Krüppel-like factors 4 and 5: the yin and yang regulators of cellular proliferation

Amr M. GHALEB[1], Mandayam O. NANDAN[1], Sengthong CHANCHEVALAP[1], W. Brian DALTON[1], Irfan M. HISAMUDDIN[1], Vincent W. YANG[1,2,*]

[1]*Division of Digestive Diseases, Department of Medicine, Emory University School of Medicine, Atlanta, Georgia, USA.*
[2]*Department of Hematology and Oncology, Winship Cancer Institute, Emory University School of Medicine, Atlanta, Georgia, USA*

ABSTRACT

Krüppel-like factors (KLFs) are evolutionarily conserved zinc finger-containing transcription factors with diverse regulatory functions in cell growth, proliferation, differentiation, and embryogenesis. KLF4 and KLF5 are two closely related members of the KLF family that have a similar tissue distribution in embryos and adults. However, the two KLFs often exhibit opposite effects on regulation of gene transcription, despite binding to similar, if not identical, *cis*-acting DNA sequences. In addition, KLF4 and 5 exert contrasting effects on cell proliferation in many instances; while KLF4 is an inhibitor of cell growth, KLF5 stimulates proliferation. Here we review the biological properties and biochemical mechanisms of action of the two KLFs in the context of growth regulation.

Keywords: cancer, cell cycle, KLF, transcription, transformation, zinc fingers.

INTRODUCTION

The regulation of gene expression in response to intrinsic and extrinsic cues is a fundamental cellular process in the growth and development of organisms. Critical to the control of gene expression are transcription factors that bind to specific DNA sequences and subsequently modulate gene transcription [1]. A significant number of transcription factors use a conserved zinc finger domain to bind their target DNAs. In fact, the human genome encompasses over 700 genes that contain a particular C2H2-type of zinc finger, which employs two cysteine and two histidine amino acid residues to coordinate the single zinc atom in the finger-like structure [2]. A further subgroup of the C2H2-zinc finger proteins exhibits homology to the *Drosophila melanogaster* segmentation gene product, Krüppel [3]. Members of this subgroup are termed Krüppel-like factors (KLFs), and many KLFs exhibit tissue-selective expression and wide-ranging regulatory functions [4]. This review will focus on two members of the KLF family of transcription factors, Krüppel-like factor 4 (KLF4) and Krüppel-like factor 5 (KLF5), which are enriched in epithelial tissues but demonstrate contrasting biological activities.

Identification and Initial Characterization of KLF4 and KLF5

A full-length mouse cDNA clone encoding KLF4 (also called gut-enriched Krüppel-like factor or GKLF) was initially isolated from a NIH3T3 cDNA library by reduced-stringency screening with a DNA probe containing the zinc finger region of an immediate early gene product, Zif268 or Egr1 [5]. Mouse KLF4 contains 483 amino acids, has a predicted molecular weight of 53 kD, and is 90% identical to human KLF4. The carboxyl terminus of KLF4 contains three C2H2-zinc fingers that are most closely related to another member of the family, KLF2 [4]. KLF4 is a nuclear protein whose cellular address depends on two nuclear localization signals [6]. A survey of the tissue distribution in adult mice revealed that *KLF4* is highly expressed in terminally differentiated, post-mitotic epithelial cells of the intestinal tract [5], a finding consistent with the anti-proliferative effect of KLF4 (see below).

KLF5 (also called intestinal-enriched Krüppel-like factor or IKLF) was initially isolated based on close homology to KLF2 [7]. The coding region of mouse KLF5 contains 446 amino acids and is 88% identical to the human sequence, previously identified as basic transcription element binding

*Correspondence: Vincent W. Yang,
Tel: +404-727-5638; Fax: +404-727-5767
E-mail: vyang@emory.edu

Abbreviations: BTE, basic transcription element; BTEB2, basic transcription element binding protein 2; CYP1A1, cytochrome P-450IA1; KLF, Krüppel-like factor; MAPK, mitogen-activated protein kinase.

protein 2 or BTEB2 [8]. Like *KLF4*, *KLF5* is expressed at a relatively high level in the intestinal tract, its expression is concentrated at the base of the crypt epithelium where active cell division occurs [7].

Expression of both *KLF4* and *KLF5* genes are developmentally regulated, with a higher level of expression occurring toward the later stage of fetal development [9, 10]. Depending on the tissues, both overlapping and mutually exclusive patterns of expression have been observed for the two genes. The respective expression of *KLF4* and *KLF5* in differentiated, post-mitotic villus and proliferative crypt epithelial cells of the adult intestinal tract also occurs during embryonic development [10]. The cellular distribution of *KLF4* and *KLF5* transcripts in the epidermis of the skin in the late stage embryo mirrors that of intestinal epithelium, wherein *KLF4* is more highly expressed in terminally differentiated, quiescent suprabasal layer and *KLF5* in the proliferative basal layer of the epidermis [10].

KLF4 and KLF5 bind to similar DNA sequences but exert opposing transcriptional regulatory activities

Like many other zinc finger-containing transcription factors, KLF4 and KLF5 bind to *cis*-DNA elements that are GC-rich. A consensus DNA binding sequence was empirically determined for KLF4 [11]. This sequence is present in many gene promoters and includes the CACCC element and the basic transcription element (BTE). Indeed, KLF4 was subsequently shown to inhibit the promoter of the cytochrome P-450IA1 (*CYP1A1*) gene in a BTE-dependent manner [12]. In contrast, BTEB2, the human homolog of KLF5, activates transcription through the BTE element [8]. KLF4 and 5 have also been shown to antagonize each other in controlling expression of other genes, despite the two sharing very similar, if not identical, *cis*-DNA sequences [13]. An example is the gene encoding KLF4 itself. Here KLF4 is an activator of the *KLF4* promoter, while KLF5 is an inhibitor, even though both proteins interact with the same *cis*-element [13]. Moreover, KLF5 abrogates the activating effect of KLF4 on the *KLF4* promoter and KLF4 abrogates the inhibitory effect of KLF5 on the same promoter. A potential reason for this competing effect is due to physical competition of the two proteins in binding to a cognate sequence in the *KLF4* promoter [13]. A similar competing effect between KLF4 and KLF5 in the transcription of several other genes has also been described, including those encoding smooth muscle α-actin [14] and laminin-1 [15].

KLF4 and 5 exhibit contrasting effects on cellular proliferation

The specificity of *KLF4* expression for terminally differentiated, post-mitotic intestinal epithelial cells, rather than proliferating crypt cells, prompted Shields *et al* to examine the behavior of *KLF4* in cultured cell systems [5]. *In vitro*, the level of *KLF4* mRNA correlates with the proliferative state of cells in a manner similar to that seen *in vivo*; serum-deprived quiescent NIN3T3 cells contain a significant amount of *KLF4* mRNA, while actively proliferating cells express little, if any *KLF4*. When serum-deprived cells are stimulated into proliferation by the addition of fresh serum, the level of *KLF4* mRNA decreases. Conversely, *KLF4* mRNA levels are increased as proliferating NIH3T3 cells become growth-arrested by either serum starvation or contact inhibition. Finally, forced expression of *KLF4* by transfection of proliferating cells results in an inhibition of DNA synthesis [5]. From these observations, it was concluded that *KLF4* is a growth arrest-associated gene.

A telling mechanism by which KLF4 inhibits DNA synthesis came from studies that examined the response of *KLF4* expression to DNA damage-induced growth arrest. DNA damage caused by methyl methanesulfonate (MMS) [16] or γ irradiation [17] leads to the induction of *KLF4* expression in a p53-dependent manner. The increase in *KLF4* mRNA level parallels the increase in the level of $p21^{WAF1/Cip1}$ mRNA, a major cyclin-dependent kinase inhibitor [16]. Importantly, KLF4 was shown to transactivate the $p21^{WAF1/Cip1}$ promoter by binding to a specific Sp1-like *cis*-element in the proximal $p21^{WAF1/Cip1}$ promoter. This same element is also needed for p53 to activate $p21^{WAF1/Cip1}$ transcription, although p53 does not directly bind to it. Instead, p53 and KLF4 physically interact with each other, thus allowing p53 to gain access to the $p21^{WAF1/Cip1}$ promoter and activate $p21^{WAF1/Cip1}$ transcription [16]. A consequence of the p53-dependent activation of $p21^{WAF1/Cip1}$ expression following DNA damage is an arrest in the cell cycle at both the G_1/S and G_2/M transition points. KLF4 was shown to be necessary and sufficient in mediating the checkpoint function of p53 at both of these transition points [17, 18]. KLF4 accomplishes this task both through its transcriptional activation of $p21^{WAF1/Cip1}$ and through direct suppression of *cyclin D1* [19] and *cyclin B1* expression [18], which are required for the G_1/S and G_2/M transitions, respectively. Expression profiling of KLF4 using cDNA microarray analysis confirmed that KLF4 activates transcription of many additional genes encoding inhibitors of the cell cycle and suppresses those encoding promoters of the cell cycle [20]. Recent studies from our laboratory indicate that KLF4 is also involved in preventing centrosome amplification after γ irradiation [21] and possibly in preventing aneuploidy after spindle damage (Dalton and Yang, unpublished observations). Taken together, these studies point to a wide-ranging and crucial effect of KLF4 in maintaining the integrity of the cell cycle. The essential

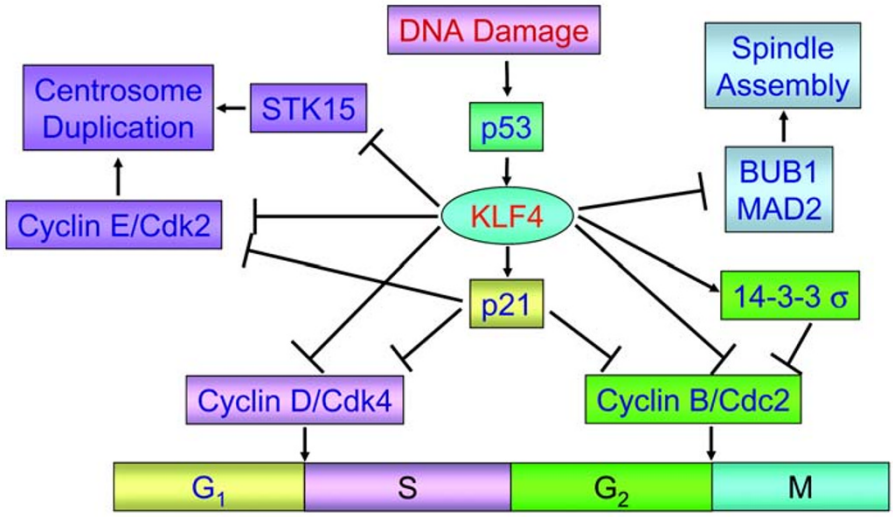

Fig 1. A model by which KLF4 mediates the cell cycle checkpoint functions of p53 in response to DNA damage. The tumor suppressor p53 is activated in response to DNA damage, which in turn activates KLF4. KLF4 then exerts a multitude of effects on expression of downstream genes by activating (→) or inhibiting their expression (⊣). References in support of the specific pathways are as follows: p53→KLF4→p21 [16]; KLF4⊣ cyclin D/Cdk4 [19]; KLF4⊣ cyclin B/Cdc2 [18]; KLF4⊣ cyclin E/Cdk2 [21]; KLF4⊣ STK15 [20]; KLF4→14-3-3 σ ; KLF4⊣ BUB1/MAD2 [20].

role of KLF4 in mediating the DNA damage response of the cell cycle is summarized in Fig. 1.

The observation that KLF4 exerts an important checkpoint function suggests that KLF4 may have a tumor suppressor effect. Indeed, the levels of *KLF4* mRNA are significantly reduced in intestinal adenomas in the $Apc^{MIN/+}$ mice and familial adenomatous polyposis (FAP) patients when compared to matched normal tissues [22]. A similar reduction in *KLF4* mRNA levels is seen in colorectal cancer specimens when compared to matched normal colonic tissues [23]. Moreover, there is evidence for loss of heterozygosity (LOH) in the *KLF4* locus in a subset of colorectal cancer specimens [23]. Conversely, re-expression of *KLF4* in a colorectal cancer cell line results in diminished tumorigenecity [24]. Collectively, these findings indicate that KLF4 is a potential tumor suppressor in colorectal cancer. A similar down-regulation and growth suppressive effect of KLF4 has also been described in bladder cancer [25].

In contrast to the growth inhibitory effect of KLF4, KLF5 has a growth promoting effect in cultured cells based on a number of studies. The level of *KLF5* mRNA is low in serum-deprived, quiescent NIH3T3 cells, but is acutely and significantly increased upon addition of serum or phorbol ester [26]. Constitutive expression of *KLF5* in NIH3T3 [26] and intestinal epithelial cells, IEC6 [27], results in an increased rate of proliferation and, eventually, a transformed phenotype as characterized by anchorage-independent growth [26] (Chanchevalap and Yang, unpublished observations). The level of *KLF5* transcript is also elevated in NIH3T3 cells transformed by oncogenic H-Ras, and this increase mediates the transforming effect of oncogenic H-Ras [28]. Fig. 2 illustrates the mechanism by which KLF5 mediates this growth-promoting function of oncogenic H-Ras. The increase in *KLF5* expression is a direct result of Egr1, which is activated by mitogen-activated protein kinase (MAPK), which is itself stimulated by oncogenic H-Ras. The increase in KLF5 then stimulates expression of the genes encoding cyclin D1 [28], cyclin B1, and Cdc2 (Nandan and Yang, unpublished observations), a change which culminates in accelerated cell proliferation and subsequent transformation. The importance of KLF5 in mediating cell proliferation is further demonstrated by the recent finding that the inhibitory effect of all-*trans* retinoic acid (ATRA) on proliferation of the intestinal epithelial cells, IEC6, is mediated by suppression of *KLF5* expression [27].

KLF4 and KLF5 exert distinct physiologic functions *in vivo* as revealed by gene knockout experiments

Transgenic experiments involving targeted deletion of *Klf4* and *Klf5* genes in mice have demonstrated that the two genes exhibit distinct physiologic functions *in vivo*. *Klf4* is normally expressed in the differentiating layers of the epidermis. $Klf4^{-/-}$ mice die shortly after birth and exhibit a defect in skin barrier function [29]. Conversely, ectopic overexpression of *Klf4* in the epidermis results in accelerated formation of the skin permeability barrier [30]. Additional studies have revealed that the critical role of

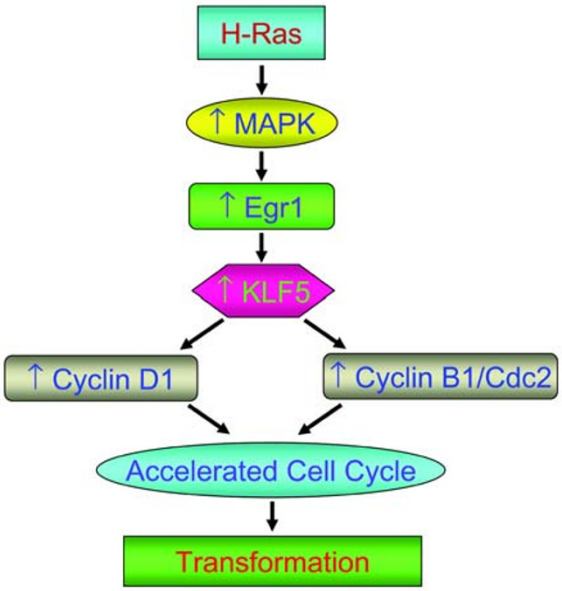

Fig. 2 KLF5 is central in mediating the transforming effect of oncogenic H-Ras. The sequence of events from oncogenic H-Ras to KLF5 and subsequent target gene expression is shown. MAPK is mitogen-activated protein kinase. See reference [28] for detail. The effect of KLF5 on cyclin B1/Cdc2 expression is unpublished.

KLF4 in maintaining barrier function is probably based on its ability to coordinately regulate gene clusters that are involved in such function including the Sprr [31] and keratin families [20]. KLF4 is also needed for terminal differentiation of goblet cells in the colon. *Klf4*$^{-/-}$ mice demonstrate a 90% reduction in the number of colonic goblet cells, as well as abnormal expression of goblet cell-specific markers [32].

Klf5$^{-/-}$ mice are embryonic lethal, but experiments using *Klf5*$^{+/-}$ mice showed that KLF5 is an important mediator of cardiovascular remodeling upon external stress. *KLF5* is normally markedly induced in activated vascular smooth muscle cells and fibroblasts. In response to external stress such as injury induced by a vascular cuff, *Klf5*$^{+/-}$ mice showed diminished levels of arterial wall thickening, angiogenesis, cardiac hypertrophy and interstitial fibrosis [33]. This physiologic effect of KLF5 is in part mediated by its ability to activate expression of genes encoding platelet-derived growth factor-A (PDGF-A) and transforming growth factor-β (TGF-β) [33].

Can KLF4 and KLF5 reverse their biological behavior in certain tumors?

The studies above demonstrate that KLF4 and KLF5 exhibit tumor suppressor and oncogenic activities, respectively, in a number of experimental systems. Can their effects be reversed in different settings? Indeed, several studies have indicated that they can. For example, expression of *KLF4* is increased in dysplastic oral squamous epithelium [34]. KLF4 mRNA and protein levels are also increased during progression of breast cancer [35], and nuclear localization of KLF4 is associated with an aggressive phenotype in early stage breast cancer [36]. In contrast to the pro-proliferative effect of KLF5 shown above, loss of *KLF5* expression has been observed in prostate [37] and breast cancer [38]. Moreover, KLF5 has been shown to reduce colony formation in transformed intestinal epithelial cells [39]. Therefore it appears that the biological behavior of KLF4 and 5 may change in the context of different tumor models, although the reasons for this are not well understood.

CONCLUSIONS

KLF4 and KLF5 are two members of the Krüppel-like factor family of transcription factors that exert important biological effects on cellular proliferation and differentiation *in vivo* and *in vitro*. Despite a close homology and similar developmental and tissue patterns of expression, the two KLFs exert very different, often opposing, effects on regulation of gene transcription and cellular proliferation. Both proteins also play a significant role in the process of tumorigenesis. Further characterization of KLF4 and 5 may advance the understanding of the molecular mechanisms regulating cellular proliferation and tumor formation.

ACKNOWLEDGEMENTS

This work was in part supported by grants from the National Institutes of Health (DK52230, DK64399 and CA84197). Vincent W.YANG is the recipient of a Georgia Cancer Coalition Distinguished Cancer Clinician Scientist Award. W. Brian. DALTON is a member of the Medical Scientist Training Program of Emory University School of Medicine.

REFERENCES

1 Yang VW. Eukaryotic transcription factors: identification, characterization and functions. J Nutr 1998; **128:**2045-51.
2 Lander ES, Linton LM, Birren B, et al. Initial sequencing and analysis of the human genome. Nature 2001; **409:**860-921.
3 Schuh R, Aicher W, Gaul U, et al. A conserved family of nuclear proteins containing structural elements of the finger protein encoded by Krüppel, a *Drosophila* segmentation gene. Cell 1986; **47:**1025-32.
4 Dang DT, Pevsner J, Yang VW. The biology of the mammalian Krüppel-like family of transcription factors. Int J Biochem Cell Biol 2000; **32:**1103-21.
5 Shields JM, Christy RJ, Yang VW. Identification and characterization of a gene encoding a gut-enriched Krüppel-like factor expressed during growth arrest. J Biol Chem 1996; **271:**20009-17.

6 Shields JM, Yang VW. Two potent nuclear localization signals in the gut-enriched Krüppel-like factor define a subfamily of closely related Krüppel proteins. J Biol Chem 1997; **272:**18504-7.
7 Conkright MD, Wani MA, Anderson KP, Lingrel JB. A gene encoding an intestinal-enriched member of the Krüppel-like factor family expressed in intestinal epithelial cells. Nucleic Acids Res 1999; **27:**1263-70.
8 Sogawa K, Imataka H, Yamasaki Y, et al. cDNA cloning and transcriptional properties of a novel GC box-binding protein, BTEB2. Nucleic Acids Res 1993; **21:**1527-32.
9 Ton-That H, Kaestner KH, Shields JM, Mahatanankoon CS, Yang VW. Expression of the gut-enriched Krüppel-like factor gene during development and intestinal tumorigenesis. FEBS Lett 1997; **419:**239-43.
10 Ohnishi S, Laub F, Matsumoto N, et al. Developmental expression of the mouse gene coding for the Krüppel-like transcription factor KLF5. Dev Dyn 2000; **217:**421-9.
11 Shields JM, Yang VW. Identification of the DNA sequence that interacts with the gut-enriched Krüppel-like factor. Nucleic Acids Res 1998; **26:**796-802.
12 Zhang W, Shields JM, Sogawa K, Fujii-Kuriyama Y, Yang VW. The gut-enriched Krüppel-like factor suppresses the activity of the CYP1A1 promoter in an Sp1-dependent fashion. J Biol Chem 1998; **273:**17917-25.
13 Dang DT, Zhao W, Mahatan CS, Geiman DE, Yang VW. Opposing effects of Krüppel-like factor 4 (gut-enriched Krüppel-like factor) and Krüppel-like factor 5 (intestinal-enriched Krüppel-like factor) on the promoter of the Krüppel-like factor 4 gene. Nucleic Acids Res 2002; **30:**2736-41.
14 Liu Y, Sinha S, Owens G. A transforming growth factor-beta control element required for SM alpha-actin expression *in vivo* also partially mediates GKLF-dependent transcriptional repression. J Biol Chem 2003; **278**:48004-11.
15 Piccinni SA, Bolcato-Bellemin AL, Klein A, et al. Krüppel-like factors regulate the Lama1 gene encoding the laminin alpha1 chain. J Biol Chem 2004; **279:**9103-14.
16 Zhang W, Geiman DE, Shields JM, et al. The gut-enriched Krüppel-like factor (Krüppel-like factor 4) mediates the transactivating effect of p53 on the p21WAF1/Cip1 promoter. J Biol Chem 2000; **275:**18391-8.
17 Yoon HS, Chen X, Yang VW. Krüppel-like factor 4 mediates p53-dependent G1/S cell cycle arrest in response to DNA damage. J Biol Chem 2003; **278:**2101-5.
18 Yoon HS, Yang VW. Requirement of Krüppel-like factor 4 in preventing entry into mitosis following DNA damage. J Biol Chem 2004; **279:**5035-41.
19 Shie JL, Chen ZY, Fu M, Pestell RG, Tseng CC. Gut-enriched Krüppel-like factor represses cyclin D1 promoter activity through Sp1 motif. Nucleic Acids Res 2000; **28:**2969-76.
20 Chen X, Whitney EM, Gao SY, Yang VW. Transcriptional profiling of Krüppel-like factor 4 reveals a function in cell cycle regulation and epithelial differentiation. J Mol Biol 2003; **326:** 665-77.
21 Yoon HS, Ghaleb AM, Nandan NO, et al. Krüppel-like factor 4 prevents centrosome amplification following γ irradiation-induced DNA damage. Oncogene, in press.
22 Dang DT, Bachman KE, Mahatan CS, et al. Decreased expression of the gut-enriched Krüppel-like factor gene in intestinal adenomas of multiple intestinal neoplasia mice and in colonic adenomas of familial adenomatous polyposis patients. FEBS Lett 2000; **476:**203-7.
23 Zhao W, Hisamuddin IM, Nandan MO, et al. Identification of Krüppel-like factor 4 as a potential tumor suppressor gene in colorectal cancer. Oncogene 2004; **23:**395-402.
24 Dang DT, Chen X, Feng J, et al. Overexpression of Krüppel-like factor 4 in the human colon cancer cell line RKO leads to reduced tumorigenecity. Oncogene 2003; **22:**3424-30.
25 Ohnishi S, Ohnami S, Laub F, et al. Downregulation and growth inhibitory effect of epithelial-type Krüppel-like transcription factor KLF4, but not KLF5, in bladder cancer. Biochem Biophys Res Commun 2003; **308:**251-6.
26 Sun R, Chen X, Yang VW. Intestinal-enriched Krüppel-like factor (Krüppel-like factor 5) is a positive regulator of cellular proliferation. J Biol Chem 2001; **276:**6897-900.
27 Chanchevalap S, Nandan MO, Merlin D, Yang VW. All-trans retinoic acid inhibits proliferation of intestinal epithelial cells by inhibiting expression of the gene encoding Krüppel-like factor 5. FEBS Lett 2004; **578:**99-105.
28 Nandan MO, Yoon HS, Zhao W, et al. Krüppel-like factor 5 mediates the transforming activity of oncogenic H-Ras. Oncogene 2004; **23:**3404-13.
29 Segre JA, Bauer C, Fuchs E. Klf4 is a transcription factor required for establishing the barrier function of the skin. Nat Genet 1999; **22:**356-60.
30 Jaubert J, Cheng J, Segre JA. Ectopic expression of Krüppel like factor 4 (Klf4) accelerates formation of the epidermal permeability barrier. Development 2003; **130:**2767-77.
31 Patel S, Kartasova T, Segre JA. Mouse Sprr locus: a tandem array of coordinately regulated genes. Mamm Genome 2003; **14:** 140-8.
32 Katz JP, Perreault N, Goldstein BG, et al. The zinc-finger transcription factor Klf4 is required for terminal differentiation of goblet cells in the colon. Development 2002; **129:**2619-28.
33 Shindo T, Manabe I, Fukushima Y, et al. Krüppel-like zinc-finger transcription factor KLF5/BTEB2 is a target for angiotensin II signaling and an essential regulator of cardiovascular remodeling. Nat Med 2002; **8:**856-63.
34 Foster KW, Ren S, Louro ID, et al. Oncogene expression cloning by retroviral transduction of adenovirus E1A-immortalized rat kidney RK3E cells: transformation of a host with epithelial features by c-MYC and the zinc finger protein GKLF. Cell Growth Differ 1999; **10:**423-34.
35 Foster KW, Frost AR, McKie-Bell P, et al. Increase of GKLF messenger RNA and protein expression during progression of breast cancer. Cancer Res 2000; **60:**6488-95.
36 Pandya AY, Talley LI, Frost AR, et al. Nuclear localization of KLF4 is associated with an aggressive phenotype in early-stage breast cancer. Clin Cancer Res 2004; **10:**2709-19.
37 Chen C, Bhalala HV, Vessella RL, Dong JT. KLF5 is frequently deleted and down-regulated but rarely mutated in prostate cancer. Prostate 2003; **55:**81-8.
38 Chen C, Bhalala HV, Qiao H, Dong JT. A possible tumor suppressor role of the KLF5 transcription factor in human breast cancer. Oncogene 2002; **21:**6567-72.
39 Bateman NW, Tan D, Pestell RG, Black JD, Black AR. Intestinal tumor progression is associated with altered function of KLF5. J Biol Chem 2004; **279:**12093-101.