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The Transcription Regulator Krüppel-Like Factor 4 and Its Dual Roles of Oncogene in Glioblastoma and Tumor Suppressor in Neuroblastoma

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Abstract

The *Krüppel-like factor 4* (*KLF4*) gene is located on chromosome 9q31. All of the currently known 17 KLF transcription regulators that have similarity with members of the specificity protein family are distinctly characterized by the Cys_2/His_2 zinc finger motifs at their carboxyl terminals for preferential binding to the GC/GT box or the CACCC element of the gene promoter and enhancer regions. KLF4 is a transcriptional regulator of cell proliferation, differentiation, apoptosis, migration, and invasion, emphasizing its importance in diagnosis and prognosis of particular tumors. KLF4 has been implicated in tumor progression as well as in tumor suppression, depending on tumor types and contexts. Different studies so far strongly suggest that KLF4 acts as an oncogene in glioblastoma, which is the most malignant and prevalent brain tumor in human adult. It is now well established that the presence of glioblastoma stem cells (GSCs) in glioblastoma causes therapy resistance and progressive growth of the tumor. Because KLF4 is one of the key stemness factors in GSCs, it is likely that KLF4 contributes significantly to the survival of GSCs and the recurrence of glioblastoma. On the other hand, recent studies show that KLF4 can act as a tumor suppressor in human malignant neuroblastoma, which is a deadly tumor mostly in children, by inhibiting the cell cycle and activating the cell differentiation and death pathways. Our increasing understanding of the molecular mechanisms of the contrasting roles of KLF4 in glioblastoma and neuroblastoma is useful for superior diagnosis, therapy, and prognosis of these tumors of the nervous system.

Keywords

KLF4; oncogene; glioblastoma; tumor suppressor; neuroblastoma

I. INTRODUCTION

All of the Krüppel-like factors (KLFs), which are transcription factors, have homology with their launching member Krüppel (‘crippled’ in Germany), the deletion of which causes a crippled phenotype in *Drosophila melanogaster*.[1] Phylogenetic studies show that all members of the KLF family have similarity with members of the specificity protein (SP) family, because both protein families harbor zinc finger (ZnF) motifs at their carboxyl terminals.[2] However, KLF family members are structurally different due to the presence of

their three highly conserved and distinct ZnF motifs with extra conserved residues between each ZnF.[3] For their transcriptional functions, all KLF family members use their conserved ZnF motifs to identify and bind to almost identical GC/GT box or CACCC element consensus sequences, but the specificity of their activities is determined by their differing amino terminals and/or their expression in specific tissues.[4] The transcription regulator KLF4, also known as the gut-enriched KLF protein,[5] is highly expressed in epithelial tissues. Notably, it is highly expressed in terminally differentiated epithelial cells of the intestinal mucosa. Considering its traditional roles and abundant expression in epithelial cells of the gut, KLF4 clearly has some key roles in maintaining cellular homeostasis in the intestinal epithelium. Indeed, the altered expression of KLF4 has been reported in colon cancer[6,7] and many other solid tumors.[8,9] The pathobiology of KLF4 is very complicated because its oncogene or tumor suppressor functions depend on the cellular, tissue, and genetic contexts.[10] Accumulating evidence almost certainly suggests that KLF4 has dual roles as an oncogene in human glioblastoma and a tumor suppressor in human neuroblatoma.

Glioblastoma multiforme, which according to the World Heath Organization (WHO) is a grade IV brain tumor and simply called glioblastoma, is the deadliest primary brain tumor in humans. A solid tumor of abnormal astroglial origin, it is highly heterogeneous at the cellular and molecular levels and harbors exceptionally invasive and aggressive tumor cells that are generally resistant to currently available therapeutic agents.[11] Unfortunately, treatment strategy for any glioblastoma patient is palliative and never curative. Currently, the standard treatment strategy for a newly diagnosed glioblastoma patient with sufficient working ability first includes surgical resection of the tumor, then radiotherapy, followed by concurrent and adjuvant chemotherapy with temozolomide (TMZ), a DNA alkylating agent.[12] Although the tumor can be removed surgically and residual tumor cells can be targeted therapeutically, the reality is the presence of a population of glioblastoma stem cells (GSCs) that can survive radiotherapy and chemotherapy and ultimately regrow the tumor.[13] Human GSCs are capable of unlimited growth as multicellular spheres in defined cell culture medium, differentiation into multiple lineages, and initiation of tumor growth in immunodeficient mice.[14] Indeed, GSCs can have a critical role in conferring therapeutic resistance and tumor recurrence.[15,16] Currently, human GSCs are characterized by the presence of the human embryonic stem cell (hESC) and induced pluripotent stem cell (iPSC) markers such as KLF4, c-Myc, Nanog, Oct4, and Sox2 that support human GSCs for retaining their stemness, self-renewal ability, and tumorigenicity.[17–21] A recent study using patient-derived specimens showed that induction of KLF4 provided the self-renewal and tumorigenic potentials of GSCs. Treatment of intracranial orthotopic xenografts with KLF4 short hairpin RNA (shRNA) plasmid transfection prevented lethal potency of KLF4 in the xenografts.[22]

Human malignant neuroblastoma, the most lethal childhood solid tumor, is responsible for 15% of all cancer deaths in children. Neuroblastoma has an unpredictable clinical course because some tumors can regress either spontaneously or by way of treatment modalities, but other tumors confer drug resistance and grow relentlessly, resulting in poor therapeutic outcomes and ultimately death in pediatric patients. Thus, recognition of reliable prognostic markers would help clinical practitioners to devise the most appropriate treatment strategies.

Several prognostic markers such as age of patient at diagnosis, N-Myc amplification, and TrkA expression can predict clinical outcomes of some neuroblastoma patients. Poor prognostic markers include age of more than 1 year[23] and N-Myc amplification;[24] both of which are clearly associated with progressive disease stages and negative therapeutic outcomes. Conversely, functional TrkA and ephrin receptor type-B (EphB) signaling pathways indicate good prognosis for neuroblastoma patients,[25,26] because they suppress neuroblastoma growth, respectively, by promoting terminal differentiation[27] and inhibiting proliferation, angiogenesis, and metastasis of neuroblastoma cells.[26] A recent study shows that a potent repression complex containing N-Myc recruits histone deacetylase 1 (HDAC1) at the TrkA promoter to directly repress TrkA expression and neuroblastoma cell response to nerve growth factor, resulting in inhibition of terminal differentiation and promotion of the aggressive growth of neuroblastoma.[28] A more recent study demonstrates that low expression of KLF4 in primary neuroblastoma in patients is associated with unfavorable outcomes, and overexpression of KLF4 in human malignant neuroblastoma SH-SY5Y cells not only profoundly inhibits cell proliferation due to direct upregulation of the cell cycle inhibitor protein p21Waf1/Cip1 but also promotes loss of their neuroblastic phenotype. This makes them resemble epithelial cells, become strongly adherent to substrate, and express smooth muscle marker and non-tumorigenic cells, suggesting that overexpression of KLF4 causes terminal differentiation in neuroblatoma cells, presaging the path to their spontaneous death.[29] This study strongly suggests that KLF4 acts as a potential tumor suppressor in human malignant neuroblastoma.

II. KLF4 STRUCTURE AND ITS MECHANISMS OF TRANSACTIVATION AND REPRESSION

All KLF members form a family of transcription factors that have a highly conserved carboxyl terminal DNA-binding domain (DBD) consisting of three Cys_2/His_2 ZnF motifs to identify and bind to GC/GT-rich sequences of the target genes in the genome.[30] These motifs exhibit sequence similarity with the DBD of the SP family members.[31] Although the carboxyl terminals of KLF family members are highly conserved, their amino terminals are highly variable for their different functions. Structural homologies and functional similarities of KLF family members are correlated, and this correlation is probably due to their homologous protein interaction motifs at the amino terminals. Currently, 17 known mammalian KLF members can be grouped into three major subfamilies based on similarities in their amino terminals.[32] Group 1 subfamily members (KLF3, 8, and 12) generally act as transcriptional repressors by recruiting chromatin-modifying enzymes to add repressive signals to histones.[33] Group 2 subfamily members (KLF1, 2, 4, 5, 6, and 7) have acidic activation domains to usually act as transcriptional activators. Group 3 subfamily members (KLF9, 10, 11, 13, 14, and 16) can repress transcription of target genes but also act as transactivators.[34] It should be noted that KLF15 and KLF17 are vaguely related as revealed from phylogenetic analysis, but they have no defined protein interaction motifs and thereby excluded from any subfamily.

The human *KLF4* gene locus, consisting of a 6.3-kb region, is located on chromosome 9q31 and has five exons to produce ~3.5 kb messenger RNA (mRNA) of KLF4 as detected by

northern blotting of total RNA from human umbilical vein endothelial and other cells.[35,36] Human and mouse KLF4 proteins have ~91% similarity in their amino acid sequences. The complementary DNA (cDNA) of human KLF4 has been predicted to encode 513 amino acids for the KLF4 protein, which has a molecular weight of 54 kDa.[37] The molecular structure of human KLF4 and its distinct functional domains are well known (Fig. 1). The KLF4 protein can be divided, more or less, into four separate functional domains: (1) an amino terminal activation domain with 1–157 amino acids,[35,38] (2) a middle repression domain with 158–385 amino acids,[35] (3) a nuclear localization sequence (NLS) having hexapeptide PKRGRR (386–401 amino acids) repeats, and (4) a carboxyl terminal DBD with 81 highly conserved 402–483 amino acids.[30] The presence of both activation and repression domains probably gives advantages to KLF4 in switching between its positive and negative transcriptional activities on its target genes, depending on its expression in the tissue types and contexts.[36,38,39] The carboxyl terminal DBD consists of 81 highly conserved amino acids to form three consecutive ZnF motifs, each of which having an antiparallel β sheet and afterward a short loop and an α-helix. Two cysteines (Cys_2) within the β sheet and two histidines (His_2) within the α-helix collaborate to coordinate a single zinc ion (Zn^{2+}) for stability of the fold. The amino acid sequence of three ZnF motifs at the carboxyl terminal of KLF4 indicates that they (ZnF1, 2, and 3) are distinct.[37] Both ZnF1 and ZnF2 have 23 amino acids, but ZnF3 has 21 amino acids. The ZnF motifs are connected by the seven-amino-acid TGEKP(Y/F)X sequence, which is the typical Krüppel-link and a highly conserved spacer among this family members.[3] Each ZnF of KLF4 binds to three consecutive nucleotides on a target gene, and the specificity of this ZnF protein for binding to a nucleotide sequence is dramatically increased due to the presence of two additional ZnF motifs.[39] In general, KLF4 binds to the GC/GT box or to CACCC elements on the target genes.[35] Resembling many other transcription factors, KLF4 is localized entirely in the nucleus of the cells. Indeed, KLF4 contains two disconnected NLSs for its transportation to the nucleus: one basic hexapeptide (PKRGRR) NLS, as mentioned earlier, on the amino terminal adjacent to the three carboxyl terminal ZnF motifs and another discrete NLS within the first two ZnF motifs.[40] In addition, a potential sequence of a peptide sequence rich in proline (P), glutamic acid (E), serine (S), and threonine (T) (PEST) is located between the transcriptional activation and transcriptional repression domains, suggesting that KLF4 is probably subject to degradation via an ubiquitin-proteasome pathway.

Because KLF4 controls transcription of a large number of genes, it is highly expected that the expression of KLF4 needs to be tightly regulated. In general, the half-life of KLF4 is only 2 h and is subject to rapid ubiquitination and degradation by the proteasome pathway.[41] The induction of expression of KLF4 occurs by various stimuli such as serum starvation,[42] interferon-γ,[43] sodium butyrate,[44] DNA damage,[45] and oxidative stress.[46] The precise mechanisms of how these stimuli induce the expression of KLF4 are unknown. Probable mechanisms may include increased transcription of the *KLF4* gene and increased stability or decreased degradation of its mRNA or its protein. Some transcription factors can regulate the KLF4 promoter and its expression. For example, transactivation of the *KLF4* gene occurs by the transcription factor p53 in response to DNA damage.[45] Interestingly, KLF4 can bind to its promoter for its own expression, but KLF5 inhibits KLF4 expression and also blocks the binding of KLF4 to its promoter.[47] Although KLF4 and KLF5 are closely related

transcription factors, their expression patterns are completely different,[48] and they have several opposite roles[49] in the intestinal epithelium.

The KLF4-mediated mechanism of transactivation is highly regulated because KLF4 has major functions in activation of transcription of many target genes (e.g., *KLF4, Nanog, Oct4, p21, p27, Rb, Sox2)* and in repression of transcription of many other target genes (e.g., *Bax, cyclin B1, cyclin D1, cyclin E, histidine decarboxylase* [*HDC*]*, p53, Sp1*).[39] The amino terminal of KLF4 has a transactivation domain that, alone, when directly fused to its three carboxyl terminal ZnF motifs, is sufficient to activate transcription of a target gene.[38] The amino terminal activation domain also interacts with the transcriptional co-activators p300/cyclic adenosine monophosphate response element-binding protein (CBP) that affects its ability to activate transcription.[38,50] Both p300 and CBP are histone acetyltransferase proteins, and their recruitment results in increased histone acetylation at the promoter and recruitment of other transcription factors to create its functional transcriptional machinery. Furthermore, acetylation of two lysines at the middle domain of KLF4 by p300/CBP is important for its function in transactivating target genes.[50] KLF4 interacts with the p65/RelA subunit of nuclear factor-kappa B (NF-κB) and synergistically activates expression of inducible nitric oxide synthase,[51] suggesting that the mechanism of transactivation by KLF4 may be dependent on other gene products.

The KLF4-mediated passive mechanism of transcriptional repression is a simple competition with an activator for binding to a DNA sequence of a target gene. For example, KLF4 binds to a DNA sequence of the *HDC* gene (notably, the *HDC* gene is a common binding site for the activator Sp1) by ousting Sp1 from the HDC promoter, resulting in the repression of transcription of the *HDC* gene and, thus, no HDAC activity.[52] Sp1 is ubiquitously expressed for positive transcription of many genes.[53] KLF4 is likely to use this passive mechanism of competition to repress transcription of many of its target genes. In addition, KLF4 contains a middle repressive domain,[35] as mentioned earlier, that may actively repress transcription of some of its target genes, in addition to or instead of its passive mechanism of repression via competition with a transcriptional activator. KLF4 represses cyclin B1 by specifically recruiting HDAC3[50] and represses transcriptional targets of Wnt signaling by direct interaction with the β-catenin/T cell factor 4 (TCF4) complex.[54]

III. KLF4 AS AN ONCOGENE IN GLIOBLASTOMA

Glioblastoma is the most malignant form of primary brain tumor and defies all current therapeutic strategies because of its ample abnormal molecular characteristics and highly heterogeneous cell populations including GSCs that can grow aggressively to cause death of the patient within a short time.[11,13] It is the capability of self-renewal and unlimited growth that makes the GSC almost indestructible. Unlike many other glioblastoma cells, GSCs can confer a high degree of resistance to radiotherapy and chemotherapy.[15,16] Even a few GSCs surviving radiotherapy and chemotherapy can regrow the tumor.[15,16] There are many stemness factors (aldehyde dehydrogenase 1 [ALDH1], CD44, CD133, c-Kit, KLF4, Nanog, Nestin, Oct4, and Sox2) that promote self-renewal and survival pathways in glioblastoma; therefore, identifying and targeting a key stemness factor that drives the most aggressive cell proliferation is crucial for combating the growth of glioblastoma.[55–57] A recent study

showed that inhibition of epidermal growth factor receptor (EGFR) in a genetically engineered mouse model of EGFR-driven glioblastoma inadvertently induced c-MET signaling, leading to increases in expression of the stemness transcriptional regulators KLF4, Nanog, and Oct4 in the GSC population for their survival and therapy resistance.[58] Results from this investigation that used the EGFR-driven glioblastoma model suggest that inhibition of the EGFR pathway in glioblastoma may produce little beneficial outcome and actually promote selective proliferation of the GSC population to regrow the tumor. It is therefore imperative to inhibit the molecular signaling that otherwise increases the specific stemness factors in GSCs. Indeed, this investigation further showed that the pharmacological inhibition of c-MET signaling potently abrogated stemness factors (KLF4, Nanog, and Oct4) in GSCs.[58] This and many other recent reports now strongly favor the concept that KLF4 is an oncogene in maintaining the survival and ability of cell proliferation in the GSC population and, thus, perpetuating the growth and regrowth of the tumor (Fig. 2).

Hypoxia (low oxygen level) in human glioblastoma promotes self-renewal of GSCs, leading to aggressive tumor growth and poor clinical outcome. A study shows that hypoxia upregulates the hypoxia inducible factor (HIF) and that the HIF, in turn, promotes expression of *KLF4, c-Myc, Nanog, Oct4*, and *Sox2* genes, which are signatures of hESCs and iPSCs, in human brain tumors.[17] This study also showed that hypoxia was able to induce hESC markers in primary glioblastoma-derived CD133 negative cells as well, suggesting that targeting the HIF could indirectly impair KLF4 oncogenic function and the dynamic state of stemness in glioblastoma. Recurrence of glioblastoma is regrettably an expected phenomenon, because GSCs that survive therapeutic treatment can cause regrowth of the tumor. A recent study investigated the contribution of the signal transducer and activator of transcription 3 (STAT3), a member of a family of DNA-binding factors, to various oncogenic signaling pathways in GSC lines derived from recurrent glioblastoma patients. This study also tested the efficacy of the novel small molecule STX-0119, an inhibitor of STAT3 dimerization, in these GSC lines, which harbored many stem cell markers (CD133, EGFR, Nanog, Olig2, and Nestin) and Yamanaka factors (KLF4, c-Myc, Oct3/4, and Sox2).[59] Treatment of the GSC lines with STX-0119 strongly inhibited expression of STAT3 target genes (*c-Myc, survivin, cyclin D1, HIF-1α, and VEGF*) and many stemness genes (*CD44, CD133, Nanog, and Nestin*), leading to induction of apoptosis in one cell line *in vitro* and the growth inhibition of two cell lines *in vivo*. This study suggested that the use of STX-0119, which could target STAT3 signaling in GSCs, in combination with TMZ plus radiotherapy, would serve as a novel therapeutic strategy against TMZ-resistant glioblastoma patients. Immunohistochemical studies show that both low-grade (astrocytoma, WHO grade II) and high-grade (anaplastic astrocytoma, WHO grade III; glioblastoma, WHO grade IV) human brain tumors have expression of all hESC marker proteins (KLF4, c-Myc, Nanog, Oct4, and Sox2), but levels of their expression are highly upregulated in the high-grade tumors.[60] Interestingly, KLF4 maintains the blood-tumor barrier (BTB) function, because it is a key transcriptional regulator for the expression of tight junction proteins in endothelial cells associated with glioblastoma.[61] Many small-molecule chemotherapeutic agents cannot cross into the brain tumor due to the integrity of the BTB. The shRNA plasmid that was targeting KLF4 expression impaired BTB integrity and increased BTB permeability to small molecules,[61] indicating KLF4 as a potential therapeutic target in glioblastoma. It is now

known that microRNAs (miRs), which have approximately 22 nucleotides, have important roles in negative regulation of expression levels of almost all human genes, including oncogenes, by targeting their mRNAs for cleavage or translational repression. A specific miR can act as a tumor suppressor when it directly binds and degrades mRNA of an oncogene. The tumor suppressor miR-152, which can directly target KLF4 mRNA, is down regulated in human GSCs. Restoration of its expression significantly reduced cell proliferation, migration, and invasion; induced apoptosis in GSCs in cell culture; and showed the least tumor volume and the longest survival in nude mice.[62] All of these results strongly indicate that KLF4 is a prospective oncogene in glioblastoma and targeting it pharmacologically or genetically could provide beneficial outcomes in the treatment of human glioblastoma.

IV. KLF4 AS A TUMOR SUPPRESSOR IN NEUROBLASTOMA

Neuroblastoma is an extracranial solid tumor that occurs mostly in children. It demonstrates extreme heterogeneity in outcomes, ranging from spontaneous regression to aggressive disease and ultimately death.[63,64] Prognosis for patients with malignant neuroblastoma is still disheartening, despite the use of aggressive treatment strategies including surgery, radiotherapy, and chemotherapy.[64,65] Limitations of current therapeutic strategies in the treatment of malignant neuroblastoma warrant development of innovative therapeutic avenues, based on knowledge of emerging novel molecular targets in this malignancy.

Recent advances in whole-genome profiling are providing new opportunities in stratification of neuroblastoma patients, identification of unique gene expression signatures that relate to specific abnormalities, prediction of therapeutic outcomes, and overall prognosis of patients.[66,67] Accumulating evidence also indicates that malignant neuroblastoma that harbors unique genetic complexion may drive tumor cells to differentiate into different lineages and contribute to paradoxical clinical outcomes.[68] For example, differentiation of neuroblastoma cells to fibromuscular lineage may advance tumor regression, but neuroblastoma cells committed to differentiate to the neuronal lineage may acquire additional genetic changes that lead to tumor progression and, therefore, an unfavorable outcome.[69,70] However, we still do not clearly know the molecular factors and pathways that mediate these differentiation processes for tumor regression or progression. It is expected that a clear understanding of the specific molecular mechanisms for tumor regression or progression will help in the near future to design innovative therapeutic interventions and improve patient outcomes.

Only a few reports are currently available about the roles of KLF4 in malignant neuroblastoma. Most of these reports strongly suggest that KLF4 serves as a tumor suppressor in human malignant neuroblastoma (Fig. 3). We and other investigators have recently reported that high levels of KLF4 are associated with glioblastoma and breast cancer, because KLF4 is crucial for maintaining cancer stem cells in these tumors.[55,56,71] In contrast, many other studies strongly suggest that KLF4 acts as a tumor suppressor in other malignancies such as cervical cancer,[72] colon cancer,[73] gastric cancer,[74] and medulloblastoma.[75] The transformative roles of KLF4 are identified by its ability to co-operate with c-Myc, Oct3/4, and Sox2 to reprogram fibroblast cells into iPSCs.[76] Usually, a

combination of several of the transcription factors such as KLF4, c-Myc, Oct3/4, and Sox2 is required to reprogram somatic cells into iPSCs that can be useful in drug discovery and cell transplantation therapies. The well-known tumor-suppressive roles of KLF4 are manifested in the inhibition of tumor cell proliferation by inducing cell cycle arrest at the G_1/S phase and promotion of p53-dependent activation of the cell cycle inhibitor p21 Waf1/Cip1.[45,77] It should be noted that our understanding of the roles of KLF4 in tumor progression or tumor suppression in human malignant neuroblastoma and molecular mechanisms associated with these processes still remains in its infancy. However, some recent results from our and other laboratories strongly indicate that the roles of KLF4 in human malignant neuroblastoma currently point toward tumor suppression as its most likely function.[78,29] Low levels of expression of KLF4 are associated with poor outcomes in neuroblastoma patients. Overexpression of KLF4 in different human neuroblastoma cell lines (neuroblastic [N] type, stemcell-like type I, epithelial-like substrate-adherent S-type, and mixed phenotypes) shows inhibition of cell proliferation due to direct upregulation of the cell cycle inhibitor p21 Waf1/Cip1 and suppression of their tumorigenicity in vitro and in vivo. KLF4 down regulation drastically reduces the ability of neuroblastoma cells to induce tumor *in vivo*, and KLF4 expression in neuroblastoma cells is required for their differentiation to a non-tumorigenic lineage.[29] All of these results from patient-derived primary tumors, cell culture, and animal models are consistent with the roles of KLF4 as a tumor suppressor in human malignant neuroblastoma. In addition, results of our recent investigation strongly suggest that KLF4 functions as a tumor suppressor (Fig. 3), and its overexpression potentiates the anticancer activities of a flavonoid in two different human malignant neuroblastoma cell lines.[78] However, we still do not know the precise transcriptional targets of KLF4 in inducing apoptosis and inhibiting invasiveness in malignant neuroblastoma. Our results do raise provocative thoughts that KLF4 may modulate the transcription of some members of the Bcl-2 family and matrix metalloprotease family. Further studies may shed light on these possibilities to account for the tumor suppressor function of KLF4 in human malignant neuroblastoma.

V. CONCLUSIONS

The human transcription factor KLF4 has dual roles in many cancers and can act as an oncogene or tumor suppressor. The chromosomal location and molecular organization of the human *KLF4* gene and KLF4 protein are almost completely known. We also know a great deal about the molecular mechanisms of KLF4's roles as a transcriptional activator or repressor. Whether it acts as oncogene or tumor suppressor depends on tumor types and contexts. Emerging data from our and other laboratories demonstrate that the functions of KLF4 are highly contrasting and interesting in the pathogenesis of human glioblastoma and neuroblastoma. High levels of KLF4 expression are associated with poor outcomes in glioblastoma preclinical models and patients. In its role as an oncogene, KLF4 promotes cell survival and proliferation of the GSC population and regrowth of glioblastoma, even after aggressive radiotherapy and chemotherapy. On the other hand, low levels of KLF4 expression are associated with poor prognosis in neuroblastoma patients. Therefore, inhibition and promotion of KLF4 expression in glioblastoma and neuroblastoma, respectively, may provide therapeutic advantages and improve patient outcomes.

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ABBREVIATIONS

BTB	blood-tumor barrier
DBD	DNA-binding domain
EGFR	epidermal growth factor receptor
GSCs	glioblastoma stem cells
HDC	histidine decarboxylase
hESCs	human embryonic stem cells
HIF	hypoxia inducible factor
iPSCs	induced pluripotent stem cells
KLF4	Krüppel-like factor 4
NLS	nuclear localization sequence
SP	specificity protein
STAT3	signal transducer and activator of transcription 3
TMZ	temozolomide
WHO	World Health Organization
ZnF	zinc finger

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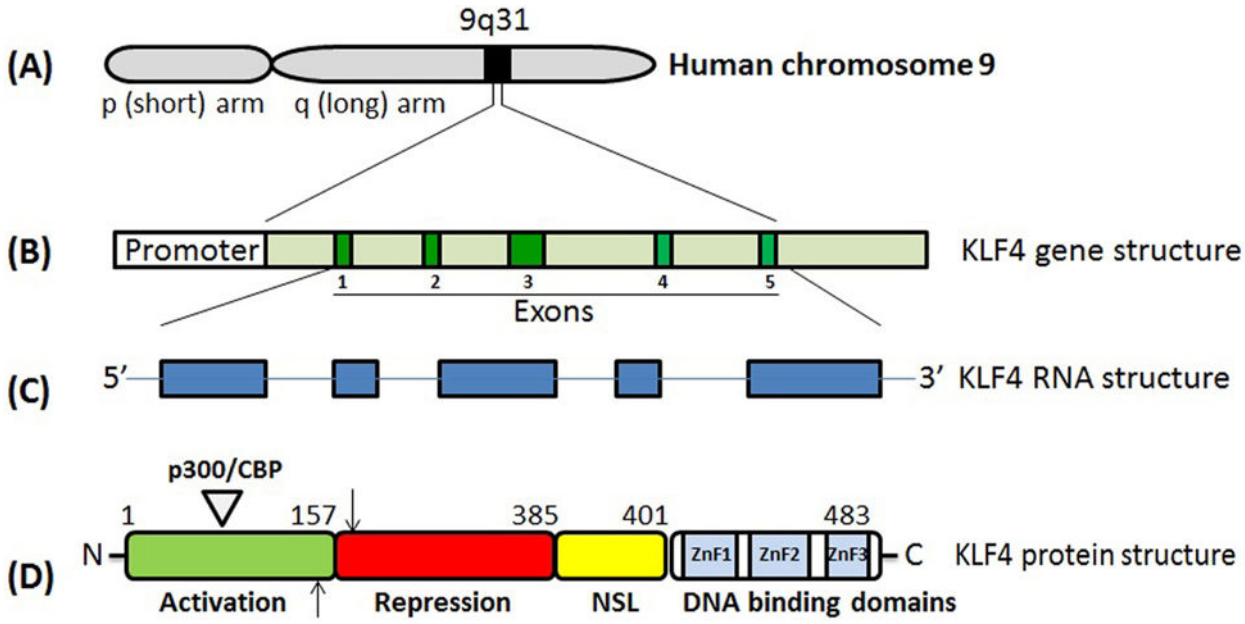

FIG. 1.

Molecular structure of human KLF4 and its functional domains. (A) Chromosomal location of the *KLF4* gene. Human chromosome 9 has the *KLF4* gene at its 9q31 location. (B) Molecular organization of the *KLF4* gene, which has a promoter region and five exons (dark green box/rectangles). (C) Molecular organization of KLF4 RNA transcript. Five RNA segments (dark blue) that result from the corresponding five exons contain the open reading frame for encoding the KLF4 protein, with the predicted 513 amino acids and a molecular mass of 54 kDa. (D) Distinct functional domains of the KLF4 protein. The protein sequence of human KLF4 has four distinct functional domains. The transactivation domain (green) at the amino terminal interacts with the co-activators p300/CBP. A repression domain (red) contains two lysines that can be acetylated by p300/CBP. Afterward, it contains a hexapeptide NLS (yellow). The potential PEST sequences are also shown (arrows) for their degradation by ubiquitin-proteasome pathway. Finally, the DBD at the carboxyl terminal has three consecutive ZnF motifs (ZnF1, 2, and 3) that participate in binding to the specific DNA sequence of a target gene.

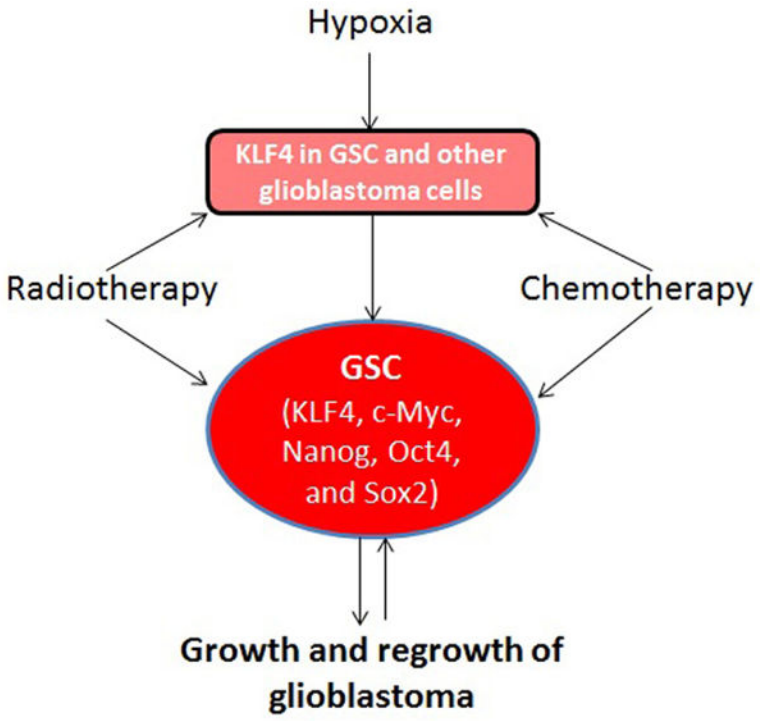

FIG. 2.

Roles of KLF4 as oncogene in human glioblastoma. KLF4 is highly expressed to function as an oncogene in heterogenic glioblastoma cells, especially in GSCs, in this solid tumor. Hypoxia has an important role in promoting expression of KLF4 in GSCs and other glioblastoma cells. Radiotherapy and chemotherapy fail to kill every GSC in the tumor, and only a few GSCs remaining after therapeutic treatments can regrow the tumor that can, in turn, produce more GSCs to perpetuate malignant tumor growth.

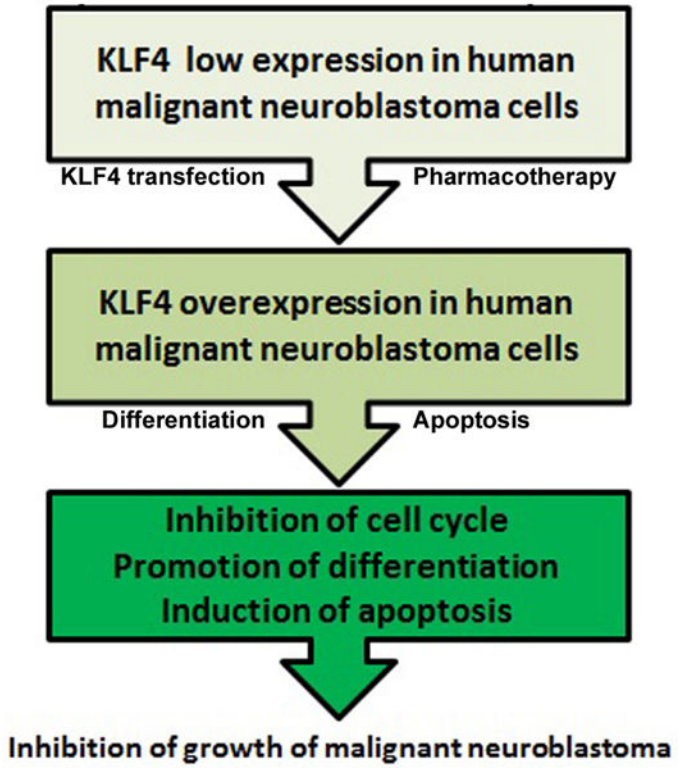

FIG. 3.

Roles of KLF4 as tumor suppressor in human malignant neuroblastoma. KLF4 functions as a tumor suppressor in neuroblastoma, because no or negligible expression of KLF4 is associated with maintenance of cell proliferation, lack of terminal differentiation, and absence of apoptosis in neuroblastoma cells. Overexpression of KLF4 by an appropriate therapeutic strategy (e.g., KLF4 cDNA transfection and pharmacological treatment alone or in combination) can inhibit cell proliferation, angiogenesis, and invasion; promote terminal differentiation; and induce apoptosis to control the growth of malignant neuroblastoma.