okuda et al. (50) found a significant correlation of KLF4 expression with brain metastasis-free survival in breast cancer. Intriguingly, we concluded that high concomitant KLF4/ S100A14 is significantly associated with worse metastasis-free survival for patients with breast cancer (Fig. 8C). Hence, the predictive capability of S100A14 improved by KLF4 reinforces the notion that KLF4 participates in transcriptional regulation of S100A14 and functions as an important mediator of the aggressive behavior of breast cancer.

In summary, our results are of significance in finding that TPA modulates the expression level of S100A14 via enhancing KLF4 expression and increasing its binding directly to the two consensus CACCC motifs within the S100A14 promoter, results in the elevation of cell motility in breast cancer. Additionally, our results facilitate the development of innovative anticancer strategies and provide considerable insight into understanding the underlying molecular mechanisms by which KLF4 and S100A14 are involved in breast cancer cell biology.

REFERENCES


12-O-Tetradecanoylphorbol-13-acetate Promotes Breast Cancer Cell Motility by Increasing S100A14 Level in a Kruppel-like Transcription Factor 4 (KLF4)-dependent Manner

Huan He, Sheng Li, Hongyan Chen, Lin Li, Chengshan Xu, Fang Ding, Yun Zhan, Jianlin Ma, Shuguang Zhang, Yaoting Shi, Chunfeng Qu and Zhihua Liu

doi: 10.1074/jbc.M113.534271 originally published online February 14, 2014

Access the most updated version of this article at doi: 10.1074/jbc.M113.534271

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 51 references, 23 of which can be accessed free at http://www.jbc.org/content/289/13/9089.full.html#ref-list-1