Cyclin-Dependent Kinase Inhibition by the KLF6 Tumor Suppressor
Protein through Interaction with Cyclin D1

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ABSTRACT

Kruppel-like factor 6 (KLF6) is a tumor suppressor gene inactivated in prostate and colon cancers, as well as in astrocytic gliomas. Here, we establish that KLF6 mediates growth inhibition through an interaction with cyclin D1, leading to reduced phosphorylation of the retinoblastoma protein (Rb) at Ser780. Furthermore, introduction of KLF6 disrupts cyclin D1-cyclin-dependent kinase (cdk) 4 complexes and forces the redistribution of p21cip1/p16 onto cdk2, which promotes G1 cell cycle arrest. Our data suggest that KLF6 converges with the Rb pathway to inhibit cyclin D1/ cdk4 activity, resulting in growth suppression.

INTRODUCTION

Kruppel-like factor 6 (KLF6) is a zinc finger tumor suppressor protein inactivated in sporadic prostate (1, 2) and colon cancers (3) and astrocytic gliomas (4). Furthermore, KLF6 promoter methylation has been identified in esophageal cancer cells (5). KLF6 is a member of a growing family of transcription factors that share a COOH-terminal C2H2 DNA binding domain but have widely divergent NH2-terminal activation domains (6, 7). Growth suppressive activity of KLF6 is linked to p53-independent transactivation of p21WAF1/Cip1, a key cyclin-dependent kinase (cdk) inhibitor (1). Some tumor-derived mutants of KLF6 have been identified that can no longer transactivate p21WAF1/Cip1 (1). Other KLF6 mutants, however, preserve p21 transactivation activity, yet these still fail to suppress growth (3). This finding suggests that KLF6 might provoke growth arrest through functionally parallel circuits, a property that is typical of other tumor suppressor genes such as p53 (8–10) or p16 (11).

Direct interaction with cell cycle regulators characterizes growth suppression by several tumor suppressor genes. The INK4a gene, for example, encodes the p16INK4a tumor suppressor protein that binds to cdk4 and alters the binding of D-type cyclins, thus, reducing the kinase’s affinity to ATP (12). By preventing the formation of cdk4/6-cyclin D complexes, INK4 proteins force the redistribution of the Cip/Kip family of inhibitors (e.g., p21) from cdk4/6-cyclin D1 kinases toward binding and down-regulating the kinase activity of cdk2-cyclin E complexes, thereby blocking exit from G1 (12).

Evasion of the cyclin-dependent and the retinoblastoma (Rb) pathways are common alternative mechanisms by which tumor cells escape the G1 restriction point (13). Cyclin D1/cdk complexes are part of a more extensive cell cycle regulatory pathway that includes the Rb protein, a nuclear phosphoprotein that is differentially phosphorylated on serine and threonine residues during the cell cycle. Phosphorylation of Rb by cdkS leads to S-phase entry and marks the irreversible commitment of the cell to exit the G1 (resting) phase. Key to the control of cell growth at this G1-S junction is the cyclin D-p16INK4a-Rb pathway.

Loss of cell cycle control is a common event in malignancies (14). The D-type cyclins (D1, D2, D3) are responsible for integrating extracellular signals into the cell (14). The cyclinD1/CCND1 oncogene (PRAD1), for example, is amplified in a number of primary cancers. Cyclin D1 overexpression, however, has been detected at a higher frequency than can be accounted for by gene amplification alone (15). Thus, mechanisms other than DNA amplification may lead to deregulated expression of cyclin D1 in cancer. Additionally, because deregulated expression of cyclin D1 directly contributes to tumorigenesis in a number of animal model systems (16, 17), inhibition of cyclin D1 function may be an important target of cancer prevention and therapy (18).

Because the KLF6 sequence predicts consensus sites for both a cdk phosphorylation site at Ser780 (19) and a proximal cyclin E/A consensus binding site at the COOH terminus (‘ZRXL’ motif, Z = basic residue, at amino acids 279–283), we have explored the possibility that KLF6 interacts directly with cell cycle components. Here, we describe an interaction between the tumor suppressor protein KLF6 and cyclin D1, defining a novel mechanism of KLF6-mediated growth suppression and identifying this protein as a putative cdk inhibitor (CKI).

MATERIALS AND METHODS

Cell Lines. Cell lines were obtained from the American Tissue Culture Collection. 293T, Hep3B, and HCT116 cell lines were cultured in DMEM (BioWhittaker) supplemented with 10% (v/v) fetal bovine serum (Hyclone), antibiotics (penicillin, streptomycin 100 mg/ml; Cellog, and l- glutamine (30 mg/ml). The NIH3T3 cell line was cultured in DMEM supplemented with calf serum. All cell lines were grown in 5% CO2 at 37°C.

Plasmids. pCneo-KLF6 (human) expression vector was constructed as previously described (19) by fusing a full-length KLF6 cDNA cloned into the EcoRI and Xhol sites of the pCneo vector. FLAG-tagged full-length KLF6 was constructed by restriction digest and subcloning into a pcDNA3 vector expressing the M5 (FLAG) epitope at the NH2 terminus. The cyclin D1 expression vector was a gift from Dr. Charles Sherr (St. Jude Cancer Center, Nashville, TN).

Retrovirus Construction, Propagation, and Infection. Human KLF6 cDNA (19) was cloned into pBabe-puro expression vector. Constructs containing the KLF6 sequence (pBabe-KLF6) or empty vector control (pBabe) were transiently transfected by lipofection (Lipofectamine 2000; Invitrogen) into 50% confluent Phoenix A packaging cells producing nonreplicating forms of amphotropic virus [a generous gift from Dr. Gary Nolan (Stanford, CA)] in 15-cm plates used according to the manufacturer’s protocol. Transfection medium was removed after 4 h and replaced with fresh medium. The supernatant from the packaging cells was collected three times daily for 3 days after transfection and filtered through a 0.45-μm membrane to remove contaminating packaging cells. For infection, 4 × 107 cells HCT116 target cells were seeded in 10-cm plates and were exposed to 5 ml of viral supernatant containing viable virus in the presence of 8 μg/ml Polybrene (Sigma, St. Louis, MO) for 4 h, twice in succession. Depending on the experiment, HCT116 cells were selected with 5 μg/ml puromycin.
Antibodies. The polyclonal anti-KLF6 (R-173), the polyclonal anti-cdk4, and the monoclonal anti-cyclin D1 antibodies were obtained from Santa Cruz Biotechnology. The anti-KLF6 monoclonal antibody was generated in the monoclonal antibody core facility at Mount Sinai School of Medicine using as the immunogen the NH\textsubscript{2}-terminal transactivation domain (residue 1–201) expressed as a GST-fusion protein. The anti-Rb and anti-pSer795-Rb antibodies were obtained from Cell Signaling (Beverly, MA). The anti-HA, anti-FLAG, and anti-β-tubulin antibodies were obtained from Sigma.

Western Blot Analysis. Unless otherwise indicated, whole cell protein extracts were obtained by lysing cells in lysis buffer [50 mM HEPES (pH 7.5), 150 mM NaCl, 1 mM EDTA, 0.1% NP40, 10% glycerol, 20 mM\textsuperscript{–} was neutralized by addition of 50 °C Western blot, rather than the characteristic smear when multiple isoforms are.

Cruz, CA) or a control antibody (as described in preceding section). Next, incubating with primary antibodies [α-KLF6-1:250 (polyclonal), α-cyclin D1-1:250, cdk4-1:250, α-Rb- and α-pSer\textsuperscript{795}-Rb-1:1000, and α-β tubulin-1:10000] and processed by the standard enhanced chemiluminescence detection method under conditions recommended by the manufacturer (Amersham). Nitrocellulose membranes were then subjected to autoradiography.

For repeated blots using the same membranes, they were stripped and reprobed after being submerged in stripping buffer [100 mM β-mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl (pH 6.7)] and incubated at 50 °C for 30–45 min with shaking approximately every 10 min. The membranes were then washed twice for 10 min in Tris-buffered saline-Tween at room temperature using large volumes of wash buffer. After blocking for 1 h at room temperature in 5% dry milk/Tris-buffered saline-Tween, immunodetection was performed as described above.

Immunoprecipitation. All protein extracts were precleared by neutrality in the presence of protease G-agarose beads and a corresponding control immunoglobulin for 30 min at 4 °C. Supernatant was then collected, and 20–40 μg of either protein G-, HA-, or M2-FLAG-agarose beads were added for immunoprecipitation with nutation overnight at 4 °C of the desired protein. The next day, beads were washed four times with lysis buffer, and proteins were subsequently boiled off the beads in 1× SDS protein loading buffer and separated on SDS-PAGE gels for subsequent Western analysis. The same procedure was used for immunoprecipitation of endogenous KLF6 from NIH3T3, Hep3B, and HCT116 cells. Endogenous KLF6 was incubated for 1 h with a monoclonal antibody specific to KLF6 (2A2) before protein G-agarose beads were added, and immunoprecipitation followed with nutation overnight at 4 °C.

Nonradioactive cdk4 Immunoprecipitation Kinase Assay. Immunoprecipitation/kinase assays were performed using a modification of the method described by Jinyo et al. (20). In brief, 200–400 μg of whole cell extracts immunoprecipitated with antibody to cdk4 (Santa Cruz Biotechnology, Santa Cruz, CA) or a control antibody (as described in preceding section). Next, beads were washed twice with lysis buffer and twice in 1× Rb kinase buffer before they were incubated in Rb kinase reaction buffer [50 mM HEPES (pH 7.5), 1 mM EGTA, 10 mM KCl, 10 mM MgCl\textsubscript{2}, 1 mM DTT, and 10 mM ATP] containing 0.5 μg of recombinant Rb protein (QED Biosciences, San Diego, CA). The reaction mixture was left for 30 min at 30 °C with gentle shaking and was neutralized by addition of 50 μl of 1× SDS protein loading buffer before Western analysis. For this assay, antibody recognizing Ser\textsuperscript{95}-phosphorylated forms of Rb (Cell Signaling) was used. This site on Rb is efficiently phosphorylated by cyclin D/cdk4 complexes (21) and yields a single band on Western blot, rather than the characteristic smear when multiple isoforms are detected using total Rb antibody.

Transfection. Transient transfections were performed using Lipofectamine 2000 reagent (Invitrogen). Lipofectamine was removed 4–6 h after transfection and cells were allowed to recover overnight in complete 10% fetal bovine serum medium. Twenty-four h after transfection, cells were washed three times with cold PBS, and cell lysates were prepared.

Small Interfering RNA (siRNA) KLF6 Silencing. The specific sequence of small interference RNA for KLF6 consists of a 19-nucleotide (nt) sequence of exon 2 separated by a 9-nt spacer from the reverse complement of the same sequence (sense 5′-GGAGAAGAGCUCUAGCATTT-3′ and antisense 3′- TTCCUCUUUCGGAAGUGCUA-5′). The oligonucleotide was cloned and expressed in a pSuper plasmid (22). The resulting pSuper-KLF6 plasmid was transfected into HCT116 cells, and cells were harvested 24 h after transfection. The pSuper vector expressing an irrelevant protein, pSuper-Luciferase (pLUC), was used as a control.

RESULTS

KLF6 Binds Cyclin D1. We have identified a cyclin E/A consensus-sus-binding motif at the COOH terminus (amino acids 279–283) in KLF6. Within this motif is the sequence ZRXL, where Z and X are typically basic (23, 24). The NH\textsubscript{2} terminus of KLF6 contains a

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number of leucine-rich repeats, including a highly conserved 11-residue stretch with the consensus sequence LxxLxLxxNxL that typically contributes to protein-protein interactions (25, 26). Taken together, these features suggested that KLF6 has the potential for protein-protein interactions, possibly binding one or more cyclins.

We tested binding to cyclins D1, E and A by coimmunoprecipitation assays after cDNA transfection of FLAG-KLF6 and the respective cyclins into 293T human embryonic kidney cells. These cells were chosen initially because of their undetectable levels of endogenous KLF6 and cyclin D1 protein, thus minimizing non-specific background interactions. Only cyclin D1 coimmunoprecipitated with FLAG-tagged KLF6 (Fig. 1A). There was no specific binding of KLF6 to cyclins E or A under identical experimental conditions (data not shown). Specificity of binding was additionally confirmed in 293T cells stably expressing FLAG-tagged cyclin D1 were transfected with increasing concentration of KLF6 (1–10 μg). Immunoblots of transfected KLF6 and endogenous cyclin D1 extracts were obtained 24 h after transfection for Western blot analysis of transfected KLF6 and endogenous cyclins in HCT116 (colon carcinoma; Fig. 1C). Immunoblot analyses showed dose-responsive manner, corroborating binding specificity.

The physiological nature of the KLF6-cyclin D1 interaction was supported by evidence of binding between endogenous KLF6 and cyclin D1 proteins in HCT116 (colon carcinoma; Fig. 1C, top panel) and U2OS osteosarcoma cells (Fig. 1C, bottom panel) [additionally validated in Hep3B (hepatocellular carcinoma) and NIH3T3 cells; data not shown]. For each cell type, whole cell extracts were harvested 24 h after cells were seeded, and endogenous KLF6 protein was immunoprecipitated using anti-M2-FLAG agarose beads. As shown in Fig. 1B, KLF6 was co-immunoprecipitated with cyclin D1 in a dose-responsive manner, corroborating binding specificity.

KLF6 Inhibits Cyclin D1/cdk4 Activity. We next examined whether the binding of KLF6 to cyclin D1 was associated with decreased cyclin D1 kinase activity. Cyclin D1/cdk4 complexes phosphorylate Rb at Ser795 (21). Thus, disruption of this interaction by KLF6 should lead to reduced phosphorylation of Rb-S795. HCT116 (colon cancer) cells that lack functional p16INK4a, were used in these experiments to avoid background inhibition of Rb phosphorylation by p16INK4a. In

**Fig. 2.** KLF6 reduces Rb phosphorylation at Ser795. A, cyclin D1 and cyclin-dependent kinase (cdk) 4 were cotransfected into HCT116 cells in the presence or absence of KLF6 or p16. cdk4 was immunoprecipitated from 500 μg of whole cell extract (WCE). Reombinant retinoblastoma (Rb) protein (0.5 μg) was added to the washed beads, and the reaction mixture was incubated in the presence of ATP for 30 min. at 30°C with gentle shaking. Samples were then subjected to Western blot analysis using antibody specific to the phosphorylated Ser795 form of Rb. Rb phosphorylation at Ser795 was quantitated by densitometry (in both A and B), and values are expressed in arbitrary intensity units relative to control (relative intensity unit = 1.0). The results shown are representative of three independent experiments. B, KLF6 was transfected into HCT116 cells and protein extracts were obtained 24 h after transfection for Western blot analysis of transfected KLF6 (left) and endogenous proteins (right). Overexpression of KLF6 results in reduced Rb phosphorylation at Ser795 in vivo.
Silencing of endogenous KLF6 by small interference RNA (siRNA) restores binding of endogenous cyclin D1 to cdk4 and reverses suppression by KLF6 of retinoblastoma (Rb) phosphorylation at Ser\(^{795}\). Twenty-four h after transfection, cells were harvested, and cdk4 was immunoprecipitated from whole cell extracts. An immunoblot of ectopically expressed cdk4 and endogenous cyclin D1 protein, increased levels of cyclin D1/cdk4 holoenzymes were detected. Therefore, in the presence of markedly reduced levels of KLF6 protein, increased levels of cyclin D1/cdk4 holoenzymes were detected.

The biological effects of silencing endogenous KLF6 by siRNA were most pronounced in PC3 prostate cancer cells. PC3 cells are characterized by high transfection efficiency relative to HCT116 cells (as assessed by fluorescence-activated cell sorting analysis of green fluorescent protein-transfected cells; data not shown). Moreover, PC3 cells express reduced levels of endogenous KLF6 protein in comparison to HCT116 cells. Thus, PC3 cells were used to obtain higher phosphorylation following silencing of endogenous KLF6 mRNA using RNA interference. KLF6-specific siRNA (see “Materials and Methods”) was expressed after transient transfection into HCT116 cells to determine the impact of silencing endogenous KLF6 on cyclin D1-cdk4 complex formation. For these experiments, cdk4 alone was ectopically expressed by transient transfection, and its interaction with endogenous cyclin D1 was assessed by immunoprecipitation from whole cell extracts, as shown in Fig. 4A. Following immunoprecipitation of cdk4, its complex formation with endogenous cyclin D1 was increased when endogenous KLF6 was reduced by specific siRNA. In other words, in the presence of markedly reduced levels of KLF6 protein, increased levels of cyclin D1/cdk4 holoenzymes were detected.
KLF6 is eliminated by posttranscriptional gene silencing, phosphorylation at this site (Fig. 2). Moreover, when the wild-type function of KLF6 expression on cyclin D1/cdk4 holoenzyme formation.

KLF6 Inhibits Cell Proliferation and Promotes Growth Arrest in HCT116 Colon Carcinoma Cells. To assess whether KLF6 expression reduces proliferation of HCT116 cells, as previously shown in PC3 cells (1) that are also null for p16, DNA synthesis was determined by measuring [3H]thymidine incorporation into DNA. A total of 100–150 × 10^6 HCT116 cells were seeded in 12-well plates for transfection the next day with 1.6 μg of wild-type KLF6, p16^INK4a−/−, or empty vector DNA as a control. Four to 6 h after transfection, cells were transferred to 10% FBS overnight, then starved for 24 h in 0.2% FBS. [3H]Thymidine was added at 1 μCi/ml (1 μCi = 37 kBq) during the last 2 h of serum starvation. Disintegrations/min (dpm) were measured in a liquid scintillation counter. KLF6 has a statistically significant antiproliferative effect relative to expression vector alone (⁎, P < 0.05, two-way ANOVA, Bonferroni correction applied, n = 5). The effect of p16^INK4a−/− is highly statistically significant (⁎⁎⁎, P < 0.001, two-way ANOVA, Bonferroni correction applied, n = 4) and may be due to lack of functional endogenous p16 in this cell line.

To corroborate these results and to further study the impact of KLF6 on cell cycle distribution, we introduced KLF6 into HCT116 cells by retroviral infection. Western blot analysis (in HCT116 cells; Fig. 6A) and immunofluorescence (in U2OS cells chosen for their lowest background autofluorescence relative to either HCT116 or PC3 cells; Fig. 6B) confirmed KLF6 expression and nuclear localization upon transduction of KLF6.

Expression of KLF6, relative to pBabe empty vector control, led to a 50% increase of HCT116 cells in G1 (Fig. 6C) with a concomitant decrease of cells in both S and G2-M phases of the cell cycle. These results substantiated our observations obtained by [3H]thymidine incorporation.

KLF6-Mediated Induction of p21 Results in Increased Redistribution of p21 to cdk2. Having established that KLF6 binds to cyclin D1, thereby inhibiting cyclin D-associated kinase activity, we next explored the effect of KLF6 on cdk2 complexes, as has been described for p16 (29–31). KLF6-mediated tumor suppression has been linked to its induction of the CKI, p21. Thus, we sought to correlate the effect of KLF6-mediated p21 induction and the redistribution of p21 to cdk2 as a result of KLF6 binding to cyclin D1. Retroviral expression of KLF6 stimulated the induction and redistribution of p21 on cdk2 complexes (Figs. 6 and 7). These findings are consistent with prior studies with keratinocytes in which p15 led to redistribution of Cip/Kip inhibitors from cdk4/6 to cdk2 (32, 33), including two independent studies demonstrating redistribution of p21 and/or p27 upon induction of p16 in U2OS cells (30, 34).

DISCUSSION

A key role of cyclin D1 is to bind cdk4 and form an active complex that can phosphorylate Rb. The Rb protein or the gatekeeper of the G1-S transition (also referred to as the restriction point) is active when hypophosphorylated. Hypophosphorylated Rb blocks the transcriptional activity of E2F and represses genes involved in S-phase progression. Rb phosphorylation is inactivating such that phosphorylated Rb can no longer bind its co-repressor E2F, allowing transcription of growth stimulatory genes and progression of cells from G1 into S phase (35, 36).

Inhibition of cyclin D1/cdk4 occurs through interactions with cdk regulatory proteins of which there are two categories—the INK4 family (p16, p15, p18, and p19) that solely inhibit cdk4/6 kinase activity and the more promiscuous Cip/Kip family (p21, p27 and p57) that inhibit a broader range of cyclin–cdks in a concentration-dependent manner (37). For example, the p21 family of proteins enhances cyclin D1 kinase activity at low concentrations and inhibits this activity at high concentrations (38, 39). Of the INK4 family of CKIs, overexpression of the tumor suppressor p16 has been shown to reduce cyclin D1 holoenzyme levels and to cause G1 cell cycle arrest as has been shown for CKIs identified to date (40–42).

Approximately 90% of human neoplasms have abnormalities in some component of the Rb pathway, with detectable lesions identified in either INK4a, cyclin D1, or Rb (43). Hyperactivation of cdks, overexpression of cyclins, or down-regulation of inhibitory factors such as the CKIs promote deregulated S-phase progression and loss of the G1 checkpoint (37).

Having identified a physiological interaction between the tumor suppressor KLF6 and cyclin D1 (Fig. 1), we tested the hypothesis that KLF6 functions as a CKI via its interaction with cyclin D1. The tumor suppressor protein and prototypical CKI p16 functions by binding to cdk4 and disrupting cyclin D1/cdk4 complexes. Similarly, the KLF6 protein reduces cyclin D1/cdk4 complexes via its interaction with cyclin D1 (Fig. 3). Conversely, silencing the endogenous KLF6 gene by KLF6-specific siRNAs results in increased cyclin D1/cdk4 complexes as detected by immunoprecipitation-Western analysis (Fig. 4A).

We examined the biological consequences of these interactions in relation to the Rb pathway, testing phosphorylation of Rb at S795, a residue specifically phosphorylated by the cyclin D1/cdk4 complex (28). Expression of wild-type KLF6 resulted in reduced Rb phosphorylation at this site (Fig. 2). Moreover, when the wild-type function of KLF6 is eliminated by posttranscriptional gene silencing, phosphorylation of Rb at S795 is increased relative to wild-type KLF6 expression (Fig. 4B).

Taken together, the data support the hypothesis that KLF6 function resembles that of the tumor suppressor p16. Because p16 is nonfunctional in the cell lines chosen—HCT116 cells (44) PC3 and U2OS cells—it is possible that lack of p16, which normally disrupts cyclin D1/cdk4 complexes, potentiates the effect of wild-type KLF6 on cdk activity and accentuates the consequences of silencing endogenous KLF6 expression on cyclin D1/cdk4 holoenzyme formation.

The Cip/Kip family of CKIs has an additional function at low concentrations that includes promoting cyclin/cdk assembly (38, 39). Indeed, at low concentrations, the Cip/Kip family member, p21, stabilizes cyclin D1/cdk4 complexes and promotes cdk4 kinase activity (39, 45). Interestingly, as shown in Fig. 3, incremental expression
of KLF6 by transient transfection similarly suggests that at low concentrations KLF6 promotes cyclin D1/cdk4 complexes but disrupts them at higher concentrations. It is possible, therefore, that KLF6 functions in a concentration-dependent manner, as demonstrated for the CKI p21.

As a tumor suppressor, the ability of KLF6 to transcriptionally up-regulate p21 (1) may not be mutually exclusive from its ability to physically interact with the cyclin D1 oncogene. Indeed, a number of growth suppressive mechanisms can be simultaneously triggered upon tumor suppressor activation. For example, p53-mediated apoptosis is triggered both by transcription-dependent (e.g., up-regulation of bax, GADD45) and transcription-independent pathways (46). Expression of KLF6 by transient retroviral infection in HCT116 cells induces expression of the p21 CKI. Furthermore, as has been shown for the binding of p16 to cdk4, KLF6 expression results in the forced redistribution of p21 toward cdk2 complexes, likely enhanced by KLF6 binding to and catalytic inhibition of cyclin D1-cdk4 complexes in HCT116 cells. Thus, KLF6 targets cyclin/cdk complexes from two converging KLF6-mediated growth suppressive pathways—binding and disruption of cyclin D1-cdk4 holoenzyme, coupled to induction and titration of p21 onto cdk2 complexes (Fig. 7).

Regulation of cell growth by KLF6 was demonstrated after its transient transfection in HCT116 colon cancer cells leading to inhibition of DNA synthesis (15%), as well as G1-S arrest after transfection in these cells. Because HCT116 colon cancer cells are null for the prototypical CKI and tumor suppressor protein p16, these cells were used as a control to confirm effects on Rb phosphorylation and [3H]thymidine incorporation. A greater inhibition in [3H]thymidine incorporation was observed in KLF6-expressing cells compared to control cells.
dine incorporation (~35%) in the presence of overexpressed exogenous p16 might be explained by HCT116’s p16-null background, wherein its reintroduction has a more profound effect on DNA synthesis. Because HCT116 cells express relatively higher levels of endogenous KLF6 protein in comparison with PC3 (prostate cancer) as well as other colon cancer cell lines tested (1, 2), the effects of KLF6 overexpression might be more pronounced (or equal to that of p16) in a KLF6-null background.

In summary, our studies provide an additional mechanism of growth suppression by KLF6, which combined with its ability to induce p21, highlights its potential role in the complex regulatory network of cell cycle progression. These findings could have broader implications for further understanding mechanisms of growth dysregulation in human cancer(s) where KLF6 is inactivated.

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