

Research Paper

KLF4 and KLF5 Regulate Proliferation, Apoptosis and Invasion in Esophageal Cancer Cells

Yizeng Yang

Bree G. Goldstein

Hann-Hsiang Chao

Jonathan P. Katz*

Department of Medicine; Gastroenterology Division; University of Pennsylvania; School of Medicine; Philadelphia, Pennsylvania USA

*Correspondence to: Jonathan P. Katz; Department of Medicine; Gastroenterology Division; University of Pennsylvania School of Medicine; 600 Clinical Research Building; 415 Curie Boulevard; Philadelphia, Pennsylvania 19104-6144 USA; Tel.: 215.746.7780; Fax: 215.573.2024; Email: jpkatz@mail.med.upenn.edu

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KEY WORDS

KLF4, GKLF, KLF5, IKLF, TE cells, anoikis, proliferation, apoptosis, invasion, esophageal cancer

ABBREVIATIONS

ECM extracellular matrix
MTT 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide

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ABSTRACT

KLF4 and KLF5, members of the KLF family of transcription factors, play key roles in proliferation, differentiation, and carcinogenesis in a number of gastrointestinal tissues. While *KLF4* is expressed in differentiating epithelial cells, *KLF5* is found in proliferating cells of the gastrointestinal tract, including the esophagus. *KLF4* regulates a number of genes vital for esophageal epithelial differentiation, and decreased expression of *KLF4* is seen in esophageal squamous cancers. Nonetheless, the roles of *KLF4* and *KLF5* in esophageal tumor progression are not known. Here, using TE2 cells stably infected with retroviral vectors to express *KLF4* or *KLF5*, we demonstrate that *KLF4* and *KLF5* are key players in a number of cellular processes critical for esophageal carcinogenesis. TE2 cells, derived from a patient with poorly differentiated esophageal squamous cancer, normally lack *KLF4* and *KLF5*. Expression of *KLF5* in TE2 cells inhibits proliferation, and both *KLF4* and *KLF5* decrease viability after treatment with hydrogen peroxide and increase anoikis. In response to DNA damage from UV irradiation, viability is decreased in *KLF5* but not *KLF4* infected cells. Both *KLF4* and *KLF5* upregulate the cdk inhibitor *p21^{waf1/cip1}* following UV irradiation, but the pro-apoptotic protein BAX is markedly induced only by *KLF5*. Thus *KLF4* may preferentially activate DNA repair pathways while *KLF5* induces both DNA repair and apoptosis after UV irradiation. Expression of *KLF4* or *KLF5* in TE2 cells also inhibits invasion, consistent with a role for each in preventing tumor metastasis. In summary, *KLF4* and *KLF5* regulate esophageal carcinogenesis by affecting proliferation, apoptosis, and invasion.

INTRODUCTION

Esophageal cancer is the sixth leading cause of cancer deaths in the world and is nearly universally fatal, with long-term survival rates below 15%, despite improvements in preoperative management and surgical therapy.¹⁻³ The development of human esophageal squamous cell cancer is a multi-step process including esophageal basal cell hyperplasia, dysplasia, carcinoma in situ, and advanced carcinoma.⁴ The molecular aspects of this progression are related to a series of disorders in cell polarity, proliferation, differentiation and apoptosis with genetic alterations of cell adhesion molecules, growth factors, cell cycle regulators, and pro- and anti-apoptotic factors.⁵ Nonetheless, the specific epithelial transcription factors which regulate these processes in esophageal carcinogenesis are not fully established.

Recently, the *Krüppel*-like factors (KLF) family member *KLF4* (previously known as *GKLF*) was found to be downregulated in esophageal squamous cell cancer.^{6,7} KLFs are DNA-binding transcriptional regulators that play a diverse role during differentiation and development in a broad array of tissues.⁸ *KLF4* and *KLF5* (previously known as *IKLF*) are highly expressed in the epithelia of the gastrointestinal tract,⁹⁻¹¹ and both are critical regulators of gastrointestinal proliferation and differentiation.^{12,13} While *KLF4* is expressed in the differentiated compartment of gastrointestinal epithelia, *KLF5* is found in proliferating cells, such as the basal layer of the esophagus or the intestinal crypts.^{14,15} Interestingly, both *KLF4* and *KLF5* bind similar "GT-box" or "CACCC element" sites¹⁶ and have been shown to exert opposing effects on a number of genes, including *KLF4* itself.^{17,18} Thus *KLF4* and *KLF5* may coordinately regulate normal gastrointestinal homeostasis.

The effects of *KLF4* and *KLF5* on epithelial proliferation and differentiation suggest that these factors may also play key roles in gastrointestinal carcinogenesis. Consistent with this, *KLF4* transcriptionally regulates a number of genes critical for gastrointestinal tumor formation, including ornithine decarboxylase, *p21^{WAF1/CIP1}*, the *cyclin D1* oncogene, and *keratin 19*, which is linked to tumor progression in esophagus and pancreas.¹⁹⁻²² In addition, loss of *KLF4* is seen in gastrointestinal and other tumors, suggesting a function as a tumor

suppressor.²³⁻²⁵ *KLF5* is a downstream target of the Wnt pathway, shows transforming properties in NIH3T3 cells, and mediates the transforming properties of oncogenic H-ras.^{12,26,27} In nontransformed cells but not in transformed intestinal cells, *KLF5* activates *cyclin D1*.²⁸ Surprisingly, given its pro-proliferative function in a number of cell types,¹⁵ *KLF5* has been implicated as a tumor suppressor in breast, prostate, and intestinal cancers.²⁸⁻³⁰

A number of models systems exist which permit investigations of the molecular mechanisms of esophageal cancer. The TE-series of cells is a particularly valuable resource, comprising a total of 15 esophageal cancer cell lines derived from individual patients with well- to poorly-differentiated cancers.³¹ These cell lines have been well-characterized and exhibit various degrees of tumorigenicity in nude mice.³² In addition, the genetic alterations, including *p53* status, vary within these TE cell lines.^{33,34} TE cells can be readily transfected and have been utilized for studies of expression, gene regulation, and functional analyses.³⁵⁻³⁸

In the present study, we investigate the roles of KLF4 and KLF5 in esophageal carcinogenesis using poorly-differentiated TE-2 esophageal squamous cancer cells. TE-2 cells, which are wild-type for *p53*,³³ express high levels of EGFR,³⁷ and form tumors in nude mice,³² do not normally express KLF4 or KLF5. Expression of *KLF4* or *KLF5* in TE-2 cells by stable retroviral infection alters DNA damage response and apoptotic pathways, possibly through activation of p21^{waf1/cip1} and/or BAX, and inhibits cell invasion. These data suggest that KLF4 and KLF5 may play critical roles in esophageal tumor formation and progression.

MATERIALS AND METHODS

Cell culture and generation of Stable Cell Lines. Human esophageal squamous cancer cells (TE1, TE2 and TE11)³¹ were cultured at 37°C and 5% CO₂ in DMEM/F-12 (Invitrogen) supplemented with 5% bovine fetal serum (Invitrogen), 100 units/ml penicillin, and 100 µg/ml streptomycin. Human primary esophageal squamous cell lines, EPC1 and EPC2 (gift of Dr. Anil Rustgi), have been described previously.³⁹ EPC cells were grown at 37°C and 5% CO₂ in keratinocyte-SFM (KSFM) medium (Invitrogen), supplemented with 40 µg/ml bovine pituitary extract (Invitrogen), 1.0 ng/ml EGF (Invitrogen), 100 units/ml penicillin, and 100 µg/ml streptomycin.

Construction of retroviral expression vectors. pWZL- α -*KLF4* and pWZL- α -*KLF5* were constructed from the pWZL- α -*neo* retrovirus⁴⁰ (gift of Dr. Morris Birnbaum). Human *KLF4* and *KLF5* were amplified by PCR from human cDNA and sequenced and included the complete coding sequences for *KLF4* (NM_004235, #236-1821) or *KLF5* (NM_009769, #136-1577). We transfected pWZL- α -*neo*, pWZL- α -*KLF4* and pWZL- α -*KLF5* into HEK 293 cells by Lipofectamine 2000 (Invitrogen) following manufacturer's instructions, filtered culture supernatants from individual HEK 293 cells, and used these supernatants to infect TE-2 cells. Following infection, TE-2 cells were selected with G418 (Invitrogen) at 300 µg/ml for four weeks.

Western blotting. Cells were lysed with 150 mM NaCl, 50 mM Tris pH 7.5, 1%NP40, 0.5% sodium deoxycholic acid, and Complete Protease Inhibitor Cocktail Tablets (Roche) and protein was isolated. Thirty micrograms of total protein were separated on a NuPAGE 4–12% bis-tris acrylamide gel (Invitrogen) and transferred onto PVDF membrane (Millipore Corp) in 1X NuPage transfer buffer (Invitrogen) for 75 min at 4°C. Membranes were blocked with 5% nonfat dry milk in PBS and TBST for at least two hours at room temperature and incubated overnight at 4°C with the following primary antibodies: rabbit anti-KLF4 (generated against amino acids 84–98 of human KLF4 by Biosource International/QCB) at 1:5000 dilution; rabbit anti-KLF5 (generated against amino acids 106-122 of human KLF5 by Biosource International/QCB) at 1:5000; mouse anti-p21^{waf1/cip1} (BD) at 1:200; rabbit anti-BAX (Cell Signaling) at 1:1000; and mouse anti- β -actin

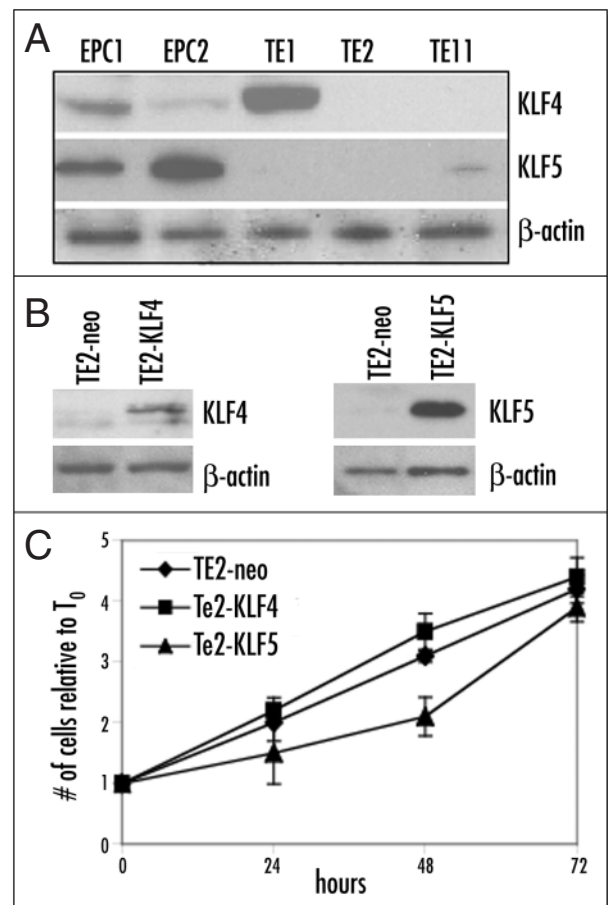


Figure 1. KLF4 and KLF5 were not expressed in TE2 esophageal cancer cells, and expression of KLF5 in TE2 cells altered proliferation. (A) Western blotting of human primary esophageal squamous cells (EPC1 and EPC2) revealed expression of both KLF4 and KLF5. Variations in levels of KLF4 and KLF5 was likely due to differing proportions of proliferating and differentiating cells in these cultures. KLF4 was highly expressed in TE1 cells, derived from a well-differentiated esophageal squamous cancer, and low levels of KLF5 were seen in the moderately-differentiated TE11 cells, while poorly-differentiated TE2 cells expressed neither KLF4 nor KLF5. β -actin served as loading control. (B) Western blot confirmed that the pWZL- α retroviral vector was sufficient to drive expression of KLF4 or KLF5 in TE2 cells. β -actin served as loading control. (C) By MTT assay, expression of KLF5 but not KLF4 inhibited proliferation of TE2 cells significantly at 48 hours ($p < 0.05$). The effects of KLF5 on proliferation were no longer significant by 72 hours.

(Sigma) at 1:2000. Membranes were then incubated for 45 min at room temperature with 1:3000 dilution of anti-rabbit/horseradish peroxidase (Amersham Pharmacia Biotech) for KLF4, KLF5, and BAX or anti-mouse/horseradish peroxidase (Amersham Pharmacia Biotech) for p21^{waf1/cip1} and β -actin, washed twice for 10 min, and developed with the Enhanced Chemiluminescence Plus Western blot analysis kit (Amersham Pharmacia Biotech).

MTT assay. After four weeks of selection in G418, 1×10^5 of TE-2 cells infected with pWZL- α -*neo*, pWZL- α -*KLF4* and pWZL- α -*KLF5* were plated per well in triplicate on a 6-well plate. To assay relative rates of proliferation, cells were observed for 0, 24, 48 and 72 hours after plating. H₂O₂ treatments were performed in cells at 80% confluence with dosages at 0, 50 and 100 µM for 24 hours. For studies of UV irradiation, cells at 80% confluence were exposed to UV light in a Stratalinker 2400 UV cross-linker (Stratagene) for 120, 240, and 360 mJ/cm² and incubated for 24 hours. For each treatment, media was removed and washed with PBS. MTT reagent (USB) was added at 2 mg/ml in Hank's buffer (Invitrogen) and incubated for 1 hour until

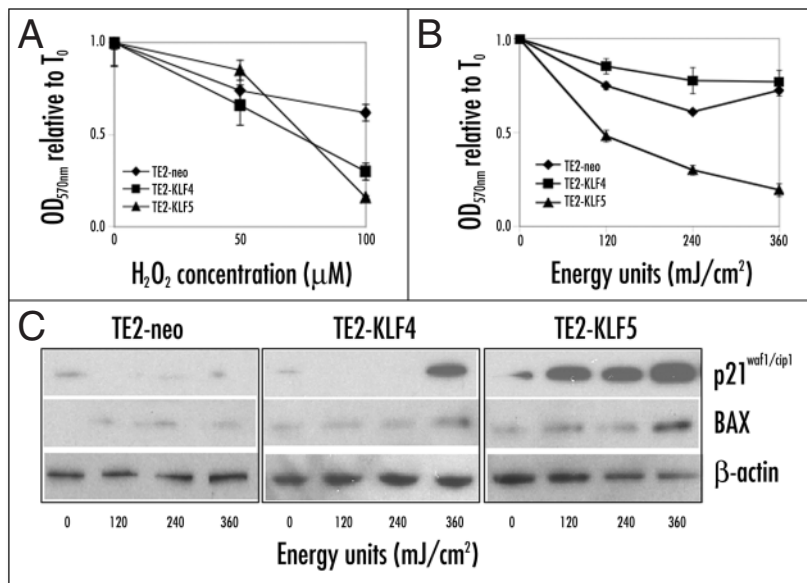


Figure 2. Expression of KLF4 or KLF5 altered cellular responses to DNA damage in TE2 cells. (A) Cell viability by MTT assay was decreased in TE2-KLF4 and TE2-KLF5 cells compared to control following treatment with H₂O₂. (B) While expression of KLF5 decreased viability of TE-2 cells by MTT assay following UV irradiation, KLF4 produced no significant effect on cell viability. (C) Western blots revealed upregulation of p21^{waf1/cip1} by both KLF4 and KLF5 in TE2 cells following UV irradiation, but the pro-apoptotic BAX was substantially increased only in TE2-KLF5 cells. β-actin served as loading control.

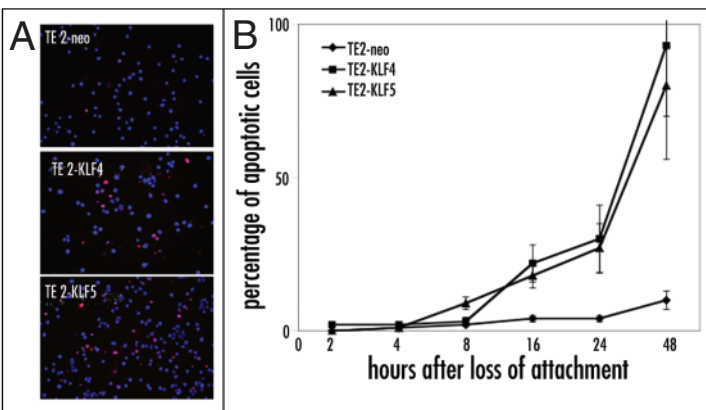


Figure 3. KLF4 and KLF5 increased anoikis in TE2 cells. (A) In control TE2-neo cells, only rare TUNEL positive (red) apoptotic cells were seen 24 hours after detachment. In contrast, numerous TE2-KLF4 and TE2-KLF5 cells were TUNEL positive 24 hours after detachment. Cells were counterstained with DAPI, in blue. (B) The percentage of apoptotic cells per field (200X) were counted at different time points. By 48 hours after loss of attachment, nearly all TE2-KLF4 and TE2-KLF5 cells had undergone apoptosis, compared to only 10% of control cells.

brown crystals were seen in the cytoplasm under light microscopy. The crystals were dissolved in DMSO and the absorbance measured at 570 nm with reference at 650 nm in a DU 600 spectrometer (Beckman). Results for each condition were expressed as the mean of A₅₇₀ relative to T₀ ± SEM.

Cell migration and invasion assay. Cell migration and invasion assays were performed as described previously.³⁹ In brief, after serum deprivation for 24 hours, single cell suspensions containing 4 × 10⁴ cells/well in 0.5 ml KBM (BioWhittaker) were plated into 24-well inserts (Falcon cell culture inserts, 8 μm pore size) with or without Matrigel (BD). The lower chamber

was filled with 0.6 ml of KBM containing 0.5 μg/ml hydrocortisone (Sigma) and 10 ng/ml EGF (BD). After incubation for 16 hours at 37°C, the cells on the upper side of transwell membrane were removed by cotton swab and rinsed with PBS. Cells migrating to the lower side of membrane were fixed in 4% paraformaldehyde for 20 min at room temperature, stained with crystal violet (Sigma), photographed, and counted.

Anoikis assay. The anoikis assay⁴¹ was performed by coating 6-well plates with poly-2-hydroxyethylmethacrylate (Sigma) and plating 2 × 10⁶ cells in 2 ml of (DMEM/F-12 (Invitrogen) supplemented with 5% bovine fetal serum (Invitrogen), 100 units/ml penicillin, and 100 μg/ml streptomycin). Cells were incubated for 2, 4, 8, 16, 24 and 48 hours at 37°C. The suspended cells were transferred in triplicate into chamber slides coated with poly-L-lysine (Sigma), incubated for 1 hour at 37°C, washed with PBS, and fixed with 4% paraformaldehyde. Apoptotic cells were stained using the TUNEL method with the In Situ Cell Death Detection Kit (Roche) according to the manufacturer's protocol. Apoptotic cells were counted in five different fields (200X) from each chamber and expressed as the mean of the ratio of the number of apoptotic cells per field to the number of total cells ± SEM.

RESULTS

Expression of KLF4 and KLF5 in non-transformed and transformed esophageal cell lines. To investigate the expression of KLF4 and KLF5 in nontransformed and transformed esophageal epithelial cells, we performed Western blotting of

whole cell protein lysate from human primary esophageal cell lines, EPC1 and EPC2³⁹ as well as the cancer cell lines TE1, TE2 and TE11,³¹ derived from well-, poorly- and moderately-differentiated esophageal squamous cell cancers (Fig. 1A). As expected, primary esophageal keratinocytes expressed KLF4 and KLF5, although the relative ratios varied among EPC1 and EPC2 cells, likely due to differing proportions of proliferating (basal) and differentiating (suprabasal) cells in these cultures. TE1 cells expressed KLF4 but not KLF5, while TE11 cells had some KLF5 expression but lacked KLF4. In contrast, the poorly-differentiated TE2 cells expressed neither KLF4 nor KLF5. We chose, therefore, to focus further experiments on TE2 cells.

We stably infected TE2 cells with pWZL-α-KLF4 (TE-KLF4) or pWZL-α-KLF5 retroviral vectors (TE-KLF5), which were engineered for these purposes to constitutively express KLF4 or KLF5, or with pWZL-α-neo (TE-neo), control (Fig. 1B). To determine relative rates of proliferation, we performed MTT assays and measured the absorbance relative to T₀. Transduction of TE2 cells with KLF5, but not KLF4, initially decreased cell proliferation significantly compared to control cells (Fig. 1C). However, these changes were no longer significant at 72 hours.

KLF4 and KLF5 alter viability of esophageal cancer cells in response to DNA damage. The responsiveness of esophageal squamous cell cancer to chemo- and radiotherapy is influenced by the propensity of the tumor cells to undergo apoptosis, and the apoptotic index is generally higher in well-differentiated than in poorly-differentiated tumors.⁴² Radiation therapy alone is rarely curative for esophageal cancer,³ and, while a number of genes have been linked to radioresistance in esophageal cancer cells,³⁵ the mechanisms of this resistance are not clear. Given their putative roles as tumor suppressors^{23-25,28-30} and in modulating response to DNA damage^{19,43-45} in other tissues and cell types, we postulated that expression of KLF4 or KLF5 could alter the responses to DNA damage in esophageal cancer cells.

Reactive oxygen species induce DNA damage, leading to apoptosis and cell death, and have also been implicated in tumor progression by promoting proliferation and cell survival.⁴⁶ The mechanisms by which cells respond to reactive oxygen species depend on a number of interacting pathways.⁴⁷ To investigate the roles of KLF4 and KLF5 in the response of esophageal cancer

cells to DNA damage induced by reactive oxygen species, we treated TE2-neo, TE2-KLF4 and TE2-KLF5 cells with hydrogen peroxide (H_2O_2). When TE2 cells were treated with $100 \mu M H_2O_2$, expression of both KLF4 and KLF5 resulted in decreased cell viability compared to control infected cells (Fig. 2A), implicating both KLF4 and KLF5 in the regulation of cell survival following oxidative stress.

To assess cell viability in response to DNA damage by UV radiation, we treated cells with increasing doses of UV irradiation. While cell viability of TE2-KLF4 cells was not significantly altered compared to control infected cells, expression of KLF5 resulted in a dramatic decrease in cell viability, which was more marked at higher doses (Fig. 2B). To understand the mechanisms of these effects, we investigated the expression of the cdk inhibitor $p21^{waf1/cip1}$, which mediates growth arrest and repair after DNA damage,⁴⁸ and the pro-apoptotic protein BAX.⁴⁹ In response to UV irradiation, both KLF4 and KLF5 produce a dose-dependent increase in $p21^{waf1/cip1}$ (Fig. 2C), although the induction of $p21^{waf1/cip1}$ occurs at lower doses in TE-KLF5 than TE-KLF4 cells. In contrast, BAX is markedly induced only by KLF5. Thus, KLF4 may preferentially activate DNA repair pathways while KLF5 induces both DNA repair and apoptosis after UV irradiation.

KLF4 and KLF5 induce anoikis in esophageal cancer cells. Normal epithelial cells depend upon interactions with the extracellular matrix (ECM) for survival, and the loss of this interaction induces apoptosis. This type of suspension-induced apoptosis is termed anoikis.^{50,51} Anoikis plays an important role in the development of normal tissues, and resistance to anoikis has been described in many types of human malignancies including osteosarcoma, and gastric, colon, lung, and oral squamous cancers.⁵²⁻⁵⁶ This resistance to anoikis allows increased survival time in the absence of attachment to the extracellular matrix and permits eventual reattachment at secondary sites.⁵⁷ A number of signaling cascades have been implicated in anoikis, including the phosphoinositide-3 kinase (PI3K)/Akt and MAP kinase pathways, although suppression of anoikis is independent of both PI3K and MAP kinase in some cell types, suggesting the presence of other effector pathways.^{52,58,59}

To determine if expression of KLF4 or KLF5 influenced suspension-induced apoptosis in esophageal squamous cancer cells, we evaluated apoptosis following cell detachment in TE2-neo, TE2-KLF4, and TE2-KLF5 cells. By TUNEL staining, apoptotic cells were rarely seen in TE2-neo after 24 hours of detachment (Fig. 3A). In contrast, numerous apoptotic cells were seen in TE2-KLF4 and TE2-KLF5 cells. The expression of KLF4 or KLF5 dramatically increased the number of cells undergoing anoikis such that, by 48 hours after loss of attachment, nearly all TE2-KLF4 and TE2-KLF5 cells were undergoing apoptosis (Fig. 3B). Thus, expression of either KLF4 or KLF5 restores sensitivity to anoikis in esophageal cancer cells.

KLF4 and KLF5 inhibit invasion of esophageal cancer cells. Cell migration is a complex process which is essential for tissue morphogenesis in the embryo and for repair and regeneration of adult epithelia and which drives disease progression in cancer.⁶⁰ To investigate whether KLF4 and KLF5 could contribute to esophageal cancer progression by altering tumor cell migration and invasion, we performed migration and invasion assays on TE2-neo, TE2-KLF4, and TE2-KLF5 cells. When esophageal cancer cells were grown on uncoated transwell plates, the number of cells migrating through the membrane was markedly decreased in TE2-KLF4 cells compared to TE2-neo control cells (Fig. 4A–B). On transwell plates coated with Matrigel, cell invasion was inhibited significantly by expression of either

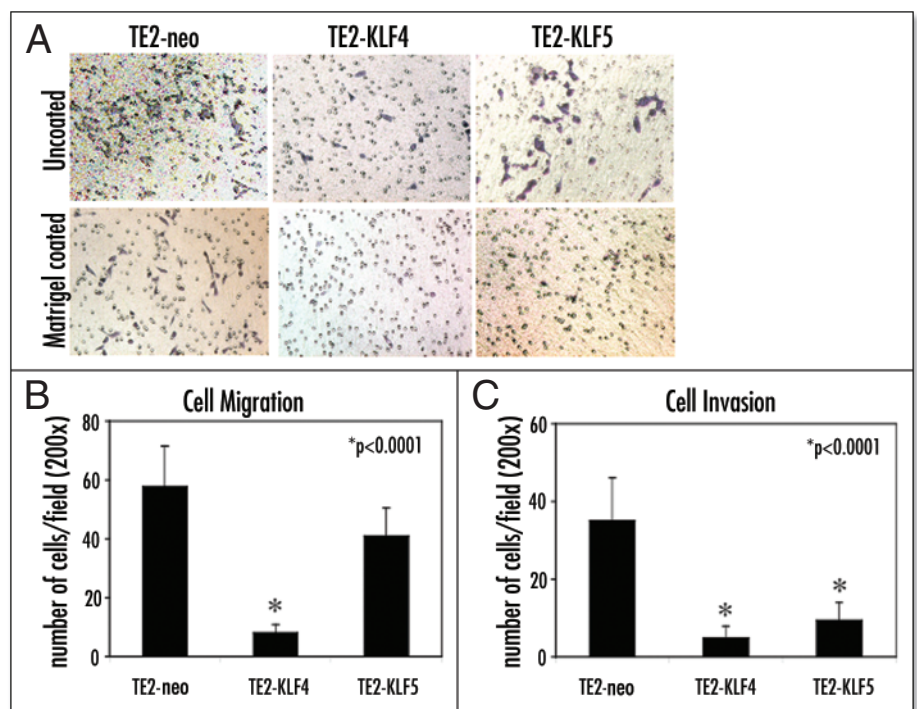


Figure 4. KLF4 and KLF5 inhibited cell migration and/or invasion in TE2 cells. (A) TE2 cells passing through the membrane of a transwell plate were stained purple with crystal violet and photographed to reveal changes in migration (uncoated plates) or invasion (Matrigel coated plates). (B and C) While only KLF4 substantially inhibited cell migration in TE2 cells (B), expression of either KLF4 or KLF5 decreased invasion of TE2 cells though Matrigel (C). Magnification = 100X (A).

KLF4 or KLF5 (Fig. 4A and C). Thus KLF4 and KLF5 alter tumor cell invasion, a hallmark of cancer progression and metastasis.

DISCUSSION

Esophageal cancer is one of the least studied and deadliest cancers worldwide.³ Once cancer develops, it usually spreads rapidly, and more than half of patients with esophageal cancer have either unresectable tumors or radiographically visible metastases at the time of diagnosis. A number of key steps occur during cancer progression, including alterations in control of cell proliferation and survival, evasion of programmed cell death, and tumor cell invasion and metastases.⁶¹ In particular, the ability of cancer cells to survive after loss of attachment to the ECM is critical for tumor cell invasion and metastases.⁵⁷

KLF4 and KLF5 are two related epithelial *Krüppel*-like factors with reportedly disparate roles in cell proliferation and differentiation.¹⁵ Nonetheless, both proteins have been implicated as tumor suppressors in a number of tissues,^{23-25,28-30} and KLF4 was recently described to be downregulated in esophageal squamous cell cancer.^{6,7} To investigate the roles of KLF4 and KLF5 in esophageal carcinogenesis, we stably infected poorly-differentiated TE2 esophageal squamous cancer cells, which normally express neither KLF4 nor KLF5, with retroviral expression vectors for KLF4 or KLF5. Using this system, we show that expression of KLF4 or KLF5 alters cell viability, apoptosis, and invasion in esophageal cancer cells and that KLF5 inhibits cell proliferation.

While KLF5 has been reported to be pro-proliferative in non-transformed cells,¹² the inhibition of proliferation in esophageal cancer cells by KLF5 is consistent with effects seen in transformed intestinal

cell lines.²⁸ In the intestine, KLF5 is normally expressed in cells of the small intestinal crypts and at the bases of the colonic crypts.⁹ However, KLF5 is downregulated in adenomas from *APC*^{min} mice and human FAP patients, as well as in Ras-transformed IEC-18 and IMCE cells.²⁸ KLF5 has been shown in a number of contexts to be both a transcriptional activator and a repressor.^{17,18,62} KLF5 activates *cyclin D1* in nontransformed intestinal cells and also in H-Ras transformed NIH3T3 cells but not in Ras transformed IEC-18 and IMCE intestinal cells.^{27,28} Thus, the function of KLF5 appears to be context dependent. In esophageal epithelial cells, KLF5 may differentially regulate *p21^{waf1/cip1}*, *BAX*, and other genes in transformed and non-transformed cells. We are currently investigating the effects of KLF4 and KLF5 on proliferation, migration, apoptosis, and gene regulation in primary esophageal squamous cells.

In response to reactive oxygen species or anoikis, expression of KLF4 or KLF5 in esophageal cancer cells decreases cell survival. The role of each of these factors in anoikis is likely to be especially key in malignant progression, given the importance of resistance to anoikis in malignant progression and metastatic spread.⁵⁷ Following UV irradiation, however, the effects of KLF4 and KLF5 diverge, with only KLF5 leading to significant changes in cell viability. An explanation for these findings is the effects of KLF4 and KLF5 on *p21^{waf1/cip1}* and *BAX*. In response to DNA damage, *p21^{waf1/cip1}* mediates growth arrest and repair⁴⁸ while expression of *BAX* promotes apoptosis.⁴⁹ Both *p21^{waf1/cip1}* and *BAX* are regulated by *p53*,⁶³ which has been shown to interact with KLF4,^{19,44} and TE-2 cells are wild-type for *p53*.³³ Thus, expression of KLF4 or KLF5 may determine the response of the cell to DNA damage following UV irradiation, either growth arrest and repair or programmed cell death. Notably, *p21^{waf1/cip1}* polymorphisms and downregulation of *BAX* have been described in esophageal cancer.⁶⁴⁻⁶⁶

KLF4 and KLF5 also inhibit invasion in esophageal cancer cells. Thus both proteins influence two critical aspects of tumor cell metastasis, invasion and anchorage independent growth.⁶⁷ Interestingly, when cells are grown on uncoated plates, KLF4 but not KLF5 markedly inhibits cell migration, suggesting that attachment to the ECM is critical for the effects of KLF5 on cell migration but not for KLF4. One possibility is that KLF5 might modulate the expression of some cell surface receptors, such as the integrins which link cells to the ECM and play a key role in carcinogenesis.⁶⁸ Different integrins have different functions, and cancer cells can switch or turn-off the expression of certain integrins,⁶⁹ influencing behaviors such as motility, resistance to apoptosis, and growth arrest or entrance into the active cell cycle.⁶¹ As KLF5 alters cell migration/invasion only in the presence of the ECM, members of the integrin pathway would be ideal targets for KLF5. Investigations of the effects of KLF5 on integrin signaling are currently underway. While the mechanisms of KLF4 mediated inhibition of migration and invasion are not clear, these effects are similar to those seen in colon cancer cells.⁷⁰

In summary, we demonstrate that KLF4 and KLF5 regulate esophageal carcinogenesis by affecting proliferation, apoptosis, and cell invasion. When restored to activity in TE2 esophageal cancer cells, which normally lack KLF4 and KLF5, both proteins can reverse many of the tumorigenic properties of TE2 cells, functions consistent with tumor suppressor genes.⁷¹ However, the identification of these factors as tumor suppressor genes in esophagus will require further study. Nonetheless, given their roles in numerous cellular processes critical for esophageal carcinogenesis, both KLF4 and KLF5 may provide valuable diagnostic markers or therapeutic targets for esophageal cancer.

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