Activation of KLF8 Transcription by Focal Adhesion Kinase in Human Ovarian Epithelial and Cancer Cells*

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KLF8 (Krüppel-like factor 8) is a transcription factor downstream of focal adhesion kinase (FAK) important in the regulation of the cell cycle and also plays a critical role in oncogenic transformation and epithelial to mesenchymal transition. Here we report the mechanisms by which FAK regulates KLF8 expression in human ovarian epithelial and cancer cells. We show that the overexpression of both KLF8 and FAK in the human ovarian cancer cells as compared with the normal human ovarian surface epithelial cells is critical for cell growth. Using promoter luciferase reporter assays, we demonstrate that exogenous FAK strongly promotes the activity of the KLF8 promoter, and knockdown of FAK inhibits it. KLF8 promoter activity and mRNA levels are induced by expression of constituatively active (CA) phosphatidylinositol 3-kinase (PI3K) or CA-Akt but are repressed by dominant negative Akt or the PI3K inhibitor LY294002. Disruption of an Sp1 binding site in the KLF8 promoter abolishes the FAK- or Sp1-mediated promoter activation. Sp1 knockdown prevents the KLF8 promoter from being activated by Sp1 or CA-Akt, and expression of CA-Akt enhances Sp1 expression in SKOV3ip1 cells. Chromatin immunoprecipitation and oligonucleotide precipitation results show that Sp1 binds to the KLF8 promoter. Taken together, our data suggest that FAK induces KLF8 expression in human ovarian cancer cells by activating the PI3K-Akt signaling pathway, leading to the activation of KLF8 promoter by Sp1.

Ovarian carcinoma remains the most lethal among gynecological cancers due to the lack of early detection methods and effective treatments for late stage cancers (1). Understanding the molecular mechanisms underlying ovarian cancer progression is urgent for developing new strategies for early diagnosis and therapies required for improvement of patient survival. As found in many other types of human tumors (2, 3), overexpression or hyperactivation of focal adhesion kinase (FAK)2 has recently been found in most ovarian cancers, where it is highly associated with high aggressiveness and poor patient survival (4–7). However, the molecular mechanisms by which FAK contributes to the initiation and progression of ovarian cancer are still poorly understood.

FAK is an important protein-tyrosine kinase downstream of integrins and growth factors in the regulation of diverse cellular events, including cell adhesion, cell cycle, and migration (8–10), and plays critical roles in skin tumor initiation (11) and breast tumor growth and metastasis (12–14). Two major signaling pathways downstream of FAK are mediated by either the FAK-Src or FAK-PI3K interaction (15–17), and recent studies have suggested that the FAK-PI3K signaling pathway may play a more critical role for cancer progression (18–20). Akt is a key PI3K effector and has been shown to regulate multiple cellular processes, such as cell growth, transformation, differentiation, and survival (21). It has been reported that Akt is frequently activated in ovarian cancer, and introduction of Akt along with either c-Myc or K-Ras into p53-null ovarian surface epithelial cells was sufficient to induce ovarian tumor formation (22). These studies suggest that the FAK-PI3K-AKT signaling pathway is likely to be involved in the initiation and progression of ovarian cancer.

KLF8 (Krüppel-like factor 8) was originally described as a widely expressed transcription repressor (23) of the Krüppel-like family of transcription factors. Previously, we identified KLF8 as a FAK downstream effector that activates the cyclin D1 promoter in the regulation of cell cycle progression (24, 25). Recently, we have further shown that KLF8 is overexpressed in several types of human cancer, including ovarian, breast, and renal carcinomas, and participated in oncogenic transformation and cancer cell invasion (26, 27). Others have reported that FAK regulation of KLF8 expression is also important in glioblastoma progression (28). Understanding the mechanisms by which FAK regulates KLF8 expression in cancer cells may have important implications for the understanding of tumor progression.

In this study, we report that FAK regulates KLF8 expression at the transcriptional level in human ovarian epithelial and cancer cells. We show that FAK signaling through the PI3K-AKT pathway plays a major role in the activation of KLF8 transcription in the ovarian cancer cells, and this regulation is mediated by increased expression of Sp1 transcription factor that binds to and activates KLF8 gene promoter.
**EXPERIMENTAL PROCEDURES**

**Cell Culture, Reagents, and Transfection**—Human ovarian surface epithelial cell lines T80 and T29 were described previously (29); IOSE825 (I385) and FHIOSE118 (F118) were kind gifts from Dr. Nena Auersperg (University of British Columbia) (30, 31). These cell lines were maintained in 1:1 MCDB 105 medium with 10% fetal bovine serum. Human ovarian cancer cell line SKOV3ip1 was a kind gift from Dr. Dihua Yu (University of Texas M.D. Anderson Cancer Center) (32); Ovcar-5 and Ovcar-8 were kindly provided by Dr. Jin Q. Cheng (University of South Florida) (33, 34). These cell lines were maintained in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum. pKH3, pKH3-FAK, and pKH3-KLF8 have been described previously (25). The plasmids encoding HA-tagged constitutively active Akt (CA-Akt) and dominant negative Akt (DN-Akt) were generous gifts from Dr. Thomas F. Franke (New York University). The Sp1 cDNA kindly provided by Dr. Ceshi Chen (35) was inserted into pKH3 vector. The human bacterial artificial chromosome clone RP11-28K17 from BACPAC (Oakland, CA) was used to clone the human KLF8 promoter. Protein-specific inhibitors (Calbiochem) were included in some experiments at a concentration of 5 \( \mu \)M for U0126, or 450 nM for JNK inhibitor II).

**One day before transfection, SKOV3ip1 or T80 cells were plated at a density of 2 \( \times \) 10⁵ cells/well in 12-well plates and were grown to 80–90% confluence. Transfection of the plasmid DNAs was performed with LipofectamineTM 2000 (Invitrogen) according to the manufacturer’s instructions.** In some experiments, after 16 h of transfection, cells were replated on fibronectin (FN)- or poly-l-lysine (PLL)-coated plates and processed for luciferase assays, RT-PCR, or immunofluorescent staining. All transfections were performed in triplicate for at least three independent experiments.

**Quantitative Real Time Reverse Transcription-PCR (qRT-PCR)**—These assays were done essentially as described previously (27). Primers used for qRT-PCR were as follows: for KLF8, 5’-tctgacagactcagcaag (forward) and 5’-tacatggtggaattcgtct (reverse); for glyceraldehyde-3-phosphate dehydrogenase, 5’-aagtggcca-gagcttaaggtc-3 (forward) and 5’-gcagggatgat-cagtagag (reverse). Semiquantitative RT-PCR was based on qRT-PCR results, so that the PCR reaction was stopped within the linear range of production. The amplified DNA fragments were visualized by agarose gel electrophoresis.

**RNA Interference**—OnTargetPlus siRNAs and scramble control siRNAs specific to KLF8, FAK, or Sp1 were purchased from Dharmacon. siRNA was transfected into SKOV3ip1 cells using Oligofectamine according to Invitrogen’s instructions. 2–3 days later, cells were further treated either with or without LY294002 (5 \( \times \) IC₅₀) and processed for qRT-PCR, chromatin immunoprecipitation (ChIP), Western blotting, etc.

**Western Blotting**—Western blotting was performed essentially as described previously (25). Equal amounts of proteins were used. Anti-HA (1:2000), anti-Sp1 (1:2000), and anti-FAK (1:2500) antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

**Promoter Reporter Assays**—Luciferase reporter assays were performed essentially as described previously (24). Briefly, SKOV3ip1 or T80 cells were transfected with promoter reporter constructs or their mutants. In some experiments, expression vector encoding FAK, Sp1, CA-PI3K, CA-Akt, or DN-Akt was also included in the transfections. Control reporter expressing Renilla luciferase was used to normalize transfection efficiencies. Luciferase activity was determined using the dual luciferase reporter assay system (Promega) and 20/20n luminometer (Turner BioSystems) according to the manufacturers’ instructions.

**ChIP Assays**—These assays were performed as described previously (27). Briefly, SKOV3ip1 cells treated with Sp1 siRNA or control siRNA were cross-linked with 1% formaldehyde (Fisher) and processed for ChIP analyses using the EZ ChIP assay kits (Upstate Biotechnology, Inc., Lake Placid, NY) according to the manufacturer’s protocol. PCRs were performed with the following primers: for KLF8 promoter (167 bp), 5’-tgttcaagtagcgctttggttgc-3’ (forward) and 5’-aagtggccag-gatggtttgc-3’ (reverse). Templateless PCRs were included as negative controls.

**Biotinylated Oligonucleotide Precipitation (BOP)**—BOP was done essentially as described previously (24). SKOV3ip1 cell lysates were processed for BOP. DNA-bound Sp1 protein was examined by Western blotting using anti-Sp1 antibody, and whole cells lysates were used as a positive control.

**5-Bromodeoxyuridine (BrdUrd) Incorporation Assays**—BrdUrd incorporation assays were essentially as described previously (10). SKOV3ip1 cells were cotransfected with siRNA and pKH3 or pKH3-KLF8 before they were replated on FN. In the KLF8 rescue experiments, anti-HA antibody (1:200) was used to identify positively transfected cells.

**RESULTS**

**FAK Modulates KLF8 Expression via Transcriptional Activation**—We previously reported that inducible expression of FAK regulates cell cycle progression in NIH3T3 cells (17), and using microarray techniques, we identified KLF8 as a downstream target of FAK in this regulation (25). To determine whether FAK regulates KLF8 expression in ovarian cells, we first compared the expression of FAK and KLF8 mRNA levels in T80 and SKOV3ip1 cells. We found that the expression of both FAK and KLF8 was significantly increased in SKOV3ip1 cells compared with T80 cells (Fig. 1A, compare lanes 1 and 3). Also, although endogenous KLF8 was hardly detectable in T80 cells, overexpression of FAK in T80 cells resulted in a 3-fold increase in KLF8 mRNA expression (Fig. 1A, compare lanes 1 and 2). Conversely, in SKOV3ip1 cells, knockdown of FAK expression led to a 2-fold decrease in KLF8 mRNA expression (Fig. 1A, compare lanes 3 and 4). These results suggest that the elevated expression of FAK is responsible for the increased expression of KLF8 mRNA in ovarian cells.
RNA expression is regulated by a variety of mechanisms, such as control of mRNA turnover and transcription. mRNA turnover is mainly regulated by the interaction of AU-rich elements located in the 3′-untranslated region of an mRNA molecule with mRNA stability-regulating proteins. Transcription regulation mainly takes place through interaction of a transcription factor with a target gene promoter. Therefore, FAK could regulate KLF8 expression by stabilizing KLF8 mRNA and/or activating KLF8 transcription. We first studied the 3′-untranslated region of KLF8 mRNA sequence and found no AU-rich elements that would contribute to KLF8 mRNA turnover. We thus hypothesized that FAK regulates KLF8 expression primarily at the transcription level. To test this notion, we cloned the gene promoter of human KLF8 by PCR using the bacterial artificial chromosome clone as the template. The promoter DNA was then inserted into the pGL3basic vector for luciferase reporter assays to examine whether FAK can activate the KLF8 promoter. We found that the activity of the KLF8 promoter reporter was significantly increased in SKOV3ip1 cells compared with that in T80 cells. Also, overexpression of FAK enhanced the promoter activity in T80 cells, and FAK knockdown dramatically decreased the activity in SKOV3ip1 cells (Fig. 1B). These data suggest that FAK regulates KLF8 expression at the transcriptional level.

Regulation of KLF8 mRNA Expression by Cell Adhesion—It is well known that FAK activation and tyrosine phosphorylation are regulated by integrin-mediated cell adhesion to extracellular matrix proteins, such as FN (15–17), and FAK-mediated stimulation of KLF8 expression by FN in fibroblasts was demonstrated previously (25). To test whether cell adhesion also regulates expression of KLF8 mRNA in human ovarian cells, T80 or SKOV3ip1 cells were serum-starved, resuspended, and replated on either PLL- or FN-coated plates. Fig. 1C shows an increase in KLF8 mRNA in SKOV3ip1 cells plated on FN compared with that on PLL. Similarly, the expression level of phosphorylated FAK at Tyr397 is higher in SKOV3ip1 cells on FN than that on PLL. Although some phosphorylation of FAK at Tyr397 was detected in T80 cells on FN,
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An Sp1 Binding Site in the Proximal Promoter of KLF8 Is Vital for the Activation of KLF8 Transcription by FAK—To determine how FAK regulates KLF8 transcription, we aimed to map the region of the KLF8 promoter that responds to FAK. To do this, we generated a series of KLF8 promoter truncation mutants and analyzed their activities in SKOV3ip1 cells (Fig. 2A). Although more than 60% of the promoter activity remained when the KLF8 promoter was truncated to −97 upstream of the transcription initiation site, a truncation to −40 caused a complete loss of the promoter activity. This result suggested that the region between −97 and −40 was an essential core for the activation of KLF8 promoter. This region contains a Sp1 binding site (5′-ccaccc) located at −57 and an AP-2 binding site (5′-ccccccggc) located at −52. To test if these sites mediated the activation of KLF8 promoter, we mutated the Sp1 site (into 5′-actctc) or the AP-2 site (into 5′-ctctctc) in the context of the −97KLF8 promoter and analyzed the activity of these mutant promoters in the T80 and SKOV3ip1 cells (Fig. 2B). We found that disruption of the Sp1 binding site led to an almost complete loss of promoter activity, whereas mutation of the AP-2 binding site had no effect. Similarly, disruption of the Sp1 site in the context of the 1312KLF8 promoter led to ~70% inhibition of its activity (Fig. 2C). These results suggest that the Sp1 binding site was the major one that mediated the activation of the promoter in SKOV3ip1 cells. To test if the Sp1 binding site is crucial for FAK activation of the KLF8 promoter, we performed promoter reporter assays using the wild-type and mutant promoters in combination with overexpression of FAK in T80, or FAK knockdown in SKOV3ip1 cells (Fig. 2D). Although the wild-type promoter was sensitive to FAK expression (Fig. 2D, compare columns 1 and 2 or columns 5 and 6), disruption of the Sp1 binding site completely abolished the responsiveness of the promoter to FAK expression (Fig. 2D, compare columns 3 and 4 or columns 5 and 6). By contrast, the mutant promoter was totally unresponsive to the expression of Sp1 in either of the cells (Fig. 2E, compare columns 3 and 4 or columns 7 and 8). These results suggest that Sp1 is required for FAK activation of the KLF8 promoter, and Sp1 may directly bind to the Sp1 binding site on the KLF8 promoter.

Sp1 Binds to the KLF8 Promoter in Vivo—Since Sp1 is a transcription factor, direct interaction in vivo between Sp1 and KLF8 promoter may serve as the mechanism for the activation of KLF8 promoter. To test this, we first performed ChIP assays to determine the interaction of Sp1 to the endogenous KLF8 promoter in both T80 and SKOV3ip1 cells with or without Sp1 knockdown (Fig. 3A). The promoter fragment (−97 to +72) containing the Sp1 binding site was co-immunoprecipitated by Sp1 antibody from the cells (Fig. 3A, top, lanes 3 and 7), and notably, there is a significantly stronger binding in SKOV3ip1 cells (compare lane 7 with lane 3). In contrast, the same antibody was no longer able to co-precipitate the promoter fragment when Sp1 was knocked down (Fig. 3A, bottom, lanes 3 and 7). To confirm that Sp1 interacted with the KLF8 promoter directly and to test if this interaction is through the Sp1 binding site, we performed BOP assays (Fig. 3B). Again, we showed that Sp1 binds to the wild-type KLF8 promoter (Fig. 3B, lane 2), and mutation of the Sp1 binding site completely abolished this binding (Fig. 3B, lane 1). Taken together, these results suggest
Taken together, these data suggest that the PI3K-Akt signaling pathway is required for the activation of KLF8 transcription by FAK.

To further confirm the role of PI3K-Akt pathway in the activation of the KLF8 promoter, we manipulated the PI3K-Akt signaling in both T80 and SKOV3ip1 cells using constitutively active and dominant negative approaches and examined the effect on the activation of the KLF8 promoter (Fig. 4C) as well as expression of endogenous KLF8 mRNA (Fig. 4D). We found that the KLF8 promoter activity was enhanced by 1.5–2 times by CA-PI3K, and this increase was blocked by co-expression of the DN-Akt (Fig. 4C). Consistently, co-transfection of CA-Akt also caused an increase in the promoter activity by ~2-fold (Fig. 4C). The expression of endogenous KLF8 mRNA was regulated to a similar degree by CA-PI3K, CA-Akt, or DN-Akt in the cells (Fig. 4D). Taken together, these results strongly suggest that the PI3K-Akt pathway is indispensable for KLF8 expression in vivo.

Sp1 Serves as an Effector Downstream of PI3-Akt for the Activation of KLF8 Transcription in SKOV3ip1 Cells—Several studies have indicated that the PI3K-Akt pathway regulates gene expression via Sp1 (36–38). To determine the potential role of Sp1 in mediating the expression of KLF8 by PI3K-Akt signaling in the ovarian cancer cells, we first compared SKOV3ip1 with T80 cells for the expression of activated Akt and Sp1 by Western blotting and KLF8 mRNA levels using RT-PCR and qRT-PCR (Fig. 5A). We found that the higher expression of activated Akt (phospho-Akt) (but not total Akt) in SKOV3ip1 was highly correlated with the increased expression of Sp1 and KLF8 in the cells (Fig. 5A).

We then tested whether or not the activation of the KLF8 promoter by PI3K-Akt was through the Sp1 binding site in the promoter. We co-transfected CA-Akt or DN-Akt with the wild-type (WT) or mutant KLF8 promoter (mSp1) into SKOV3ip1 cells and performed luciferase reporter assays. We found that the wild-type KLF8 promoter was activated by CA-Akt and inhibited by DN-Akt, whereas the mutant promoter completely lost responsiveness to both CA-Akt and DN-Akt (Fig. 5B). These data suggest that Sp1 mediates the activation of KLF8 transcription by PI3K-Akt signaling in the ovarian cancer cells. To further confirm this notion, we co-transfected CA-Akt with Sp1 siRNA or control siRNA into SKOV3ip1 cells and examined this effect of CA-Akt on the expression of KLF8 mRNA. Indeed, CA-Akt increased KLF8 expression by about 2-fold in the control cells, whereas in the Sp1 knockdown cells, CA-Akt was no longer capable of enhancing KLF8 expression (Fig. 5C). We then transfected CA-Akt or DN-Akt into SKOV3ip1 cells and examined whether Akt regulated endogenous Sp1. Importantly, Sp1 protein levels were suppressed in ~90% of cells expressing DN-Akt; in contrast, CA-Akt increased Sp1 protein levels in the nuclei of SKOV3ip1 cells, as demonstrated by immunostaining and Western blotting (Fig. 5D).

Taken together, these results suggest that FAK-PI3K-Akt signaling up-regulates the expression of Sp1, which binds to and activates the KLF8 promoter, resulting in increased transcription and expression of KLF8 in the ovarian cancer cells.

The PI3K-Akt-Sp1-KLF8 Pathway Regulates Cell Cycle Progression in SKOV3ip1 Cells—Our previous studies demonstrated that KLF8 is a downstream target of FAK and mediates...
FAK regulation of the cell cycle in 3T3 cells (25). To investigate the role of PI3K-Akt-Sp1-KLF8 pathway in the regulation of cell cycle progression in SKOV3ip1 cells, we conducted BrdUrd incorporation assays (Fig. 6A). As expected, inhibition of PI3K resulted in a significant decrease in DNA synthesis (Fig. 6A, compare lanes 1 and 2), and overexpression of KLF8 partially rescued new DNA synthesis from inhibition by the PI3K inhibitor LY294002 (Fig. 6A, compare lanes 2 and 3). A low transfection efficiency of KLF8 most likely accounted for a partial rescue rather than a full rescue in these experiments. This result indicates that KLF8 is a downstream mediator of PI3K in the regulation of cell cycle progression. Knockdown of Sp1 caused a decrease in the BrdUrd intake rate similar to that by the PI3K inhibitor (Fig. 6A, compare lane 4 with lanes 1 and 2), and combination of Sp1 knockdown with PI3K inhibition did not cause an additional decrease (compare lane 5 with lanes 2 and 4). Again, overexpression of KLF8 was able to restore the BrdUrd intake rate in the Sp1 knockdown cells (Fig. 6A, compare lanes 5 and 6). Overall, these results suggest that Sp1-mediated up-regulation of KLF8 expression by the FAK-PI3K Akt signaling pathway is critical to the cell cycle progression of human ovarian cancer cells. Additionally, overexpression of KLF8 promoted BrdUrd intake rate in several independent cell lines of human ovarian surface epithelia (Fig. 6B), and KLF8 knockdown inhibited it in two other human ovarian cancer cell lines that express high levels of KLF8 (Fig. 6C) (26), suggesting a general role of FAK-KLF8 signaling in cell cycle regulation of human ovarian cancer cells.

**DISCUSSION**

Aberrant overexpression of KLF8 has been found in several types of human cancer, including ovarian, breast, renal, and brain cancer (26–28). These cancers also express abnormally high levels of FAK. Although it has been demonstrated that FAK signaling up-regulates KLF8 expression in fibroblasts (25), whether and how FAK regulates KLF8 expression in human cancer was not previously investigated. In this paper, we investigated the possible mechanisms by which KLF8 expression is regulated by FAK signaling in human ovarian cancer cells. We conclude that KLF8 expression is regulated at the transcriptional level primarily by FAK activation of the PI3K-Akt pathway, leading to increased expression of Sp1 that in turn directly activates KLF8 promoter. This study is the first to demonstrate a potential pathway for FAK regulation of KLF8 expression and the functional relevance to cell cycle progression in human ovarian cancer cells.

Our conclusions are based on the following evidence. First, the high levels of KLF8 mRNA as well as its promoter activity were well correlated with the expression and activity of FAK in SKOV3ip1 cells compared with T80 cells that show little activation of FAK and literally no KLF8 expression. Knockdown of FAK resulted in reduced expression of KLF8 mRNA and its promoter activity, and activation of FAK by FN-mediated adhesion promoted it. Second, the high levels of KLF8 mRNA and promoter activity were also well correlated with the expression...
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of activated Akt and nuclear Sp1 in SKOV3ip1 cells. CA-P13K, CA-Akt, and Sp1 all enhanced expression of KLF8 mRNA and promoter activity, and P13K inhibitor, DN-Akt, or Sp1 siRNA inhibited it. Third, nuclear expression of Sp1 was enhanced by CA-Akt but reduced by DN-Akt. Fourth, the Sp1 protein directly interacted with the KLF8 promoter at the proximal Sp1 binding site, and this site is required for the activation of the promoter by FAK, CA-P13K, CA-Akt, and Sp1. Finally, overexpression of KLF8 in SKOV3ip1 cells could prevent or restore the cell cycle progression from the P13K inhibitor- or Sp1 siRNA-mediated inhibition.

FAK plays a critical role in the regulation of cancer initiation, progression, and metastasis, and its aberrantly high expression and phosphorylation are directly correlated with the malignancy of a variety of human cancers (3, 4, 6, 7, 11–14). FAK expression or activity is barely detectable in normal human ovarian epithelium but highly elevated in ~70% of human ovarian tumors and is associated with high risk clinical features and poor patient survival (4, 7). In addition to SKOV3ip1, our recent studies have demonstrated that KLF8 is also highly overexpressed in several other human ovarian cancer cell lines and primary ovarian tumor tissues (26). Our results showing a similar cell cycle-promoting role for KLF8 in multiple ovarian normal and cancer cell lines (see Fig. 6) suggest a general role of FAK-KLF8 pathways in ovarian cancer progression. Given the similar patterns of aberrant expression of KLF8 and FAK in human ovarian as well as breast cancers and the role of KLF8 in both oncogenic transformation and invasion (26, 27), it is likely that the activation of KLF8 by FAK signaling critically contributes to the progression and metastasis of ovarian and breast cancer in vivo.

The Src-ERK and P13K-Akt pathways represent two primary signaling cascades downstream of FAK activation in many types of cells. In fibroblast cells, both pathways seem to be important for FAK-regulated expression of KLF8 (25). In this work, we found that in ovarian cells, the P13K-Akt pathway plays a dominant role in the activation of KLF8 transcription by FAK. This finding is in agreement with other reports demonstrating that the activation of Src kinases is less frequent in ovarian cancer although very common in other types of cancer, such as breast and colorectal cancers (39).

Indeed, activation of the P13K-Akt pathway is a very common and critical event in ovarian cancer malignancy (40, 41). Therefore, it is plausible that the expression of KLF8 in ovarian cancer may be primarily regulated by the P13K-Akt signaling, although it is possible that in other types of cancer, such as breast cancer, the Src-ERK signaling pathway may play a more significant role.

It is obvious that the regulation of KLF8 expression by FAK in the ovarian cells occurs at the transcriptional level, and this regulation depends on Sp1 binding to the site located at the −57-position in the KLF8 promoter (Figs. 1 and 2). Indeed, recent studies have identified Sp1 as an important mediator of gene transcription induced by diverse signaling stimuli, including oncoproteins, growth factors, and cytokines (42), despite the fact that Sp1 has been implicated in controlling the basal transcription of many “housekeeping” genes. For example, Sp1 binding sites are required for stimulation by the ERK, Akt, and JNK pathways of promoters of several genes, including the
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FIGURE 6. KLF8 promotes G1 cell cycle progression in ovarian epithelial and cancer cells. A, overexpression of KLF8 protects BrdUrd incorporation from inhibition by LY294002 or Sp1 siRNA. SKOVip1 cells were cotransfected with Sp1 siRNA plus KLF8 or empty vector, control siRNA plus KLF8, or empty vector. After 16 h, cells were replated on FN and treated with LY294002 (7 μM) as indicated. The percentage of BrdUrd cells at 12 h after serum stimulation was analyzed. The results showed the mean ± S.E. for at least three independent experiments. *p < 0.05. B, KLF8 promotes DNA synthesis in ovarian epithelial cells. HA-KLF8 was transiently expressed in the indicated cells, after 24 h of serum deprivation, and cells were stimulated with serum containing BrdUrd for 14 h before they were prepared for immunofluorescent staining with antibodies against HA and BrdUrd. 50 HA-positive cells were counted for BrdUrd-positive cells. *p < 0.01 as compared with the vector control. Data are representative of three independent experiments. C, KLF8 knockdown inhibits DNA synthesis in ovarian cancer cells. KLF8 siRNA or control siRNA was transiently transfected into Ovcar-5 (Ov5) or Ovcar-8 (Ov8) cells for 48 h. The cells were then serum-starved for 24 h and stimulated with serum for 22 h before the BrdUrd incorporation assay was done. *p < 0.01 as compared with control siRNA treatment. Data are representative of three independent experiments.

KLF8 promotes G1 cell cycle progression in ovarian epithelial and cancer cells. A, overexpression of KLF8 protects BrdUrd incorporation from inhibition by LY294002 or Sp1 siRNA. SKOVip1 cells were cotransfected with Sp1 siRNA plus KLF8 or empty vector, control siRNA plus KLF8, or empty vector. After 16 h, cells were replated on FN and treated with LY294002 (7 μM) as indicated. The percentage of BrdUrd cells at 12 h after serum stimulation was analyzed. The results showed the mean ± S.E. for at least three independent experiments. *, p < 0.05. B, KLF8 promotes DNA synthesis in ovarian epithelial cells. HA-KLF8 was transiently expressed in the indicated cells, after 24 h of serum deprivation, and cells were stimulated with serum containing BrdUrd for 14 h before they were prepared for immunofluorescent staining with antibodies against HA and BrdUrd. 50 HA-positive cells were counted for BrdUrd-positive cells. *, p < 0.01 as compared with the vector control. Data are representative of three independent experiments. C, KLF8 knockdown inhibits DNA synthesis in ovarian cancer cells. KLF8 siRNA or control siRNA was transiently transfected into Ovcar-5 (Ov5) or Ovcar-8 (Ov8) cells for 48 h. The cells were then serum-starved for 24 h and stimulated with serum for 22 h before the BrdUrd incorporation assay was done. *, p < 0.01 as compared with control siRNA treatment. Data are representative of three independent experiments.

KLF8 transcription could emerge as a novel approach for therapeutic intervention against human cancer. Importantly, recent studies have identified several other ovarian cancer-associated oncogenic signaling proteins, including BRAC1 (46), telomerase (47), claudin-4 (48), and α-folate receptor (49), as Sp1 target genes, suggesting a potentially combinatorial role of these Sp1 target proteins, including KLF8, in certain pathological situations of ovarian cancer progression.

The mechanisms by which Akt regulates Sp1 seem to depend on cell types or cellular contexts. In one study, active Akt was found to interact with Sp1, and this interaction was associated with increased phosphorylation of Sp1 in the regulation of vascular endothelial growth factor expression (37). Another study, however, suggested Akt protection of Sp1 protein from degradation as an alternative mechanism in the regulation of MMP14 transcription (50). In our experimental conditions, we failed to detect obvious changes in the serine or threonine phosphorylation on Sp1 (data not shown). Instead, the nuclear expression of Sp1 protein was clearly up-regulated by the PI3K-Akt signaling (Fig. 5). Thus, phosphorylation may not be a major mechanism underlying the up-regulation of Sp1 by Akt in the ovarian cancer cells. No matter exactly how Akt regulates Sp1, it is believed that the enhanced Sp1 capability of activating its target gene promoters is a common outcome.

In conclusion, this study identified the PI3K-Akt-Sp1 as a novel signaling mechanism responsible for the activation of KLF8 transcription by FAK in human ovarian cancer cells. This finding has advanced our understanding of the roles of the FAK-KLF8 signaling axis in the pathology of ovarian and possibly other types of cancer. Given the potential role of KLF8 in promoting tumor formation and metastasis in vivo, this is worthy of further investigation, targeting pathways involved in KLF8 transcription could emerge as a novel approach for therapeutic intervention against human cancer.

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