REVIEW

Conflicting pro- and anti-tumoral reports of the clock transcription factor BHLHE41 involvement in oncogenesis at the advent of spatiotemporal multiomics

Katalin Csép*

Genetics Department, George Emil Palade University of Medicine, Pharmacy, Science and Technology of Targu Mures, Targu Mures, Romania

The bHLH-Orange transcriptional repressor BHLHE41 is considered a member of the fifth clock gene family. Diverse mechanisms of gene expression regulation and interaction with numerous transcription factors, epigenetic modifiers and master regulators often in feedback loops characterize BHLHE41 activity. BHLHE41 may be involved in oncogenesis by various mechanisms due to its pleiotropic functions. Responsive to various signals such as hypoxia or chemotherapeutics, BHLHE1 controls cell fate as a regulator of proliferation, differentiation, DNA damage repair and apoptosis. Conflicting reports of pro- and anti-tumoral effects suggest context-dependent and tumor-specific effects. BHLHE1 involvement in key mechanisms repeatedly reported include the hypoxia response and the inhibition of apoptosis and epithelial-mesenchymal transition. The sensitive balance between BHLHE41 and its paralog BHLHE40, characterized by shared and non-redundant complementary or opposing moonlighting functions, may be critical in oncogenesis. Addressing the functional complexity and heterogeneity as well as the short and long term dynamics of BHLHE41 biology by emerging spatial and temporal omics technologies may be of practical importance for precision oncology and personalized care, drug development and selection, early diagnosis and patient monitoring, or chrono-chemotherapy.

Keywords: BHLHE41, DEC2, SHARP1, oncogenesis, apoptosis

Received 16 November 2025 / Accepted 26 January 2026

Introduction

Members of the large superfamily of bHLH (basic helix-loop-helix) transcription factors have pivotal roles in various processes including the circadian clock and cell fate decision, crucial for diverse conditions from embryonic development to oncogenesis. The bHLH transcriptional repressors BHLHE40 (DEC1, SHARP2) and BHLHE41 (DEC2, SHARP1) are encoded by the fifth clock gene family, acting as an ancillary transcriptional-translational feedback loop of the molecular clock mechanism [1]. In addition to the shared effects, the two paralogs have several non-redundant complementary or opposing moonlighting functions related to differences in their structure, gene expression pattern and interactions with other proteins [2].

Circadian disruption increases the risk of disease development, and clock transcription factors may be implicated in various pathologies including tumorigenesis [3]. Investigation of BLHLE41 involved in the regulation of the circadian rhythm, sleep homeostasis and immunity may offer insight to the relationship of the molecular clock and disease development, as succinctly phrased by Kurien et al. “Genes that make you tick can make you sick” [4]. The contradictory findings about the presumed context-dependent pro- or anti-tumoral effects remain, however, incompletely clarified. BLHLE41 structural characteristics, gene expression regulatory mechanisms and role in oncogenesis is reviewed. Possible sources of conflicting observations and research gaps, as well as opportunities for new studies associated with potential clinical implications using emerging technologies are addressed.

I. Gene and protein structure and function

The gene *BHLHE41* or *BHLHB3* encodes a basic helix-loop-helix (bHLH) transcription factor, which contains an Orange domain (*BHLHE),* and binds to E-box sequences (*BHLHB)* [5]. (Table I) Due to the presence of the Orange domain, BHLHE41 is a member of the forth subfamily of bHLH-O proteins, the repressor family of bHLH transcription factors. Unlike the other class members, however, BHLHE41 contains no WRPW (Trp-Arg-Pro-Trp) tetrapeptide sequence necessary for recruiting the co-repressor Grouchy [6].

The N-terminal half comprises the bHLH region (position 44-99) necessary for DNA-binding and homo- or hetero-dimerization. It includes also the LXXLL motif (position 67-71) responsible for the interaction with retinoid nuclear receptors [5,7]. The Orange domain is situated towards the central region (position 131-166), and possibly serves target specificity and transcriptional repression by interaction with other proteins [5,6]. The C-terminal half is required for interaction with HDAC1. It contains a proline-rich region towards the end including also the P384R mutation critical for sleep length, which is preceded by a region rich in alanine and glycine absent in BHLHE40 [2,5,7,8].

* Correspondence to: Katalin Csép
E-mail: katalin.csep@umfst.ro

Table 1. Gene and protein characteristics [5]

Symbol (Aliases)	Name	Cytogenetic locus	Genomic location (GRCh38)	Gene size	Number of exons	Protein size	Molecular mass
BHLHE41 *BHLHB3* *DEC2* *SHARP1*	Basic Helix-Loop-Helix Family, Member E41 Basic Helix-Loop-Helix Domain-Containing Protein, Class B, 3 Differentially Expressed In Chondrocytes Protein 2 Homolog Of Enhancer of Split- and Hairy-Related Protein 1	12p12.1	chr12: 26119000-26127601	5008 bases	5	482 amino acids	50498 Da

The paralogs share an overall homology of approximately 42%, with a high similarity in the conserved bHLH region (~97%) and a more diverse structure in the rest of the proteins, including moderate similarities in the Orange domain (52%) [8]. While the highly conserved bHLH domain may explain the redundant paralog functions carried out by direct DNA binding at E-box sequences, the major differences in the rest of the protein structures may explain the non-redundant effects dependent on interactions with various transcription factors and epigenetic modifiers. (Figure 1)

The conserved bHLH domain is necessary for DNA-binding and formation of homodimers or heterodimers with BHLHE40 or other bHLH proteins [9,10]. Mutation analysis demonstrated that the basic region is essential for the suppressive activity. 57Arg is conserved among the group B bHLH proteins which can bind to the CACGTG sequence characteristic for the regulatory E-box of target genes; 57Arg but not 48Pro was found essential for the BHLHE41 suppressive activity on the CLOCK:BMAL-mediated trans-activation [10]. Post-translational modifications include ubiquitination and sumoylation [2,9]. Ubiquitination leads to proteasomal degradation, while sumoylation at evolutionarily conserved sites inhibits ubiquitination. Sumoylation promotes the repressor activity by assisting the association with chromatin-modifying enzymes such as the methyl-transferase G9a, critical for specific functions [10-12].

BHLHE41 is widely expressed, though the expression pattern is more restricted compared to the ubiquitous expression of *BHLHE40* [2,8]. Expression starts during embryonic development, increasing until adulthood, and is highest in the brain [13-15]. *BHLHE41* presents circadian expression both in the central clock and the peripheral clocks [1,16].

The *BHLHE41* promoter harbors two E-boxes (5'-CACGTG-3') which allow the core clock activator binding dictating its expression with a circadian rhythmicity. By binding to the E-box regulatory sequences within the promoter, *BHLHE41* transcription is under the control of the core clock transcriptional activator CLOCK(NPAS2):BMAL1(BMAL2) heterodimer and the repressor PER:CRY inhibiting transactivation, following a repressor-precedes-activator pattern [1,17]. Creating an auxiliary transcriptional-translational feed-back loop (DEC loop), BHLHE40 and BHLHE41 repress their own and their paralog's transcription through E-box binding [18].

The promoter of *BHLHE41* contains no TATA-box, but two E-box sequences at -0.3 and -1.8 kb [2]. Various response elements (RE) render gene expression responsive to various transcription factors and stimuli, though less numerous than in case of *BHLHE40* [2,19]. Hypoxia,

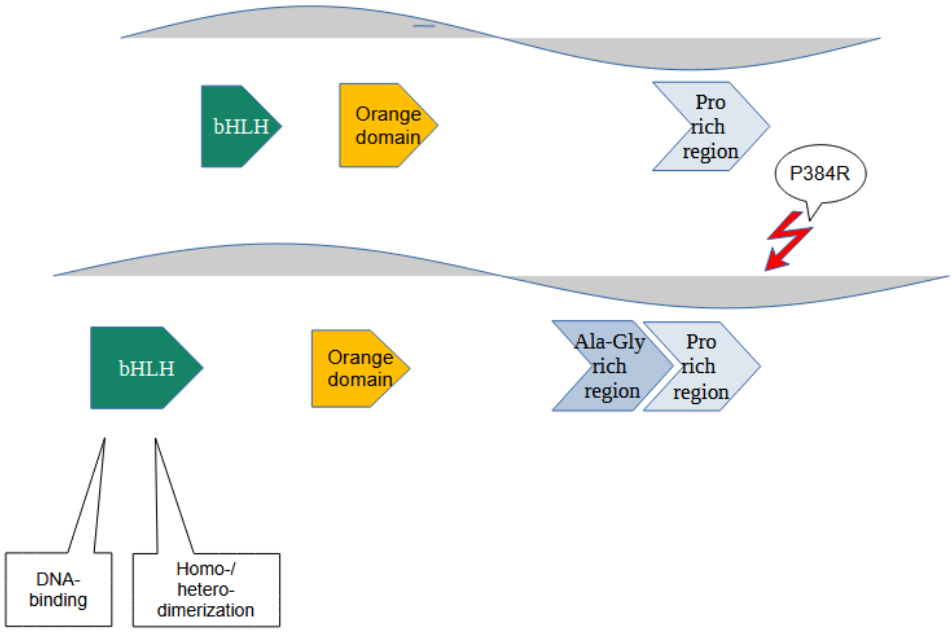

Fig. 1. BHLHE40 and BHLHE41 structure: conserved domains and major differences

nutrients, cytokines and growth factors may modulate gene expression, suggesting BHLHE41 involvement in diverse physiological responses and various moonlighting functions with pathological implications. BHLHE41 was identified in a model for neurite growth studies induced by NGF, being considered an important factor for neuronal growth, differentiation and survival [13]. The clock nuclear receptor activator ROR-alpha regulates *BHLHE41* gene activity through the regulatory sequences RORE, which gains special importance during adipogenesis [20]. The Kruppel-like zinc finger transcription factors GLI1 and GLI2 modulate *BHLHE41* expression through GLI-binding site, leading to repressed mismatch repair (MMR) in cancer cells [21]. BHLHE41 is required for the initial Th2 lineage commitment induced by GATA3 [22]. BHLHE41 plays a key role in the hypoxia response through its two HREs, binding HIF-1 rapidly, with higher affinity than BHLHE40 [23].

BHLHE41 may repress gene expression by various mechanisms. Transcriptional regulation is highly dependent on protein structure, and involves the conserved domain responsible for direct DNA binding, or less conserved domains allowing protein interactions with epigenetic modifiers and other transcription factors. Acting in the nucleus as a transcription factor, BHLHE41 may carry out the repression of gene expression in various and combined ways, by active and passive mechanisms, both by binding to DNA and interacting directly or indirectly with other transcription factors and epigenetic writers or erasers [9,11-12,18,24-30]. BHLHE41 may act, however, also at post-transcriptional level in the cytoplasm, regulating target protein stability and activity. Interestingly, it may demonstrate even earlier and higher repression activity than BHLHE40 [24].

II. BHLHE41 in oncogenesis

II.1. Reports of pro- and anti-tumoral effects

By regulating the circadian clock and sleep, hypoxia and DNA-damage response, cell cycle progression and apoptosis, EMT (epithelial-mesenchymal transition) and cell migration, angiogenesis and immunity, or even drug metabolism, BHLHE41 may contribute to tumor development, malignant progression and therapeutic response by multiple mechanisms [2,3,4,30]. BHLHE41 dysregulation was identified in various tumors, however, the numerous studies reported also conflicting observations. BHLHE41 was reported both as cancer promoting and suppressive [31]. Though considered a tumor suppressor, it may act also as a driver of tumor progression. It was also suggested that BHLHE41 might be critical for cancer development, but not always necessary for maintaining advanced tumors [32].

II.1.1. Breast, ovarian, endometrial and prostate cancer

In breast cancer, BHLHE41 may serve as a tumor suppressor. Its lower expression is associated with a more aggressive pathogenic grade and poor overall survival in ER- and HER2-positive cases [33]. In triple-negative breast cancer, BHLHE41 is considered a crucial regulator of the invasive and metastatic phenotype. Regulated by the metastasis suppressor P63, member of the P53 family, BHLHE41 inhibits cancer aggressiveness by blunting the HIF-induced malignant cell behavior [34]. It may oppose the HIF-dependent cancer cell migration through inhibition of HIF-1-alpha and HIF-2-alpha, promoting the proteasomal degradation of HIF [24,33]. Investigation of the hypoxia-induced expression of BHLHE40 and BHLHE41 in MCF-7 human breast cancer cells, however, found that the expression pattern of BHLHE41 and HIF-2-alpha was identical, and over-expression of BHLHE41 increased proliferation under both normoxic and hypoxic conditions [35]. As a target gene for the PI3K/Akt signaling pathway, BHLHE41 over-expression due to hypoxia exposure resulted in up-regulated expression of c-Myc, and thus hypoxia-induced cell proliferation [35].

In MCF-7 breast cancer cells, the expression of both BHLHE40 and BHLHE41 is increased by Paclitaxel in a dose-dependent manner, but BHLHE41 up-regulation is higher than that of BHLHE40. BHLHE41 was found having anti-apoptotic effect both with and without Paclitaxel treatment, its knock-down leading to increased apoptosis [36,37]. The therapeutic effect of the RXR-selective vitamin A analogs in ER-negative mammary tumorigenesis also involves BHLHE41. Bexarotene represses cyclin D1 by inducing the expression of BHLHE41, and BHLHE41 represses the transcription of the gene encoding cyclin D1 by HDAC1 recruitment to the promoter [38].

BHLHE41 is considered a characteristic gene of ovarian clear cell carcinoma and high-grade serous carcinoma [39]. In endometrial carcinogenesis and tumor phenotype development, activation of the HIF-1 pathway and BHLHE41 have key roles. Hypoxia causes an increase in HIF-1 alpha expression and the expression of its target genes, including *BHLHE41*. The expression of BHLHE41 was found significantly higher in atypical hyperplasia tissues and carcinoma compared to normal cells [40]. However, in endometrial cancer also suppression of EMT by BHLHE41 was described, linked to the NOTCH1 signaling pathway. The over-expression of BHLHE41 attenuated EMT, and lead to inhibited cell migration, invasion and metastasis [41]. In addition, in endometrial cancer cells BHLHE41 was found inhibiting invasion and EMT also by regulation of TWIST1, competing with SP1 for promoter binding sites [42].

In prostate cancer, BHLHE40 and BHLHE41 demonstrate opposite effects on apoptosis and TGF-beta induced EMT [43, 44]. When investigated in human prostate cancer DU145 and PC-3 cells, under Paclitaxel treatment, BHLHE40 and BHLHE41 expression decreased in DU145 cells and increased in PC-3 cells. BHLHE41 was found exerting anti-apoptotic effect on the Paclitaxel-induced apoptosis, the knock-down of BHLHE41 increasing, while that of BHLHE40 decreasing the amount of

cleaved PARP [43]. In PC-3 cells, TGFβ increased BHLHE40, but decreased BHLHE41 levels. The knock-down of BHLHE40 decreased, while that of BHLHE41 promoted a more metastatic phenotype, associated with the opposite effects on EMT, as shown by the mesenchymal marker N-cadherin and vimentin activation and epithelial marker E-cadherin suppression [44].

II.1.2. Osteosarcoma

BHLHE41 is expressed at higher levels in chondroblastic than osteoblastic osteosarcoma, and has a key role in cell proliferation and apoptosis resistance. In the human chondroblastic-like osteosarcoma cell line MNNG/HOS, over-expression of BHLHE41 affects the proliferation of the cells by activating the VEGFC/VEGFR2 signaling pathway. The enhanced BHLHE41 expression leads to increased VEGFR2 expression, as well as increased phosphorylation levels at sites Y951 and Y1175 of VEGFR2 [45]. While activation of $VEGFR2_{Y1175}$ was found increasing cell proliferation through $VEGFR2_{Y1175}$-PLCγ1-PKC-SPHK-MEK-ERK signaling, the activation of $VEGFR2_{Y951}$ decreased mitochondria-dependent apoptosis rate through $VEGFR2_{Y951}$-VARP-PI3K-AKT signaling [45].

Interestingly, while BHLHE41 is known to promote HIF-1-alpha degradation, in osteosarcoma it facilitates HIF-1-alpha stabilization and promotes HIF-1 activation. In a tumor-specific manner, in human osteosarcoma BHLHE41 expression is positively correlated with HIF-1-alpha levels and a poor prognosis, contributing to progression and metastasis though a BHLHE41-HIF-1 vicious cycle [46].

In tumorigenesis, BHLHE41 may be involved also as the target of various miRNAs, and the mutual regulation between BHLHE41 and miRNAs may have potential therapeutic implications. For instance, in osteosarcoma miR-138 or miR-301 inhibit cell proliferation and invasion at least in part by regulation of BHLHE41 [47,48].

II.1.3. Renal cell carcinoma

BHLHE41 polymorphisms were described in association with renal cell carcinoma risk.

In the development of renal cell carcinoma, HIFs play essential roles, and rs12814794 by A>G leads to a modified BHLHE41 promoter sequence introducing a novel HIF-1 binding site, which may allow increased expression by the hypoxia-induced transcription factor [49].

Associated with clear cell renal carcinoma, independent of HIF involvement, rs7132434 was described introducing an additional AP-1 binding site, while a lower frequency methylation in the 3'-UTR was reported, correlating with BHLHE41 over-expression and tumor growth promotion [32,50,51].

II.1.4. Gastrointestinal tumors

BHLHE41 may modulate tumor cell dormancy. In salivary adenoid cystic carcinoma, BHLHE41 over-expression was found limiting tumor growth, inhibiting cell proliferation and increasing the cell population arrested in G0/G1 phase. Decreased BHLHE41 levels were found associated with dormancy exit resulting in primary tumor growth and lung metastasis, possibly related to hypoxia and EMT activation [52]. In oral cancer HSC-3 but not CA9-22 cells, BHLHE41 expression was shown to be up-regulated by Cisplatin treatment, and BHLHE41 over-expression associated with the inhibition of the pro-apoptotic factor Bim, explaining the inhibition of medication-induced apoptosis [53].

In esophageal squamous cell carcinoma, besides the marked inhibition of the proapoptotic Bim, a slight increase of the antiapoptotic BclxL expression may also contribute to antagonizing the Cisplatin-induced apoptosis and improved cell viability by BHLHE41 [54]. miR-873, which acts as a tumor suppressor by targeting BHLHE41, and inhibits cancer cell growth, migration and invasion, is under-expressed in esophageal cancer [55].

In gastric cancer, BHLHE41 expression appears lower, negatively correlated with malignancy progression. BHLHE41 inhibits proliferation, induces apoptosis, shows positive correlation with the EMT regulator E-cadherin. The anti-metastatic effect is mediated by inhibition of the ERK/NF-κB pathway [56].

In pancreatic cancer, BHLHE41 levels appear low compared to the significant amounts found in the adjacent non-cancerous pancreatic tissues. BHLHE41 may inhibit the progression of pancreatic cancer involving TGF-beta and SLUG. In the presence of TGF-beta, BHLHE41 over-expression decreased the migration and invasion of BxPC-3 cells, while knock-down of BHLHE41 in the presence of TGF-beta significantly increased the expression and nuclear concentration of SLUG [57]. Activation of GLI1, a key molecule of the Hedgehog signaling pathway, represses the MMR activity, contributing to the development and progression of pancreatic cancer through the BHLHE41-dependent suppression of MLH1 [21]. Tumor hypoxia may also cause a functional loss of MMR through the HIF-induced BHLHE41 down-regulation of MLH1 expression [58].

In colon cancer models, BHLHE41 over-expression blocked the cell cycle, induced apoptosis, decreased migration and invasion, by reducing the level of HIF-1-alpha and EMT-related proteins [59].

II.1.5. Other malignancies

In lung cancer, BHLHE41 was proposed as a tumor suppressor, inhibiting colony formation mainly through the down-regulation of *CCDN1* encoding cyclin D1 [60]. Compared to the normal tissue, a decreased expression characterizes the adenocarcinoma cells, while a better prognosis may associate with higher expression. Preventing early malignant progression by inducing autophagic cell death, BHLHE41 expression constitutes a favorable prognostic factor in non-small cell lung cancer development [61].

In glioma and glioblastoma, pro-tumoral effects of BHLHE41 is suggested by promotion of proliferation in U87 and U251 cell lines through the ERK/cyclinD1 signaling pathway [62].

BHLHE41 was reported significantly down-regulated in TT and TPC-1 thyroid cancer cell lines, and up-regulated in other thyroid cancer cell lines. Over-expression in TT and TPC-1 cells inhibited cell viability, migration and invasion associated with decreased HIF-1-alpha, suggesting a tumor suppressor role through HIF-1-alpha effects [63].

According to RNA interference viability assay screening, BHLHE41 appears as an essential gene in hematological malignancies [30]. Increased BHLHE41 expression associates with poor survival of patients with leukemia and myeloma. A unique oncogenic role of BHLHE41 was described in acute myeloid leukemia with a rearrangement involving the large histone methyltransferase encoding *MLL*, by the translocation t(6;11)(q27;p23) named MLL-AF6 [32, 64]. BHLHE41 is considered an oncogenic driver in the MLL-AF6 acute myeloid leukemia characterized by the worst prognosis of *MLL* rearrangement associated forms. As a target of MLL-AF6, BHLHE41 over-expression is involved in disease promotion by activating genes critical for cell survival, crucial for maintaining clonogenic growth and preventing apoptosis. The suppression of BHLHE41 was found robustly inducing apoptosis, reducing growth and colony formation of blast cells, without affecting normal hematopoiesis [64].

II. 2. Possible sources of conflicting observations and future perspectives

II.2.1. Tumor-specific effects resulting from dynamic context-dependent changes

Interpreting the available data, the pro-tumoral and anti-tumoral effects observed in various types of neoplasia remains challenging. (Figure 2) According to current GEPIA data, the highest BHLHE41 expressions characterize ovarian serous cystadenocarcinoma, thyroid carcinoma, stomach adenocarcinoma and clear cell renal cell carcinoma [65]. Systematic analysis of knockout mice, in vitro models, genetic analysis of tumor cells and immunohistochemical studies of clinical samples demonstrate the complexity of BHLHE41 functions and involvement in oncogenesis, and suggest that the contradictory findings may be related to the context-dependent actions and the limitations of study design in case of a circadian transcription factor influenced by stress factors [31,32].

A high degree of complexity characterizes BHLHE41. BHLHE41 may be the target of multiple signaling pathways, and may have tumor-specific effects. The context-dependent BHLHE41 molecular function may be the result of environmental changes and varying interacting molecules. Situated at the intersection of key signaling pathways and fundamental mechanisms involved in oncogenesis, BHLHE41 may act as a molecular switch critical for health or disease development.

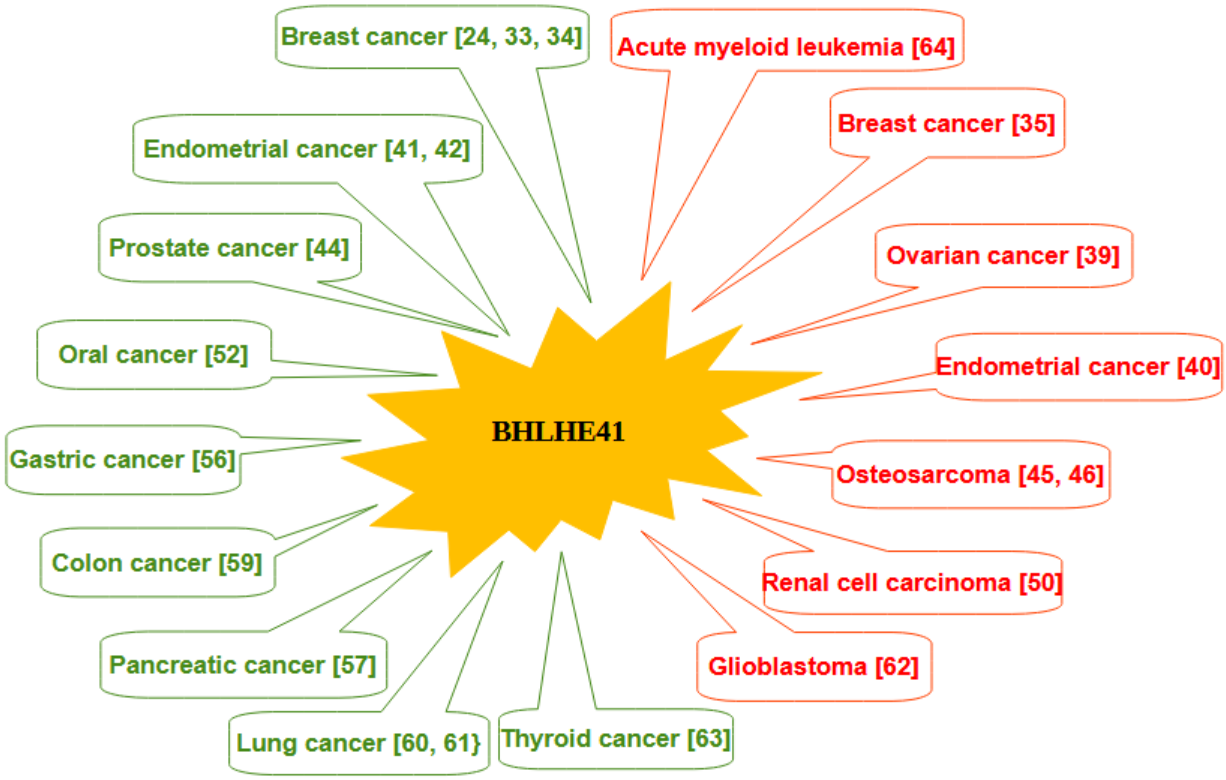

Fig. 2. Pro-tumoral (red) and anti-tumoral (green) effects of BHLHE41 reported in various tumors

Reports of involvement in oncogenesis suggest diverse mechanisms of activation and pleiotropic functions. For instance, it may act as a target for the histone methyltransferase MLL, and key pathways such as PI3K/Akt or Shh signaling [21,35,64]. In addition to cytokines and growth factors, various types of stress like exposure to hypoxia or chemotherapeutic agents affect BHLHE41 expression [16,23,24,28,38,40,53]. Finally, BHLHE41 is a multifaceted regulatory protein, and in response to the various signals, it may regulate directly and indirectly crucial elements of tumor development and progression, like the master regulators of cell proliferation, DNA damage repair, apoptosis, EMT, cell migration, invasion, angiogenesis or immunity [21,22,34-38,43,45,53,57,58,62].

BHLHE41 and apoptosis

Apoptosis appears as the major process involved in tumorigenesis affected by BHLHE41. Its complex role in apoptosis regulation may be critical for both tumor development and therapeutic response. With or without treatment, BHLHE41 has an anti-apoptotic effect demonstrated in various breast, prostate, oral and esophageal cancer cell lines; however, tumor-specific and context-dependent pro-apoptotic effects were attributed to BHLHE41 in acute myeloid leukemia or gastric cancer [36,37,43,53,54,56,64].

The tightly regulated cell death program may be modulated by BHLHE41 in various ways. Firstly, BHLHE41 may reduce the expression of such pro-apoptotic proteins as Bim [53,54]. Induced by TNF-alpha, BHLHE41 over-expression leads to reduced caspase-8 and cleaved PARP by repressed expression of the pro-apoptotic proteins Fas and Bax, possibly through the E-box elements present in their regulatory sequences [36]. BHLHE41 over-expression may also associate with a slight increase in the expression of the anti-apoptotic Bcl-xL [54]. In case of BHLHE41 knock-down, an increased amount of cleaved Bid was observed after treatment with TNF-alpha [36]. In addition, BHLHE41 may modulate apoptosis indirectly, regulating the expression of such key molecules as cyclin D1, c-myc or VEGFR involved in regulating tumorigenesis by controlling the cell cycle, proliferation or angiogenesis [35,38,45,62] (Figure 3). Moreover, BHLHE41 levels may change after the administration of chemotherapy, and the efficacy of intervention may be modulated by its anti-apoptotic effects [37,43,53,54].

BHLHE40 and BHLHE41 interplay

BHLHE40 and BHLHE41 interplay adds a further layer to complexity, and the paralog dominance could be crucial also for the cell fate. While BHLHE40 appears critical for cell senescence regulation, BHLHE41 seems important for apoptosis control. Interestingly, the differences between the paralogs become most evident during oncogenesis, when BHLHE40 and BHLHE41 may demonstrate opposite effects on processes such as apoptosis or EMT [31,43,44]. Similarities and differences of BHLHE40 and BHLHE41 structure, expression pattern, response to external and internal stimuli, protein interactions, and mutual regulation may explain the complex redundant, complementary or antagonistic effects.

The similar structure in the conserved bHLH domain may determine the shared functions, while structural differences in the C-terminal half may be responsible for the non-redundant functions related to different interactions. Coordinated interactions and complementary collaborations of the paralogs have been identified in case of the fine regulation of the circadian rhythm or hypoxia response [23,24], while functional antagonism or opposing effects

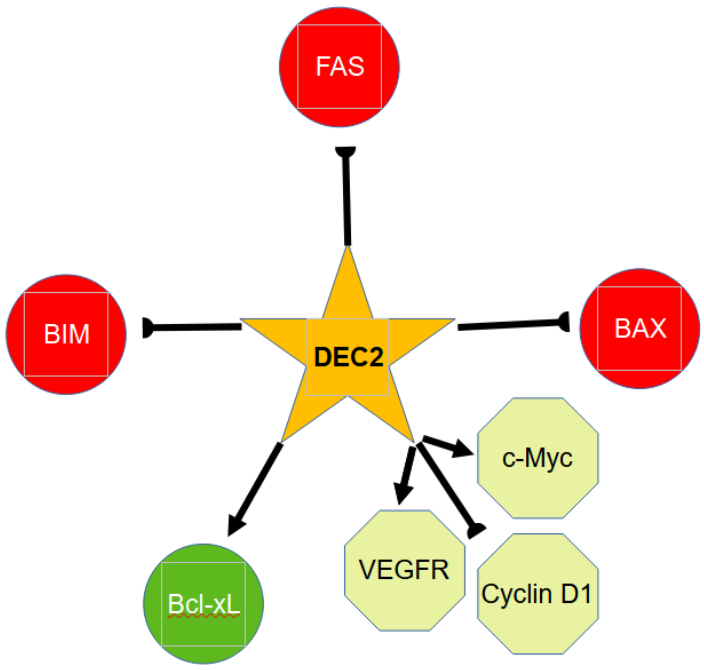

Fig. 3. BHLHE41 regulatory roles of apoptosis in oncogenesis

have been described in apoptosis and EMT regulation [43,44]. Opposing roles during tumor development could be related to different protein-protein interactions, and mutual transcriptional repression could lead to antagonistic effects.

Paralog levels present opposing tendencies in tumor cells, and the sensitive balance between BHLHE40 and BHLHE41 could have important functional consequences during carcinogenesis [31,44]. Dysregulation by different stimuli and regulatory pathways of BHLHE40 and BHLHE41 may lead to an altered balance, and the predominant form and mutual down-regulation could determine the ultimate effect on cell fate or tumor development. Paralog dominance in tumor cells, tumor micro-environment, or distant cells with indirect effects on tumor development and progression, changing over time and in constant mutual regulation could be an important aspect in oncogenesis, and needs to be addressed by further systematic analyses.

II.2.2. From investigational challenges to novel opportunities in research and patient care

Contradictory observations may be related to the limited possibilities of previous investigations, and aspects not addressed in case of sampling and analyzing a transcription factor manifesting rhythmic circadian expression and modulated by various stress factors [31,32]. At the advent of a new paradigm shift in understanding oncogenesis by using innovative technologies, addressing former analytical challenges and current research gaps in BHLHE41 biology appears promising.

BHLHE41 biology research

BHLHE41 is a transcription factor with pleiotropic functions, acting in the context of a complex gene interaction network. Perturbed communication is a key aspect of carcinogenesis, and BHLHE41 involvement could be critical in the process, since it acts as a regulatory hub for sleep, cell cycle, DNA repair, apoptosis and immunity through numerous interactions. Molecular heterogeneity is an important characteristic of neoplastic processes, and BHLHE41 changes could vary, and its involvement may be tumor-specific. BHLHE41 role could be different in the various tumor cells, in the tumor micro-environment, or distant cells associated with indirect effects on oncogenesis. In addition, BHLHE41 expression is modulated by various stress factors, so investigation results could be affected by the conditions of sampling or the analytical methods used. Novel techniques like spatial multiomics may address functional complexity and molecular heterogeneity. Single-cell multi-omics analysis of tissue-specific interactomes may elucidate the importance of the balance between BHLHE41 and its paralog or other interacting proteins. Perturbomics may allow accurate identification of gene signatures and phenotypes associated with altered interactions dependent on different signals received.

Beyond the static snapshots offered by modern genomic investigations, however, temporal changes must be also clarified. BHLHE41 is a circadian clock transcription factor characterized by dynamic changes, orchestrating the oscillating synthesis of numerous gene products often crucial for carcinogenesis. Previous investigations had very limited possibilities to address this aspect. Time notation associated with sampling is necessary, but limited. Repeated sampling is often practically and ethically unfeasible. Novel data-driven possibilities and machine learning may overcome such difficulties [66]. Transcription factor reporter assays allow monitoring transcription factor activity in individual cells in real time, and the emerging live-omics technologies may ultimately clarify the temporal changes of the complex regulatory network and the importance of BHLHE41. Nevertheless, the time factor must be addressed also on the long term, since tumors develop in time, associated with characteristic molecular changes and adaptive processes [67]. Repeated investigations during follow-up by non-invasive methods like liquid biopsy and circulating tumor cell analysis may contribute with valuable information to understanding long term changes and implications.

BHLHE41 biology and patient care

Understanding BHLHE41 biology may contribute with valuable information to personalized management and precision oncology. It may clarify its biomarker potential, contribute to drug development, and selection of the most appropriate treatment or the optimal drug administration schedule. Since BHLHE41 may be critical for cancer development, but perhaps not always required for maintaining the process at advanced stages, *BHLHE41* could act as a potential stratifying marker gene of early diagnosis [32]. Since BHLHE41 regulates DNA repair by controlling MMR, and influences microsatellite instability and the mutation burden, it could affect the response to immunotherapy, and might guide selection of the most appropriate treatment.

BHLHE41 biology may also reveal novel therapeutic strategies, or the need of personalized dosing regimens for existing therapies. To improve immune checkpoint inhibitor therapy in colorectal cancer, for instance, the possible blockade of myeloid-derived suppressor cells involves the epigenetic writer METTL3 (methyltransferase like 3) and the m^{6}A-BHLHE41-CXCL1/CXCR2 axis [68].

BHLHE41 also participates in the circadian control of CYP2D6 expression, and the effect may be relevant for the administration of drugs like Tamoxifen [69]. Chrono-modulated chemotherapy has great potential in decreasing side-effects and increasing efficacy, and BHLHE41 investigations may have special chrono-chemotherapeutic importance due to its role in the coordinated regulation of circadian rhythm, sleep length and architecture, stress response, DNA damage repair, cell differentiation, apoptosis, immunity and drug-metabolizing enzymes. For

instance, chrono-modulated chemotherapy appears especially important in case of Cisplatin, and while Cisplatin administration influences BHLHE41 levels, BHLHE41 affects the Cisplatin-induced apoptosis [53,54].

Conclusion

The circadian clock transcription factor BHLHE41 may be involved in oncogenesis and may affect therapeutic efficacy by various mechanisms due to its pleiotropic functions, such as the regulation of the cell cycle, DNA repair, apoptosis, angiogenesis, immunity, or drug metabolization. Limited possibilities of previous studies to investigate context-dependent functions or temporal changes may explain the conflicting observations of pro- and anti-tumoral effects. The dynamic tumor-specific dysregulation of BHLHE41 and the complex paralog interplay may be critical in tumor development and progression, and needs further investigations. Innovative real-time in vivo multi-omics analyses addressing the complexity and time-dependent changes characteristic for BHLHE41 may clarify the reported discrepancies. Understanding BHLHE41 biology may contribute to an improved personalized care and precision oncology by assisting early diagnosis and patient monitoring, drug development, selection and chrono-chemotherapy.

Author contributions

KC - Conceptualization, Formal Analysis, Resources, Visualization, Writing – original draft, Writing – review & editing.

Conflict of interest

None to declare.

Funding

No external funding was received.

References

1. Honma S, Kawamoto T, Takagi Y, et al. Dec1 and Dec2 are regulators of the mammalian molecular clock. Nature. 2002;419(6909):841-4.
2. Kato Y, Kawamoto T, Fujimoto K, Noshiro M. DEC1/STRA13/SHARP2 and DEC2/SHARP1 coordinate physiological processes, including circadian rhythms in response to environmental stimuli. Curr Top Dev Biol. 2014;110:339-72.
3. Ow JR, Tan YH, Jin Y, Bahirvani AG, Taneja R. Stra13 and Sharp-1, the non-grouchy regulators of development and disease. Curr Top Dev Biol. 2014;110:317-38.
4. Kurien PA, Chong SY, Ptáček LJ, Fu YH. Sick and tired: how molecular regulators of human sleep schedules and duration impact immune function. Curr Opin Neurobiol. 2013;23(5):873-9.
5. UniProt [cited 2025 October 20]. Available from: http://www.uniprot.org.
6. Sun H, Ghaffari S, Taneja R. bHLH-Orange Transcription Factors in Development and Cancer. Transl Oncogenomics. 2007;2:107-20.
7. Cho Y, Noshiro M, Choi M, et al. The basic helix-loop-helix proteins differentiated embryo chondrocyte (DEC) 1 and DEC2 function as corepressors of retinoid X receptors. Mol Pharmacol. 2009;76(6):1360-9.
8. Fujimoto K, Shen M, Noshiro M, et al. Molecular cloning and characterization of DEC2, a new member of basic helix-loop-helix proteins. Biochem Biophys Res Commun. 2001;280(1):164-71.
9. Fujimoto K, Hamaguchi H, Hashiba T, et al. Transcriptional repression by the basic helix-loop-helix protein Dec2: multiple mechanisms through E-box elements. Int J Mol Med. 2007;19(6):925-32.
10. Kondo J, Sato F, Fujimoto K, et al. 57Arg in the bHLH transcription factor DEC2 is essential for the suppression of CLOCK/BMAL2-mediated transactivation. Int J Mol Med. 2006;17(6):1053-6.
11. Liu B, Wang T, Mei W, et al. Small ubiquitin-like modifier (SUMO) protein-specific protease 1 de-SUMOylates Sharp-1 protein and controls adipocyte Differentiation. J Biol Chem 2014;289(32):22358-64
12. Wang Y, Shankar SR, Kher D, Ling BM, Taneja R. Sumoylation of the basic helix-loop-helix transcription factor sharp-1 regulates recruitment of the histone methyltransferase G9a and function in myogenesis. J Biol Chem. 2013;288(24):17654-62.
13. Rossner MJ, Dörr J, Gass P, Schwab MH, Nave KA. SHARPs: mammalian enhancer-of-split- and hairy-related proteins coupled to neuronal stimulation. Mol Cell Neurosci. 1997;10(3-4):460-75.
14. Azmi S, Taneja R. Embryonic expression of mSharp-1/mDEC2, which encodes a basic helix-loop-helix transcription factor. Mech Dev. 2002;114(1-2):181-5.
15. Chen L, Zhou J, Xu H, Xu G, Xue J. Identification and developmental expression of Dec2 in zebrafish. Fish Physiol Biochem. 2010;36(3):667-675.
16. Guillaumond F, Lacoche S, Dulong S, et al. Altered Stra13 and Dec2 circadian gene expression in hypoxic cells. Biochem Biophys Res Commun. 2008;369(4):1184-9.
17. Ueda HR, Hayashi S, Chen W, et al. System-level identification of transcriptional circuits underlying mammalian circadian clocks. Nat Genet. 2005;37(2):187-92.
18. Li Y, Xie M, Song X, Gragen S, Sachdeva K, Wan Y, Yan B. DEC1 negatively regulates the expression of DEC2 through binding to the E-box in the proximal promoter. J Biol Chem. 2003;278(19):16899-907.
19. Hamaguchi H, Fujimoto K, Kawamoto T, et al. Expression of the gene for Dec2, a basic helix-loop-helix transcription factor, is regulated by a molecular clock system. Biochem J. 2004;382(Pt 1):43-50.
20. Ozaki N, Noshiro M, Kawamoto T, et al. Regulation of basic helix-loop-helix transcription factors Dec1 and Dec2 by RORα and their roles in adipogenesis. Genes Cells. 2012;17(2):109-21.
21. Inaguma S, Riku M, Hashimoto M, Murakami H, Saga S, Ikeda H, Kasai K. GLI1 interferes with the DNA mismatch repair system in pancreatic cancer through BHLHE41-mediated suppression of MLH1. Cancer Res. 2013;73(24):7313-23.
22. Yang XO, Angkasekwinai P, Zhu J, et al. Requirement for the basic helix-loop-helix transcription factor Dec2 in initial TH2 lineage commitment. Nat Immunol. 2009;10(12):1260-6.
23. Miyazaki K, Kawamoto T, Tanimoto K, Nishiyama M, Honda H, Kato Y. Identification of functional hypoxia response elements in the promoter region of the DEC1 and DEC2 genes. J Biol Chem. 2002;277(49):47014-21.
24. Montagner M, Enzo E, Forcato M, et al. SHARP1 suppresses breast cancer metastasis by promoting degradation of hypoxia-inducible factors. Nature. 2012;487(7407):380-4.
25. Azmi S, Sun H, Ozog A, Taneja R. mSharp-1/DEC2, a basic helix-loop-helix protein functions as a transcriptional repressor of E box activity and Stra13 expression. J Biol Chem. 2003;278(22):20098-109.
26. Azmi S, Ozog A, Taneja R. Sharp-1/DEC2 inhibits skeletal muscle differentiation through repression of myogenic transcription factors. J Biol Chem. 2004;279(50):52643-52.
27. Cho Y, Noshiro M, Choi M, et al. The basic helix-loop-helix proteins differentiated embryo chondrocyte (DEC) 1 and DEC2 function as corepressors of retinoid X receptors. Mol Pharmacol. 2009;76(6):1360-9.
28. Choi SM, Cho HJ, Cho H, Kim KH, Kim JB, Park H. Stra13/DEC1 and DEC2 inhibit sterol regulatory element binding protein-1c in a hypoxia-inducible factor-dependent mechanism. Nucleic Acids Res. 2008;36(20):6372-85.
29. Gulbagci NT, Li L, Ling B, et al. SHARP1/DEC2 inhibits adipogenic differentiation by regulating the activity of C/EBP. EMBO Rep. 2009;10(1):79-86.
30. Bret C, Desmots-Loyer F, Moreaux J, Fest T. BHLHE41, a transcriptional repressor involved in physiological processes and tumor development. Cell Oncol (Dordr). 2025;48(1):43-66.
31. Sato F, Bhawal UK, Yoshimura T, Muragaki Y. DEC1 and DEC2 Crosstalk between Circadian Rhythm and Tumor Progression. J Cancer. 2016;7(2):153-9.
32. Furukawa T, Mimami K, Nagata T, Yamamoto M, Sato M, Tanimoto A. Approach to Functions of BHLHE41/DEC2 in Non-Small Lung Cancer Development. Int J Mol Sci. 2023;24(14):11731.
33. Fang W, Li Q, Wang M, Zheng M, Xu H. DEC2 Serves as Potential Tumor Suppressor in Breast Carcinoma. Dis Markers. 2020;2020:6053154.
34. Piccolo S, Enzo E, Montagner M. p63, Sharp1, and HIFs: master regulators of metastasis in triple-negative breast cancer. Cancer Res. 2013;73(16):4978-81.

35. Wu Y, Sato H, Suzuki T, et al. Involvement of c-Myc in the proliferation of MCF-7 human breast cancer cells induced by bHLH transcription factor DEC2. Int J Mol Med. 2015;35(3):815-20.
36. Liu Y, Sato F, Kawamoto T, et al. Anti-apoptotic effect of the basic helix-loop-helix (bHLH) transcription factor DEC2 in human breast cancer cells. Genes Cells. 2010;15(4):315-25.
37. Wu Y, Sato F, Bhawal UK, et al. Basic helix-loop-helix transcription factors DEC1 and DEC2 regulate the paclitaxel-induced apoptotic pathway of MCF-7 human breast cancer cells. Int J Mol Med. 2011;27(4):491-5.
38. Li Y, Shen Q, Kim HT, et al. The rexinoid bexarotene represses cyclin D1 transcription by inducing the DEC2 transcriptional repressor. Breast Cancer Res Treat. 2011;128(3):667-77.
39. Nagasawa S, Ikeda K, Horie-Inoue K, et al. Systematic Identification of Characteristic Genes of Ovarian Clear Cell Carcinoma Compared with High-Grade Serous Carcinoma Based on RNA-Sequencing. Int J Mol Sci. 2019;20(18):4330..
40. Yunokawa M, Tanimoto K, Nakamura H, et al. Differential regulation of DEC2 among hypoxia-inducible genes in endometrial carcinomas. Oncol Rep. 2007;17(4):871-8.
41. Liao Y, He X, Qiu H, C et al. Suppression of the epithelial-mesenchymal transition by SHARP1 is linked to the NOTCH1 signaling pathway in metastasis of endometrial cancer. BMC Cancer. 2014;14:487.
42. Asanoma K, Liu G, Yamane T, et al. Regulation of the Mechanism of TWIST1 Transcription by BHLHE40 and BHLHE41 in Cancer Cells. Mol Cell Biol. 2015;35(24):4096-109.
43. Liu Q, Wu Y, Yoshizawa T, et al. Basic helix-loop-helix transcription factor DEC2 functions as an anti-apoptotic factor during paclitaxel-induced apoptosis in human prostate cancer cells. Int J Mol Med. 2016;38(6):1727-1733.
44. Liu Q, Wu Y, Seino H, et al. Correlation between DEC1/DEC2 and epithelialmesenchymal transition in human prostate cancer PC3 cells. Mol Med Rep. 2018;18(4):3859-3865.
45. Tuerxun M, Zheng X, Xu J, Yang Q, Yuan T, Zhang C, Zhou S. High expression of DEC2 distinguishes chondroblastic osteosarcoma and promotes tumour growth by activating the VEGFC/VEGFR2 signalling pathway. J Cell Mol Med. 2024;28(11):e18462.
46. Hu T, He N, Yang Y, Yin C, Sang N, Yang Q. DEC2 expression is positively correlated with HIF-1 activation and the invasiveness of human osteosarcomas. J Exp Clin Cancer Res. 2015;34(1):22.
47. Jiang B, Mu W, Wang J, et al. MicroRNA-138 functions as a tumor suppressor in osteosarcoma by targeting differentiated embryonic chondrocyte gene 2. J Exp Clin Cancer Res. 2016;35:69.
48. Wu H, Yu Z, Chen Q, Li D, Chen W. MicroRNA-301a inhibits the progression of osteosarcoma by regulating DEC2. J BUON. 2020;25(2):1013-1021.
49. Grampp S, Schmid V, Salama R, et al. Multiple renal cancer susceptibility polymorphisms modulate the HIF pathway. PLoS Genet. 2017;13(7):e1006872.
50. Shen Z, Zhu L, Zhang C, Cui X, Lu J. Overexpression of BHLHE41, correlated with DNA hypomethylation in 3'UTR region, promotes the growth of human clear cell renal cell carcinoma. Oncol Rep. 2019;41(4):2137-2147. .
51. Bigot P, Colli LM, Machiela MJ, et al. Functional characterization of the 12p12.1 renal cancer-susceptibility locus implicates BHLHE41. Nat Commun. 2016;7:12098.
52. Yang X, Wu JS, Li M, et al. Inhibition of DEC2 is necessary for exiting cell dormancy in salivary adenoid cystic carcinoma. J Exp Clin Cancer Res. 2021;40(1):169.
53. Wu Y, Sato F, Bhawal UK, et al. BHLH transcription factor DEC2 regulates pro-apoptotic factor Bim in human oral cancer HSC-3 cells. Biomed Res. 2011;33:75–82.
54. Sato H, Wu Y, Kato Y, et al. DEC2 expression antagonizes cisplatininduced apoptosis in human esophageal squamous cell carcinoma. Mol Med Rep. 2017;16(1):43-48.
55. Liang Y, Zhang P, Li S, Li H, Song S, Lu B. MicroRNA-873 acts as a tumor suppressor in esophageal cancer by inhibiting differentiated embryonic chondrocyte expressed gene 2. Biomed Pharmacother. 2018;105:582-589.
56. Li P, Jia YF, Ma XL, et al. DEC2 suppresses tumor proliferation and metastasis by regulating ERK/NF-κB pathway in gastric cancer. Am J Cancer Res. 2016;6(8):1741-57.
57. Sato F, Kawamura H, Wu Y, et al. The basic helix-loop-helix transcription factor DEC2 inhibits TGF-β-induced tumor progression in human pancreatic cancer BxPC-3 cells. Int J Mol Med. 2012;30(3):495-501.
58. Nakamura H, Tanimoto K, Hiyama K, et al. Human mismatch repair gene, MLH1, is transcriptionally repressed by the hypoxia-inducible transcription factors, DEC1 and DEC2. Oncogene. 2008;27(30):4200-9.
59. Chen S, Dong QJ, Wan ZA, Gao S, Tu SL, Chai R. BHLHE41 Overexpression Alleviates the Malignant Behavior of Colon Cancer Cells Induced by Hypoxia via Modulating HIF-1α/EMT Pathway. Gastroenterol Res Pract. 2022;2022:6972331.
60. Falvella FS, Colombo F, Spinola M, Campiglio M, Pastorino U, Dragani TA. BHLHB3: a candidate tumor suppressor in lung cancer. Oncogene. 2008;27(26):3761-4.
61. Nagata T, Minami K, Yamamoto M, et al. BHLHE41/DEC2 Expression Induces Autophagic Cell Death in Lung Cancer Cells and Is Associated with Favorable Prognosis for Patients with Lung Adenocarcinoma. Int J Mol Sci. 2021;22(21):11509.
62. Wang C, Zhao N, Zheng Q, Zhang D, Liu Y. BHLHE41 promotes U87 and U251 cell proliferation via ERK/cyclinD1 signaling pathway. Cancer Manag Res. 2019;11:7657-7672.
63. Zhou ZH, Wang B, Cheng XB, et al. Roles of SHARP1 in thyroid cancer. Mol Med Rep. 2016 ;13(6):5365-71.
64. Numata A, Kwok HS, Kawasaki A, et al. The basic helix-loop-helix transcription factor SHARP1 is an oncogenic driver in MLL-AF6 acute myelogenous leukemia. Nat Commun. 2018;9(1):1622.
65. GEPIA2. http://gepia2.cancer-pku.cn. Accessed 19 October 2025.
66. Ananthasubramaniam B, Venkataramanan R. Rhythm profiling using COFE reveals multi-omic circadian rhythms in human cancers in vivo. PLoS Biol 2025;23(5): e3003196.
67. Follain G, Dibus M, Joshi O, Jacquemet G. Time, the final frontier. Mol Oncol 2025;29(8):2157-2162.
68. Chen H, Pan Y, Zhou Q, et al. METTL3 Inhibits Antitumor Immunity by Targeting m6ABHLHE41-CXCL1/CXCR2 Axis to Promote Colorectal Cancer. Gastroenterology 2022;163(4):891-907.
69. Matsunaga N, Inoue M, Kusunose N, et al. Time-dependent interaction between differentiated embryo chondrocyte-2 and CCAAT/enhancer-binding protein α underlies the circadian expression of CYP2D6 in serum-shocked HepG2 cells. Mol Pharmacol 2012;81(5):739-47.