Published in final edited form as:
J Endocrinol. 2010 March ; 204(3): 223–231. doi:10.1677/JOE-09-0329.

The emerging role of Krüppel-like factors in endocrine-responsive cancers of female reproductive tissues

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Abstract

Krüppel-like factors (KLFs), of which there are currently 17 known protein members, belong to the Specificity-protein (Sp) family of transcription factors and are characterized by the presence of Cys_2/His_2 zinc-finger motifs in their carboxy-terminal domains that confer preferential binding to GC/GT-rich sequences in gene promoter and enhancer regions. While previously regarded to simply function as silencers of Sp1-transactivity, many KLFs are now shown to be relevant to human cancers by their newly identified abilities to mediate cross-talk with signaling pathways involved in the control of cell proliferation, apoptosis, migration, and differentiation. Several KLFs act as tumor suppressors and/or oncogenes under distinct cellular contexts, underscoring their prognostic potential for cancer survival and outcome. Recent studies suggest that a number of KLFs can influence steroid hormone signaling through transcriptional networks involving steroid hormone receptors and members of the nuclear receptor family of transcription factors. Since inappropriate sensitivity or resistance to steroid hormone actions underlie endocrine-related malignancies, we consider the intriguing possibility that dysregulation of expression and/or activity of KLF members is linked to the pathogenesis of endometrial and breast cancers. In this review, we focus on recently described mechanisms of actions of several KLFs (KLF4, KLF5, KLF6, and KLF9) in cancers of the mammary gland and uterus. We suggest that understanding the mode of actions of KLFs and their functional networks may lead to the development of novel therapeutics to improve current prospects for cancer prevention and cure.

Introduction

Endocrine-responsive cancers of female reproductive tissues constitute a complex set of pathologies that arise, in part, from aberrant levels and/or activity of the ovarian hormones estradiol (E) and progesterone (P) (Pasqualini 2007, Eliassen & Hankinson, 2008). While there

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Declaration of interest
The authors declare that there are no conflicts of interest that could be perceived as prejudicing this report.

are many factors that affect sex steroid hormonal profiles, foremost of which is the control of ovarian steroidogenic activity, numerous studies have shown that defects in steroid hormone signaling prominently underlie target cell resistance to the biological actions of E and P. Evidence for the latter is provided by the noted dysregulation of uterine and mammary functions consequent to the loss or aberrant expression of proteins involved in their respective steroid signaling cascades (Lydon *et al.* 1995, Curtis-Hewitt S *et al.* 2000, Mulac-Jericevic *et al.* 2000, Spears & Bartlett 2009). E and P actions are mediated by their cognate receptors, namely estrogen receptor (ESR) and progesterone receptor (PGR) in coordination with a whole host of functionally context-dependent coregulators, to stimulate or inhibit target gene transcription. The signal transduction pathways initiated by the binding of E and P to their respective receptors are the subject of excellent recent reviews (Beato & Klug 2000, Hall *et al.* 2001). Similarly, the biochemical and biological properties of ESR and PGR, each of which exists classically in two forms, designated ESR1 and ESR2 and PGR-A and PGR-B, respectively, have been well-described (Kastner et al. 1990; Katzenellenbogen BS *et al.* 2000). By contrast, there is yet an incomplete understanding of the pathways by which ESR and PGR specify, recruit, and functionally categorize their co-regulatory proteins to optimize target cell sensitivity (Lonard DM *et al.* 2007). In this review, we consider the emerging role of a subset of nuclear proteins, namely the Krüppel-like factors (KLFs) in the regulation of steroid hormone signaling leading to appropriate responses of mammary epithelial and uterine endometrial cells to E and P. We also review findings to support the concept that maintenance of appropriate KLF expression is tightly controlled in mammary and uterine tissues and that the consequence of deregulated KLF expression is aberrant cell proliferation and differentiation leading to pre-neoplasia and cancer.

Krüppel-like Factors (KLFs)

KLFs, so named for their similarity to the Drosophila segmentation gene product Krüppel (Preiss *et al.* 1985), belong to the evolutionary conserved Sp/KLF family of which there are currently 26 members (Fig.1). The Sp family is comprised of 9 members (Sp1-9), while the KLF family consists of 17 distinct members, a few of which (e.g., KLF6, KLF10, and KLF8) exhibit splice variants (Kaczynzki *et al.* 2003; Suske *et al.* 2005; Pearson *et al.* 2008). The family is characterized by a DNA-binding domain with conserved three tandem C_2H_2 type zinc-finger motifs at the carboxy-terminus and which recognizes the GT/GC box or CACCC element sites on promoter/regulatory regions. The phylogenetic relationship depicted in Fig. 1 is based on the similarity in sequences of their DNA binding domains. While Sp-family members are distinguished by glutamine and to a limited extent, serine-threonine-rich domains at the N-terminus, there is considerable diversity in the corresponding region among KLF members, which can display acidic, proline-rich, serine-rich, or hydrophobic transactivation domains. The highly variable amino-terminus confers functional specificity to KLF interactions with distinct nuclear proteins (Bieker 2001, Suske *et al.* 2005). Most KLFs are ubiquitously expressed, while others are found to be developmentally or temporally expressed in tissue- and cell-type specific manner; in recent years, however, the latter notion has been questioned with increasing evidence to the contrary (Kaczynski *et al.* 2001; Pearson *et al.* 2008). KLF members were previously designated as xKLF, where x refers to the tissue in which the gene was first identified (e.g., EKLF for erythroid KLF; GKLF for gut KLF). A single nomenclature system (KLF1, KLF2, etc.) has now been adopted by the scientific community to describe their order of discovery. Sp-family members Sp1–4 are highly related to KLF9, although Sp1 with 717 amino acids and a mol wt of 120 kDa is at least three times larger than KLF9 with 244 amino acids and a mol wt of 30 kDa. Other family members have molecular sizes in between. To date, only Sp1–4 and Sp7 among Sp-family members have documented cellular functions (Philipsen & Suske 1999, Waby *et al.* 2008). The expression patterns of Sp5, 6, 8 and 9 in various tissues have yet to be examined, and their transactivation potential relative to Sp1 remains relatively unknown. Among KLFs, several members, including KLF4, KLF5,

KLF6, KLF8, KLF9, KLF10, KLF11, and KLF13, have been implicated in the regulation of a wide range of cellular functions including cell growth, differentiation, apoptosis, migration, and tumor formation (Black *et al.* 2001, Ghaleb *et al.* 2005, Wang *et al.* 2006, Pearson *et al.* 2008). Interestingly, family members can antagonize each other's transcriptional activity, mostly because of physical competition between them in binding to cognate sequences in target gene promoters. *In vivo*, the physiologic functions of most KLF members have been validated by use of gene targeting technologies. This has been the subject of a recent review and will not be discussed here (Pearson *et al.* 2008). Suffice it to say that these studies have confirmed the pleiotropic actions of KLFs during embryonic and postnatal development in diverse tissue and cell types, and in adult tissues during distinct physiological states such as early pregnancy, parturition, and adipogenesis. The current review will focus on a subset of KLFs (KLF4, KLF5, KLF6, and KLF9) for which experimental evidence exists for their roles in growth control and in the pathobiology of uterine endometrial and breast cancers.

KLFs in the control of cell proliferation

The regulation of genes involved in cell cycle control and cell proliferation has surfaced as a major aspect of KLF action in diverse cell types (Black *et al.* 2001, Ghaleb *et al.* 2005). KLFs interact with different promoters and with other coregulators in their capacity to function as transcriptional activators, repressors, or both to influence cell growth regulation. This duality in functions is likely dependent on the architecture of the specific promoter (e.g., presence of single or multiple GC-rich motifs); the chromatin environment; and cellular co-expression of family members. KLF members mediate cell proliferation by attenuating or enhancing the transcription of anti-proliferative genes such as p21/wif1/cip1 (*CDKNIA*), p53 (*TP53*), and E-cadherin (*CDH1*) (Simmen *et al.* 2002, Yoon *et al.* 2003, Rowland *et al.* 2005, Wang *et al.* 2007) and of pro-proliferative genes such as those encoding cyclin E1 (*CCNE1*), cyclin D1 (*CCND1*), cyclin B1 (*CCNB1*), ornithine decarboxylase (*ODC*) and IGF-binding protein 2 (*IGFBP2*) (Shie *et al.* 2000, Chen *et al.* 2002, Simmen *et al.* 2002, Yoon *et al.* 2005, Evans *et al.* 2007). Several mechanisms involved in KLF transcriptional activation or repression have been described. KLFs can directly bind to GC-rich regions within target gene promoters to alter specific gene transcription. In this capacity, KLFs may bind alone or in complex (e.g. KLF4 and p53 in the *CDKNIA* promoter) with other proteins (Simmen *et al.* 2002, Yoon *et al.* 2003). By interfering with the recruitment of or competing with Sp1 for binding to recognition motifs within gene promoter regions, KLFs can suppress the well-recognized Sp1 induction of pro-proliferative gene transcription (Lomberk & Urrutia 2005). Finally, KLFs can selectively recruit negative co-regulators such as histone deacetylase-1 (HDAC-1) and mSin3A to gene regulatory regions to support transcriptional repression (Kaczynski *et al.* 2001). Recent studies, however, indicate that KLFs may also alter proliferative signaling pathways independent of binding to gene promoters. For example, KLF6 has been shown to interact with cyclin D1, thereby disrupting the phosphorylation of Retinoblastoma protein to promote cell cycle arrest (Benzeno *et al.* 2004). In addition, KLF4 was reported to inhibit Histone H4 acetylation by interacting with histone deacetylase 3 (HDAC-3) leading to transcriptional repression of proliferation-associated genes (Evans *et al.* 2007). Further, our group recently showed that KLF9 facilitates the recruitment of ESR1 to its own promoter, thus contributing to ESR1 auto-inhibition and decreased cell proliferation in the context of a high E_2-environment (Velarde *et al.* 2007). Since the effect of KLF9 occurred without binding to DNA or ESR1, this suggests KLF9 interactions with other yet unknown nuclear proteins. Gene expression profiling has expanded the repertoire of KLF-induced or -repressed genes encoding cell cycle regulators in distinct cell types, revealing novel gene targets (Simmen RC *et al.* 2002, Goldstein *et al.* 2007, Simmen FA *et al.* 2008). It is unlikely that these up- or down-regulated genes all constitute direct targets of KLFs; however, data strongly suggest the depth and range of KLF involvement in growth signaling pathways.

KLFs and Endometrial Carcinoma

Endometrial carcinoma ranks as the fourth most frequent cancer among women in the Western world and causes significant morbidity and mortality in advanced stages (Jemal *et al.* 2008). The possible involvement of KLFs in uterine dysfunction as exemplified by endometrial carcinoma initially came from our group's studies demonstrating cell type-dependent expression of KLF9 (previously designated Basic Transcription Element Binding Protein, BTEB-1; Imataka *et al.* 1992, Ohe *et al.* 1993) in uterine endometrium during pregnancy. We found KLF9 expression predominantly in endometrial stromal cells and to a lesser extent in glandular epithelial cells, with no or undetectable expression in luminal epithelial cells of normal cycling and early pregnant mice (Simmen *et al.* 2004, Velarde *et al.* 2005; Pabona *et al.* 2009). Importantly, we observed that null mutation of *Klf9* by gene targeting in mice resulted in altered patterns of proliferation and apoptosis in all endometrial cell types, suggesting an essential role for largely stromal-derived KLF9 in uterine growth regulation (Velarde *et al.* 2005). Further, ovariectomized *Klf9* null mutant mice were refractory to the proliferative effects of estradiol-17β (E_2) in uterine cells, when compared to similarly treated ovariectomized wildtype counterparts (Pabona *et al.* 2009), documenting KLF9 involvement in E-mediated uterine proliferation. Using clonal sub-lines of HEC-1A human endometrial carcinoma cells that were stably transfected with sense and anti-sense *Klf9* expression vectors, we found distinct cell phenotypes and gene expression patterns with KLF9 over- vs. under-expression (Zhang *et al.* 2001). KLF9 over-expressing cells displayed higher DNA synthesis and promoted G1/S progression of the cell cycle, concomitant with increased expression of *CCND1*, *PCNA*, *CDKN1A*, secretory leukocyte protease inhibitor (*SLPI*) and mitosin genes, all of which (with the exception of *CDKN1A*) are associated with increased proliferation status, relative to parent cells. Conversely, KLF9 under-expressing HEC-1A cells had lower expression levels of these genes, displayed lower mitotic index and interestingly, manifested increased ability to grow in multi-layers, the latter indicative of disruption in cell adhesion and cytoskeletal organization. Subsequent gene profiling of the same cell lines demonstrated regulation by KLF9 of gene transcripts encoding additional proteins associated with proliferation (e.g., brain-derived neurotrophic factor; KLF4); extracellular matrix (ECM) formation, motility and cell adhesion (e.g., integrin, beta 8; laminin gamma 2 protein; collagen type IV; versican); and signal transduction (e.g., mitogen-activated protein kinase-activated protein kinase 3; Wnt5b receptor) (Simmen *et al.* 2008). Collectively, these findings indicated that KLF9 levels are normally tightly regulated to maintain cellular homeostasis, and that inappropriate expression of KLF9 may lead to aberrant growth regulation and loss of epithelial-mesenchymal communication, contributing to endometrial carcinoma.

Two recent analyses suggested an association of KLF9 with human endometrial tumor pathology. In one study from our group, quantitative RT-PCR analyses of human endometrium and endometrial tumors using a normalized cDNA panel demonstrated a significant increase in *KLF9* transcript levels in normal endometrium and stage I (more differentiated) endometrial tumors, when compared to tumors of more aggressive pathology (stages II, III, and IV) (Simmen *et al.* 2008; Fig. 2A). In the second study using the Cancer Microarray Data Mining Program (Oncomine.org; Compendia Biosciences, Ann Arbor, MI), we analyzed a published gene array database (Mutter *et al.* 2001) that compared the expression profiles of normal (from proliferative and secretory phases of the menstrual cycle) and malignant endometria, for *KLF9* transcript levels. We found that levels of *KLF9* transcripts were decreased in endometrial carcinoma tissues relative to normal endometria (Fig. 2B). Further studies using increased tumor sample sizes and at the level of the KLF9 protein for each tumor grade will be necessary to confirm this associational findings.

Given the observations that *KLF4* expression was induced in HEC-1A cells over-expressing KLF9 (Simmen *et al.* 2008); that KLF13 can mimic KLF9 in transactivating genes in

endometrial epithelial cells (Zhang *et al.* 2002; Zhang *et al.* 2003); and that KLF5 modulates the promoter of the uterine endometrial epithelial gene encoding lactoferrin (Shi *et al.* 1999), a protein with reported tumor-promoting activity (Albright & Kaufman, 2001), the expression of these and other KLFs in human normal endometrium (proliferative and secretory phases of the menstrual cycle) and endometrial carcinoma tissues were subsequently evaluated from a published gene array data by Mutter and colleagues (Mutter *et al.* 2001). Data mining indicated that the transcript levels of most KLFs were unaffected by malignant status (Fig. 3). The exceptions were *KLF6* and *KLF5*, whose respective transcript levels were reduced and tended to increase, respectively in endometrial tumors, albeit not to the same magnitude as for *KLF9* (Fig. 2B). These findings suggest that the physiological control of uterine epithelial proliferation may be limited to a small subset of KLFs. Moreover, since the expression of these KLFs has not been localized to specific cell types, it is not known whether the deregulated expression of these KLFs in tumors is directed from the stromal compartment or epithelium. Thus, a careful analysis of the adult uterine phenotypes of mice with conditional *Klf* null mutations will be required. Further studies will be also be needed to clarify whether in the context of normal vs. tumor cells, KLF members may have distinct, similar, or synergistic biological behaviors.

KLFs and Breast Cancer

The potential loss of growth control mediated by distinct KLFs is well-studied and better documented in mammary epithelial cells than in endometrial cells, a fact likely related to the higher incidence and hence, more wide-spread and devastating consequences, of breast than endometrial cancers in the populace. In the USA alone, an estimated 180,000 new cases of breast cancer and 50,000 deaths from this disease are reported annually (Jemal *et al.* 2008). The linkage between breast cancer and KLFs is strongest for KLF4 and KLF5, although a consensus on whether these KLFs function as tumor suppressors or oncogenes in breast cancer is lacking. In support of a tumor suppressor function for KLF4, breast cancer cells were found to exhibit loss of *KLF4* expression relative to normal mammary epithelial cells, and this was associated with markedly down-regulated expression of laminin B5, a component of the major ECM protein lamin α (Miller *et al.* 2001). However, *KLF4* was also reported to be expressed at low levels in morphologically normal (uninvolved) breast epithelium adjacent to tumor cells, but displayed increased expression in neoplastic cells (Foster *et al.* 2000). Increased *KLF4* expression in tumor cells was localized to the nucleus in the early stages of invasive ductal carcinoma of the breast, suggesting its prognostic potential for aggressive phenotype (Pandya *et al.* 2004). Similar to KLF4, KLF5 has also been reported to have a dual role as a tumor suppressor or as an oncogene. One study found that KLF5 is pro-proliferative, and the positive association between higher *KLF5* expression coincident with increased expression of HER2/neu and Ki67 on the one hand, and shorter disease-free survival and limited overall survival time on the other hand, suggest the prognostic value of this KLF for patients with breast cancer (Tong *et al.* 2006). In another study, KLF5 was implicated in breast cancer progression by inducing the expression of fibroblast growth factor-binding protein, which is over-expressed in breast tumors and found to promote tumorigenesis (Zheng *et al.* 2008). *In vitro*, knockdown of *KLF5* expression in the human mammary epithelial cell lines MCF-10A and BT20 resulted in induction of apoptosis (Liu *et al.* 2008). This effect was attributed to loss of KLF5-mediated inhibition of degradation of the pro-survival phosphatase MAPK-phosphatase-1 protein. In support of KLF5 as a tumor suppressor, elevated expression of KLF5 in non-neoplastic and normal human mammary tissues, in contrast to lower expression in breast cancer lines, has been reported (Chen *et al.* 2002). Recent studies, albeit limited, have also implicated KLF6 and KLF8 in breast cancer progression. KLF6 expression was found to be negatively associated with breast cancer status, suggesting a possible tumor suppressor function (Guo *et al.* 2007). By contrast, KLF8 is considered to be involved in the promotion of breast cancer based on its ability to increase epithelial-mesenchymal transition and to enhance motility as a consequence

of its direct binding to the E-cadherin promoter to decrease this gene's transcription (Wang *et al.* 2007). A role for KLF9 has not been specifically evaluated in normal mammary tissues or mammary tumors; however, we have found no gross morphological differences in mammary glands of young and adult *Klf9* null and wildtype mice, and observed no spontaneous mammary tumor occurrence in older (~1 year-old) *Klf9* null mutants (Simmen RCM & Velarde MC, unpublished findings). It will be interesting to further evaluate the mechanisms of mammary tumor progression mediated by KLFs in mouse models of tumorigenesis (e.g. MMTV-*Wnt* transgenic mice), for example by a comprehensive study of the different KLFs during mammary tumor development and by an extensive analyses of the mammary phenotypes of specific *Klf* mutants crossed to *Wnt*-Tg mice.

KLFs and Steroid Hormone Signaling

Endometrial and ESR1-positive breast cancers arise from dysregulated E and/or P signaling. Given the experimental data that KLFs may promote or attenuate endocrine-responsive cancers, the possibility that KLFs exert their effects through cross-talk with ESR1 and PGR signaling pathways was anticipated (Zhang *et al.* 2002). Indeed, a subset of KLF family members have now been confirmed to function as co-activators of ESR1 and PGR based primarily on *in vitro* cell culture studies, but increasingly supported from analyses of *in vivo* mouse mutant models. The major evidence to date comes from analyses of KLF9 and its interaction with PGR in the regulation of PGR-dependent gene transcription in uterine endometrial cells. In the human endometrial carcinoma cell line Ishikawa which is of glandular epithelial cell origin, KLF9 was shown to physically interact with PGR-B and to promote the PGR-B dependent transactivation of P-responsive promoters (Zhang *et al.* 2003, Velarde *et al.* 2006). Interestingly, PGR-A isoform did not recapitulate PGR-B interactions with KLF9, suggesting the selective utilization of KLF9 by PGR-B as a co-regulator of its transactivity (Zhang *et al.* 2003). KLF13 can substitute for KLF9 as a PGR-B partner in this context (Zhang *et al.* 2003); this is likely due to the structural homology between KLF9 and KLF13, which exhibit the greatest similarities among all KLF members (Philipsen & Suske, 1999) (Fig. 1). *In vivo*, functional interactions between PGR and KLF9 were confirmed by comparison of *Klf9* wildtype and null mutants for P-dependent gene expression; E+P-dependent cell proliferation and apoptotic status; and embryo implantation outcome, an E+P-dependent event (Simmen *et al.* 2004, Velarde *et al.* 2005). Similar to PGR signaling, ESR1 signaling may also involve the participation of KLF9. Evidence for this is provided by *in vivo* and *in vitro* studies describing: a) loss of responsiveness to E_2-induced proliferation of endometrial cells with *Klf9* null mutation, possibly mediated by loss of KLF9 inhibition of Repressor of Estrogen Receptor Activity (REA) expression (Pabona *et al*, 2009); b) increased *Esr1* expression in peri-implantation stromal cells of *Klf9* null mutants (Velarde *et al*, 2005); c) KLF9 transcriptional repression of ESR1 signaling in Ishikawa endometrial adenocarcinoma cells by promoting ligand-dependent ESR1 auto-downregulation (Velarde *et al*, 2007); and d) the negative association between *Klf9* and *Esr1* transcript levels in endometrial tumors (RCM Simmen, data not shown).

The recent generation of *Klf13* null mutants which are not embryo-lethal (Zhu *et al.* 2007) will now allow parallel comparison of KLF13 effects on ligand-dependent PGR transcriptional and biological events, to those of KLF9. More importantly, such studies would provide confirmation on the ability of KLF13 and KLF9 to compensate/substitute for each other's function in the uterine endometrium. Nevertheless, since initial analyses of endometrial tumor samples indicated undetectable *KLF13* expression in human endometrium and endometrial tumors (Mutter *et al.* 2001, Fig. 3), context-dependent functions of KLF13 are likely. Although no data is available regarding the participation of KLF6 and KLF5 in steroid hormone signaling, perturbations in their expression under a pathological E_2-dominated environment (endometrial carcinoma) hint of potential linkages. However, given that null mutations of *Klf4*, *Klf5*, and

Klf6 result in embryonic or perinatal lethality (Pearson *et al.* 2008), it is not currently possible to utilize knockout mice for evaluation of respective uterine and mammary gland phenotypes; such studies await the generation of mammary- and uterine-targeted gene mutations.

How may KLFs participate in steroid hormone signaling? Limited mechanistic data are available to fully describe KLF involvement, albeit insights gleaned from our data (Zhang *et al.* 2002, 2003, Velarde *et al.* 2005, 2006, 2008, Pabona *et al.* 2009) and those described for family member Sp1 (Khan *et al.* 2007, Wu *et al.* 2009) provide some directions. Given that KLFs are transcription factors, family members may modulate E- and/or P-sensitivity of target cells by: a) regulating ESR1 and PGR expression; b) by facilitating recruitment of PGR and ESR1 to steroid hormone-responsive promoters which lack canonical P-responsive elements (PRE) or E-responsive elements (ERE), through their direct binding to GC/GT boxes in gene promoters and by competing with SP factors to promote or inhibit transcription; c) by interacting with chromatin modifiers such as HAT, HDAC, and mSin3A to induce or repress recruitment of nuclear PGR/ESR1 co-regulators and components of the RNA pol II enzyme; and d) by post-translational modifications (e.g. phosphorylation) of nuclear receptors or their co-factors through control of expression and/or activity of specific kinases that modify these proteins. The latter possibility, while speculative, comes from findings that PGR phosphorylation is important for its transcriptional activity (Clemm *et al.* 2000, Knotts *et al.* 2001) and that CDK2, which has been implicated in PGR-A and PGR-B phosphorylation is a KLF9-induced gene in the human endometrial carcinoma cell line HEC-1A (Simmen *et al.* 2002). Clearly, the potential importance of KLFs in mediating multiple events (summarized in Fig. 4) necessitates a thorough understanding of the specific family member(s) involved in these and other similar yet unknown, regulatory processes.

Conclusions

Recent studies have documented KLF family members in the control of cell proliferation, differentiation, and apoptosis in steroid-responsive mammary and uterine endometrial cells. Since these processes are well-recognized as critical events regulated by ESR1 and PGR signaling, and loss of this regulation partly underlies endocrine-responsive cancers, the further understanding of the cross-talk between KLF-regulated pathways and those orchestrated by ligand-activated ESR1 and PGR may lead to the identification of common and possibly novel, gene targets that will facilitate the development of agents for the treatment of hormone-responsive cancers. Albeit Sp/KLF family member Sp1 has significant headway in the mechanistic understanding of its participation in growth control, current information predicts that KLFs may have far greater consequences on progression to neoplasia given their duality in functions (tumor suppressor or promoter) under distinct contexts even in the same target tissue. Further, given the fact that many other types of cancer (e.g. prostate cancer, colon and intestinal cancers, leiomyoma) have an endocrine component underlying their molecular pathologies, and some of these have been recently associated with loss of KLF expression (e.g., colorectal cancer and KLF9) (Kang *et al.* 2008), it is reasonable to assume that the development of effective therapies for a broad range of cancers may be well-served by further analyses of KLF signaling. In this regard, the increasing data in support of the involvement of multiple KLFs (KLF2, KLF4, KLF5) in the regulation of stem cell renewal and maintenance (Jiang *et al.* 2008, Chan *et al.* 2009) open new possibilities for the use of KLFs and signaling components to target cancer stem cells that drive tumor growth (Zhang & Rosen, 2006).

Acknowledgments

Funding

Research cited here from the Simmen's laboratories was supported by funds from the National Institutes of Health grant no. HD21961, and the Arkansas Children's Hospital Research Institute/Arkansas Biosciences Institute.

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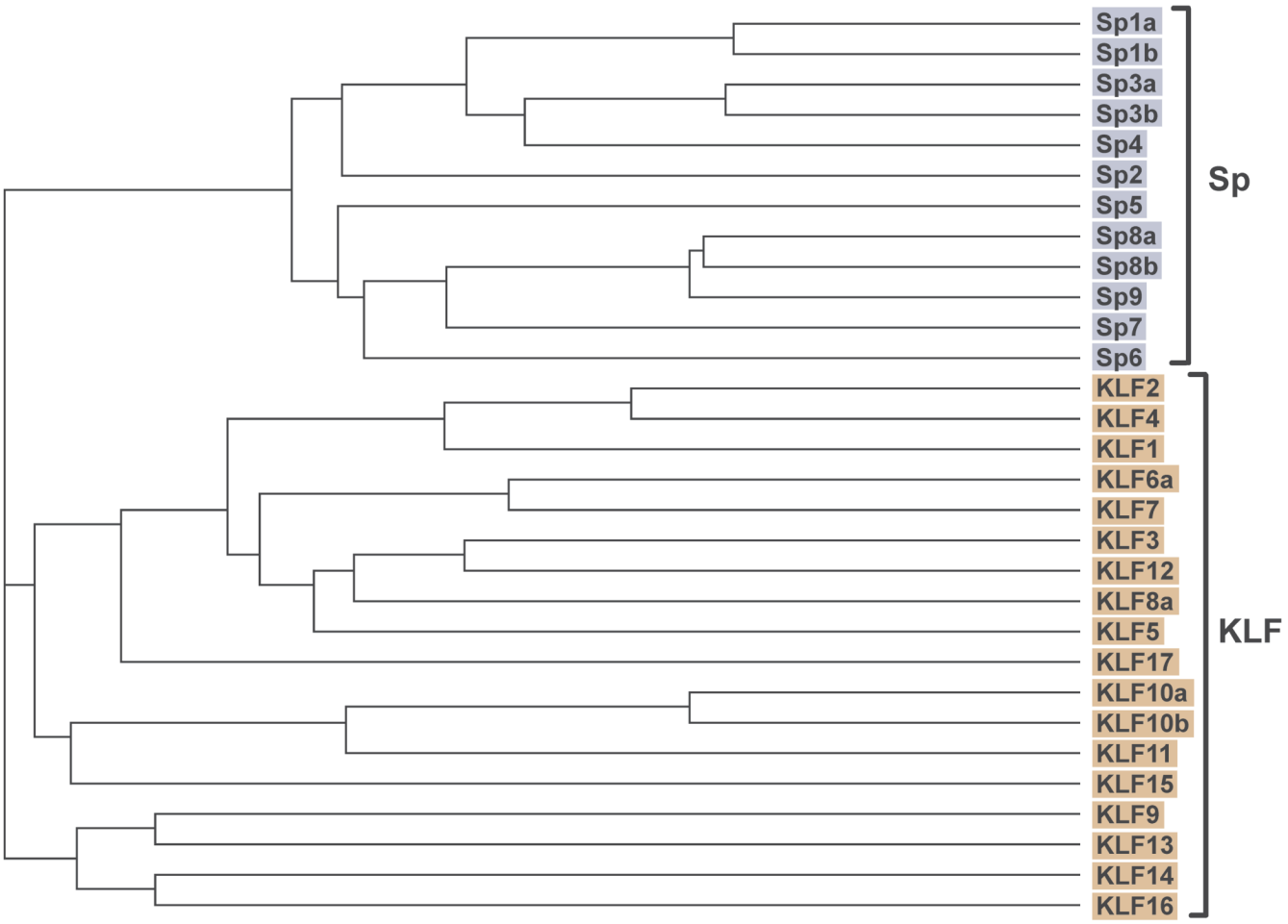

Figure 1.
Cladogram of the human Sp- and KLF-transcription factors. The 110-aa domain containing the buttonhead box (BTD)/zinc finger motifs was used for the multiple alignment with ClustalW, as described by Suske *et al.* 2005. *KLF6b, KLF6c, and KLF8b are truncated isoforms that contain deletion in the zinc finger motifs, and hence, were excluded in the alignment.

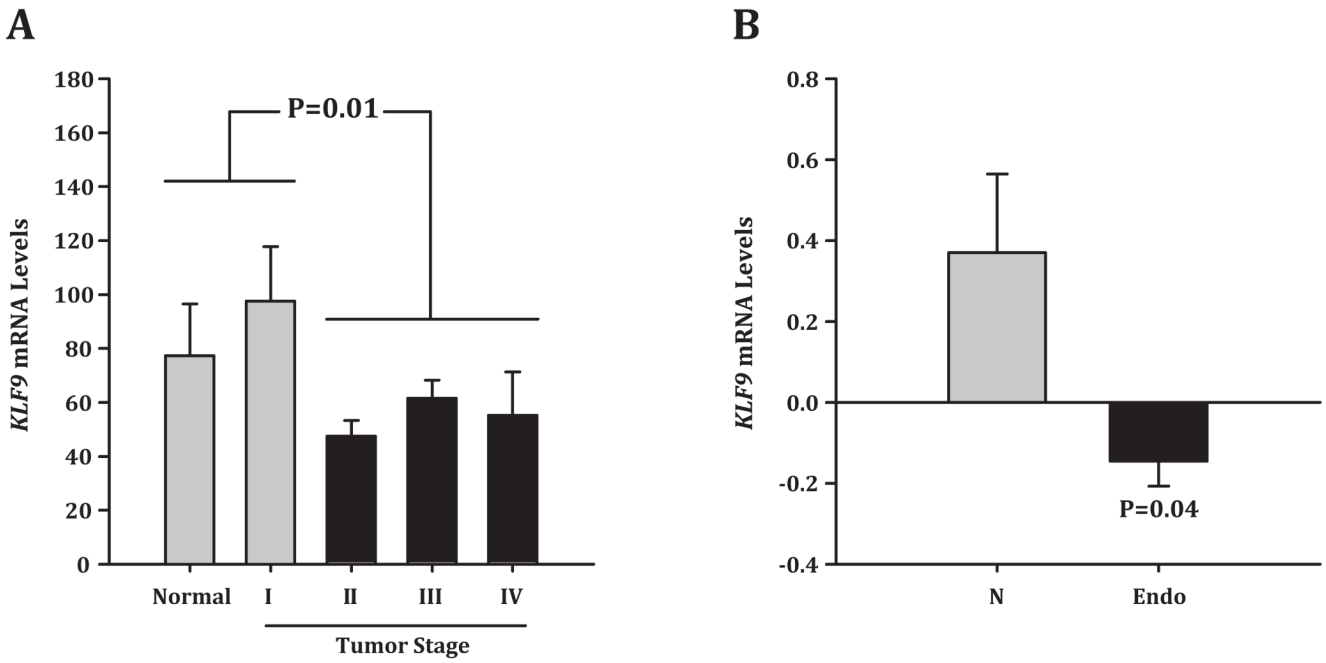

Figure 2.

KLF9 and endometrial carcinoma. (A) A normalized cDNA panel of human endometrial tumors (OriGene Technologies, Inc.) was used to probe for *KLF9* transcript levels by quantitative RT-PCR. Data were adapted from Simmen *et al.* 2008. For each stage, data from endometrioid and serous tumors were combined. Sample numbers (in parenthesis) for each tissue or tumors are: normal (6); I (9); II (8); III (19), and IV (6). (B) *KLF9* expression levels from comparison of normal (N) and malignant endometria (Endo) obtained from Affymetrix Hu6800 GeneChip probe arrays, as reported by Mutter *et al.* 2001. The normalized values shown here were obtained using the Cancer Microarray Data Mining Program (Oncomine.org) and are presented in Oncomine graphical representations. Sample numbers for N and Endo are 4 and 10, respectively. Significant difference ($P<0.05$) between groups was determined by *t*-test.

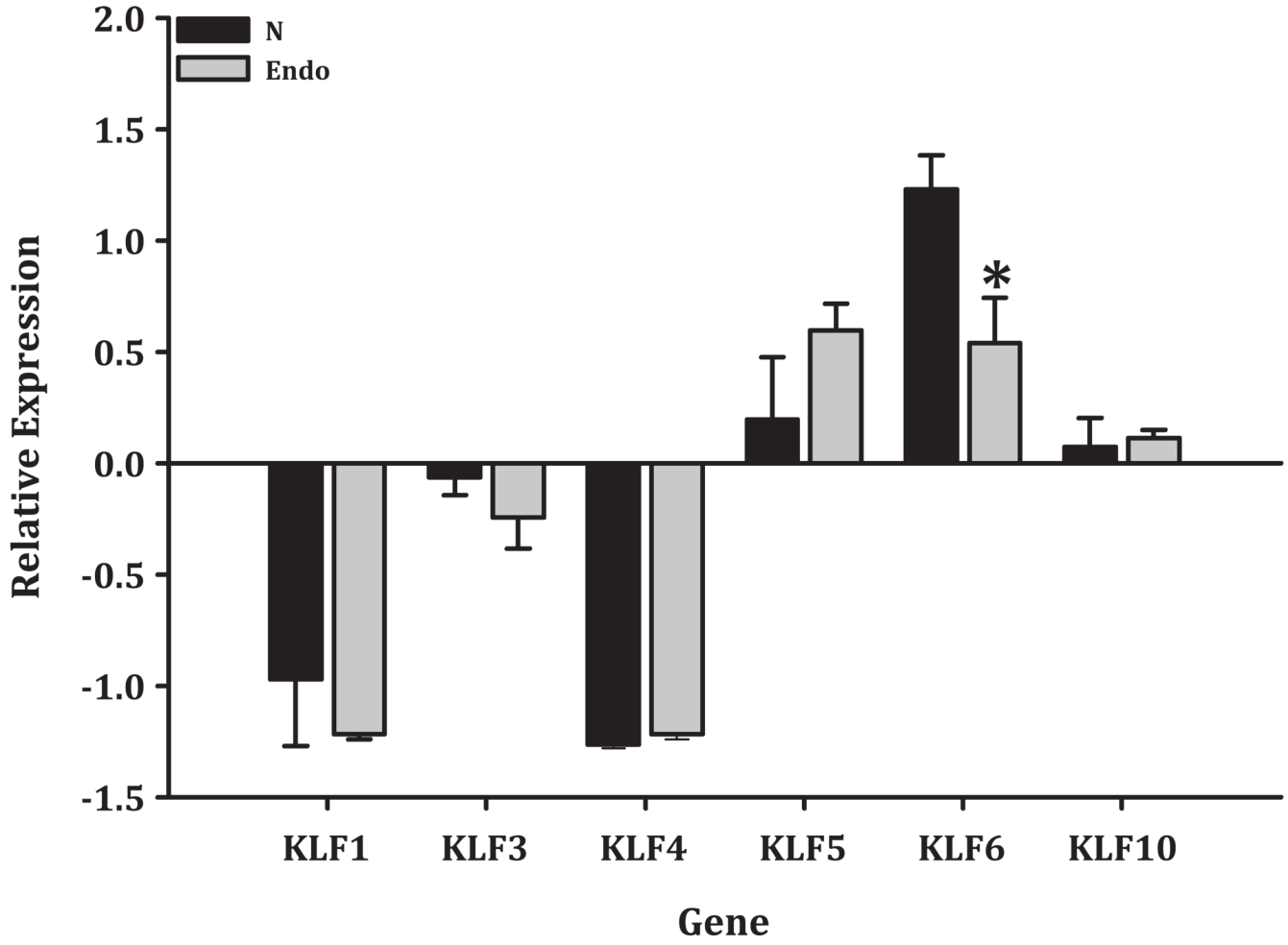

Figure 3.
Transcript levels of different KLF members in normal (N) and malignant (endo) endometria. The analysis was carried out using the same data set described by Mutter *et al.* 2001 and normalized values are presented in Oncomine graphical representations. Sample numbers for N and Endo are 4 and 10, respectively. Difference between groups was determined by t-test. **P*=0.05.

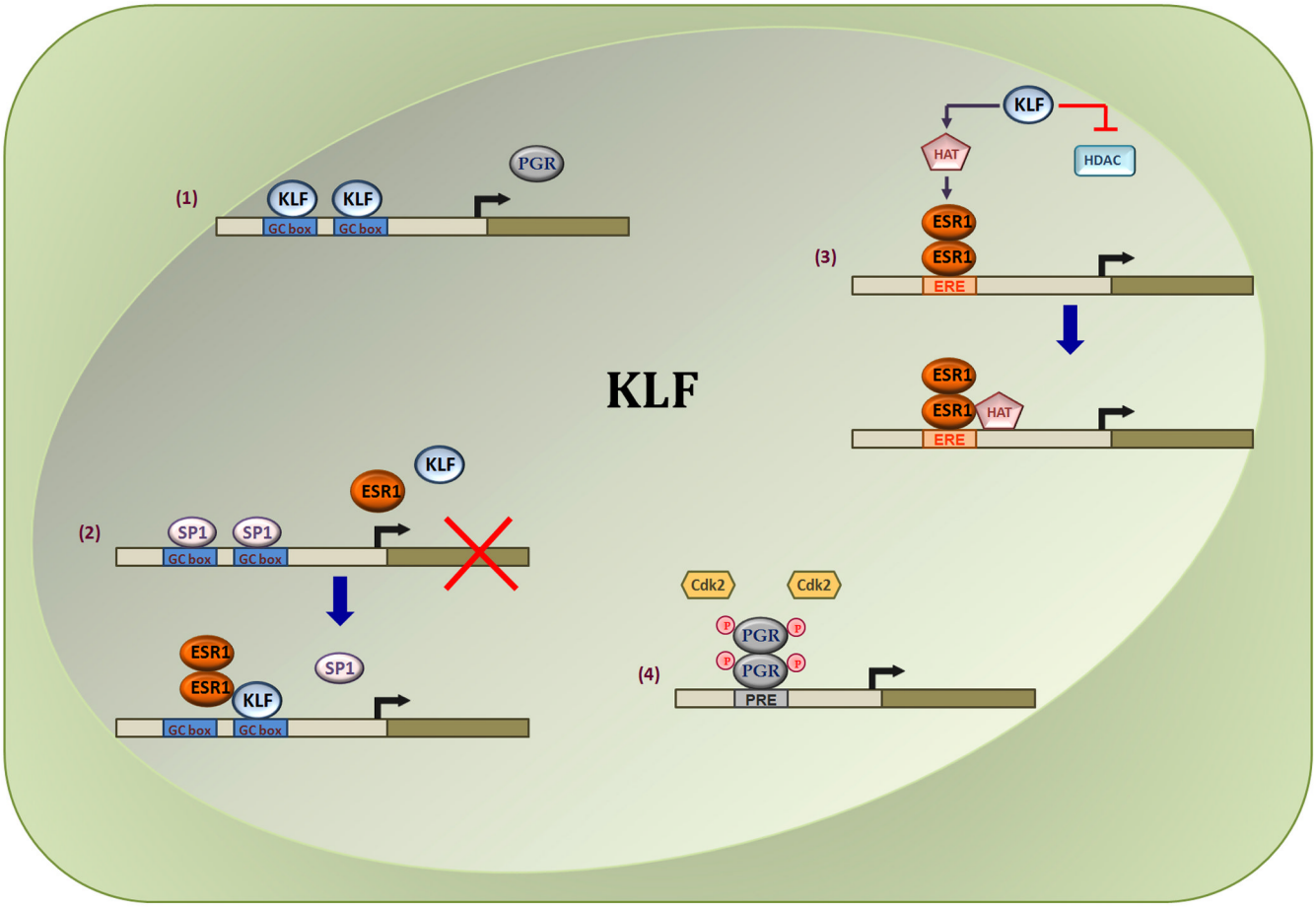

Figure 4.

Postulated model for KLF involvement in ESR and PGR transcriptional pathways. KLF members may mediate transcriptional activities of steroid hormone receptors by regulating their levels of expression (1), and/or transactivities by interfering with Sp1 binding to gene promoters (2); promoting the recruitment of nuclear co-regulators (3); and influencing post-translational modifications (e.g., phosphorylation) of nuclear receptors or co-regulators through transcriptional regulation of kinase cascades (4). ESR1, estrogen receptor-α; PGR, progesterone receptor A/B; HAT, histone acetyl transferase; HDAC, histone deacetylase; Sp1, specificity protein-1.