

Cancer stem cells and hypoxia-inducible factors (Review)

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Abstract. Cancer stem cells (CSCs), also known as tumor-initiating cells, are a subpopulation of tumor cells that exhibit properties similar to those of normal stem cells. Oxygen is an important regulator of cellular metabolism; hypoxia-inducible factors (HIFs) mediate metabolic switches in cells in hypoxic environments. Hypoxia clearly has the potential to exert a significant effect on the maintenance and evolution of CSCs. Both HIF-1 α and HIF-2 α may contribute to the regulation of cellular adaptation to hypoxia and resistance to cancer therapies. This review provides an overview of the roles of HIFs in CSCs. HIF-1 α and HIF-2 α have significant prognostic and predictive value in the clinic and the concept of personalized medicine should be applied in designing clinical trials for HIF inhibitors.

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1. Brief history of CSCs

The original hypothesis of CSCs was proposed several decades ago (Fig. 1). Virchow first proposed that some tumors may arise from embryo-like cells and that cancer cells have properties reminiscent of stem cells reported in 1855 (1). In 1937, Furth and Kahn confirmed that a single leukemic cell from a mouse tumor could initiate a new tumor in a recipient mouse (2). Southam and Brunschwig harvested recurrent

cancer cells from patients and demonstrated the differential tumor-forming ability of these cells in 1961 (3). In 1977, Hamburger and Salmon found that tumor stem cell colonies arising from different types of cancer have differing growth characteristics and colony morphology (4). Lapidot *et al* (5) defined a new leukaemia-initiating cell from human acute myeloid leukemia (AML) by using a mouse model in 1994. Bonnet and Dick first demonstrated the existence of CSCs in AML in 1997 (6). This is the most notable and definitive evidence regarding the existence of CSCs. Following research into leukemia, the first CSCs identified in solid tumors were demonstrated in breast cancer by Al-Hajj and colleagues in 2003 (7). Since then, numerous studies have provided evidence of the existence of CSCs in solid tumors of a number of tissue types, including the brain (8), breast (9) and prostate (10).

2. Origin of CSCs

According to the somatic stem cell hypothesis, mutations or chromosomal rearrangements in dormant stem cells present in organs may induce the formation of CSCs (11). It has been demonstrated that the implantation of embryonic stem cells or the induction of pluripotent stem cells in mice results in cancer (12). Another view on the origin of CSCs is that cancer cells with genetic instability may generate CSCs (13). Cancer cells transfected with Oct3/4, Sox2, Klf4 and c-Myc have been reported to transform into CSCs (14).

In most scenarios, CSC subpopulations have emerged following the accumulation of epigenetic and/or genetic alterations in normal stem cells or cancer cells. However, in 2013, Wang *et al* (15) hypothesized that CSCs develop *de novo* from the misplaced somatic stem cells and proposed a new theory of carcinogenesis; the stem cell misplacement theory. This theory stated that misplaced epithelial stem cells, which reach the wrong tissue stroma by accident undergo malignant transformation and become CSCs.

3. Properties of CSCs

CSCs, also known as tumor-initiating cells or tumor-propagating cells are a subpopulation of tumor cells that demonstrate properties similar to normal stem cells (16). Tumor-initiating cells may better describe these cells; however, we have referred to these cells as CSCs in this review. At the 2006 meeting of the American Association for Cancer Research, Reya *et al* (17) proposed the definition of a cancer stem cell as a cell within

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a tumor that possesses the capacity to self-renew and produce the heterogeneous lineages of cancer cells that comprise the tumor.

Tumorigenicity and self-renewal. The core definition of CSCs comprises their ability to induce tumor formation (17). Tumorigenicity is defined as the capacity of a cell population inoculated into an animal model to produce a tumor by proliferation (18). The process of tumorigenicity has been long known to resemble organogenesis; most tumors are heterogeneous, containing many cells that vary phenotypically and functionally (19). The current hypothesis is that tumor growth and progression are driven by minority populations of tumorigenic cells, and that other cancer cells have little or no capacity to contribute to tumor growth (20). The proportion of tumorigenic cells in cancer is very low. For example, both in the study by O'Brien *et al* (21) and Hope *et al* (22), only one cell in 10^4 - 10^6 unsorted human cancer cells was able to generate a tumor following xenotransplantation into immunosuppressed mice. In the study by Xia *et al* (23), they also found that the tumorigenic cell fraction comprised only 0.28% of Lewis lung carcinoma cells.

Two main approaches have been used to identify tumorigenic cells in published studies: One method is termed 'spheroid colony formation; and is considered the most appropriate *in vitro* assay to detect the malignant transformation of cells (25), and the other one is an *in vivo* method involving implantation of tumorigenic cells in immunodeficient mice (26).

The hallmark of stem cells is their dual ability to self-renew and to generate multiple cell lineages with more differentiated characteristics (26). Self-renewal is the ability of a CSC to sustain itself and continue to give rise to cells with equal abilities of tumorigenicity (27). CSCs can self-renew through asymmetric cell division in which one daughter cell possesses stem cell properties (28). Prior to asymmetric division, unequally distributed cellular components are differentially enriched at either the apical or basal pole, in which the mitotic spindle apparatus and centrosomes are unequally aligned (29).

Drug resistance. Anticancer drugs have been applied alone or in combination to prolong life or to alleviate the symptoms of cancer for decades (30,31). However, these drugs have failed to completely eradicate cancers. Multidrug resistance (MDR) plays an important role in preventing drug absorption (32). Various factors can contribute to MDR, including the existence of CSCs (33). CSCs possess multiple mechanisms of drug resistance: A high expression of ABC transporters and anti-apoptotic factors, and the maintenance of a quiescent state to avoid the induction of apoptosis (34). The ABC transporter family acts by pumping drugs into the extracellular domain (35). To date, 49 human ABC genes have been identified and are clustered in seven subfamilies (ABCA-ABCG) (36). There are three major transporters correlated with MDR, including P-glycoprotein (MDR1/ABCB1), MDR-associated protein (MRP/ABCC1) and breast cancer resistance protein (BCRP/ABCG2) (37).

P-glycoprotein (ABCB1). P-glycoprotein (P-gp) is a 170 kDa phosphoglycoprotein constituting two transmembrane

domains and two cytosolic nucleotide-binding domains (Fig. 2) (38). P-gp overexpression is related to negative clinical outcomes, including treatment failure, relapse and survival. An increased P-gp expression has been observed in breast tumor biopsies treated with conventional chemotherapy (39). In a previous study on AML, the relapse rates were associated with elevated P-gp expression levels (40). A similar observation was reported in a study on multiple myeloma, in which 6% of patients expressed P-gp at diagnosis, and >43% of patients exhibited overexpressed P-gp following treatment (41). The patients with osteosarcoma that did not overexpress P-gp had significantly better relapse-free rates and improved survival rates of 5 to 14 years (42). P-gp plays a significant role in transporting a diverse array of molecules, including anionic, and neutrally charged drugs and toxins (43-45).

MRP/ABCC1. MRP1/ABCC1 was the first gene to be identified in the ABCC subfamily and was cloned from an MDR small cell lung cancer cell (46). Numerous studies have demonstrated the upregulation of MRP1 in a variety of solid tumors, such as those of the lung, breast and prostate (47-49). MRP1 is potentially an important target for reversing chemotherapy resistance in many cancers (50).

Breast cancer resistance protein (BCRP/ABCG2). Human BCRP is encoded by the ABCG2 gene which is located on chromosome 4q22 (51). ABCG2 is the second member of subfamily G within the large human ABC transporter superfamily (49). BCRP is believed to exhibit important physiological and pathophysiological functions in tissues and is involved in cellular protection and in mediating the homeostasis of physiological substrates (52).

4. The roles of HIFs in cancer

Hypoxia is an important factor that affects clinical outcomes by promoting genetic instability, tumor cell metastasis and invasiveness (53). The HIF protein is a heterodimeric complex formed by an oxygen-dependent α subunit and an oxygen-insensitive β subunit (53). The three HIF α subunits (HIF-1 α , HIF-2 α and HIF-3 α) with a HIF-1 β subunit act as key mediators of cellular adaptation to low oxygen (54). The carboxy-terminal domain of HIF-1 α and HIF-2 α consists of domains that regulate its stability (the oxygen-dependent degradation domain, ODD) and transcriptional activity (two transactivation domains (TADs), N-TAD and C-TAD (Fig. 3) (55). Furthermore, both the C- and N-termini of the α subunits have nuclear localization signals (N-NLS and C-NLS, respectively) that direct them to the nucleus (Fig. 3) (56). The stability of HIF-1 α and HIF-2 α is regulated by oxygen tension (57). HIF-1 α and HIF-2 α have been extensively studied and are ubiquitously expressed in normal tissue (58). C-TAD regulates most hypoxia-induced genes, although a subset of genes depended solely on N-TAD initiation, and N-TAD contributes to target gene specificity of HIF-1 α and HIF-2 α (59). An increased HIF-1 α or HIF-2 α expression has been observed in many types of cancer, such as breast (60), colon (61), lung (62), pancreatic (63) and ovarian cancers (65) (Figs. 4 and 5). Upon exposure of the cells to hypoxia, the HIF α subunits accumulate in the

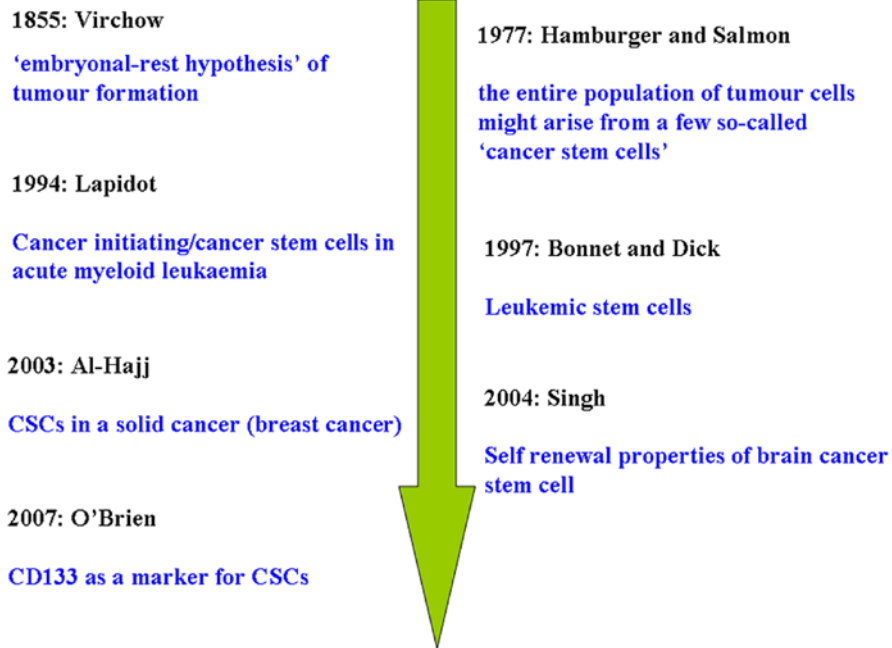


Figure 1. The timeline of cancer stem cell research.

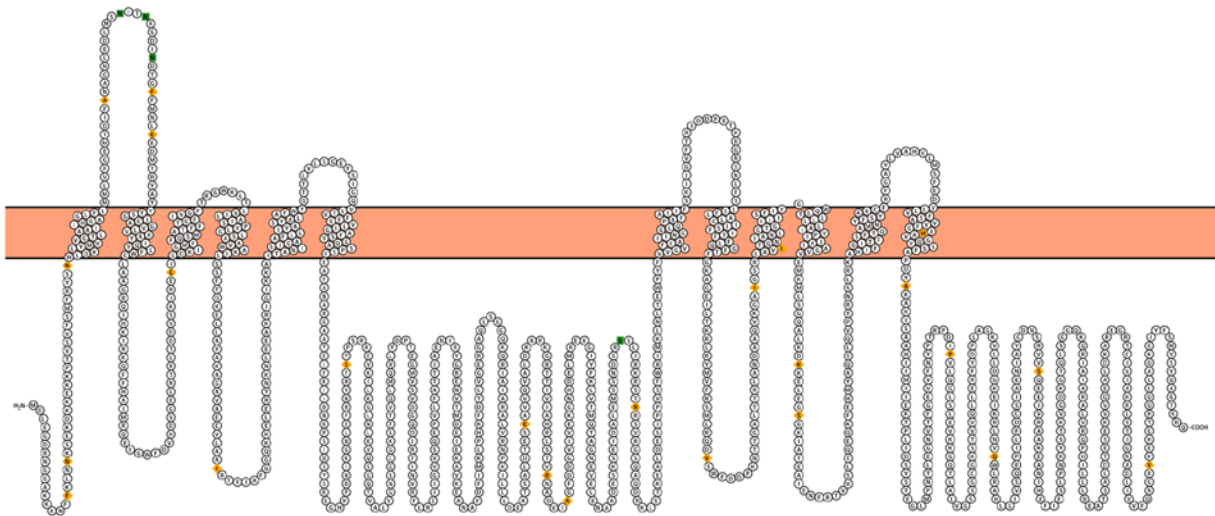


Figure 2. Ideograph of ABCB1 protein generated using Peptide Atlas (<https://db.systemsbiology.net/sbeams/cgi/PeptideAtlas/Search>). P-glycoprotein (P-gp) is a 170 kDa phosphoglycoprotein constituting two transmembrane domains and two cytosolic nucleotide-binding domains.

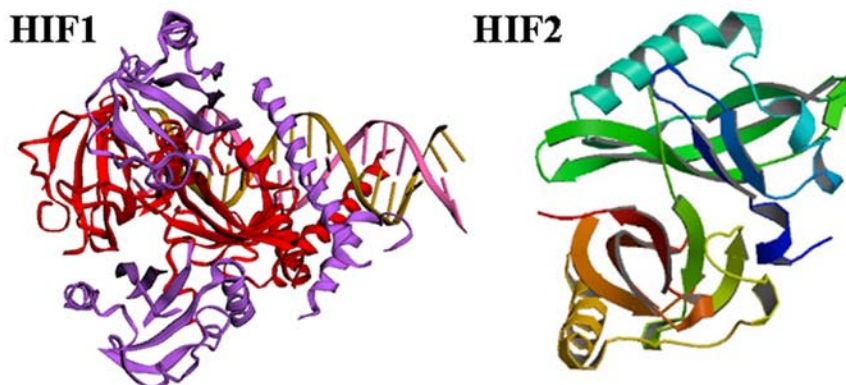


Figure 3. Structure of HIF-1 α and HIF-2 α was downloaded from PDB. HIF-1 α and HIF-2 α protein contain a bHLH region, a PAS region and an ODD domain. HIF, hypoxia inducible factor; bHLH, basic-helix-loop-helix; PAS, Per/Arnt/Sim; ODD, oxygen-dependent degradation; PDB, protein database.

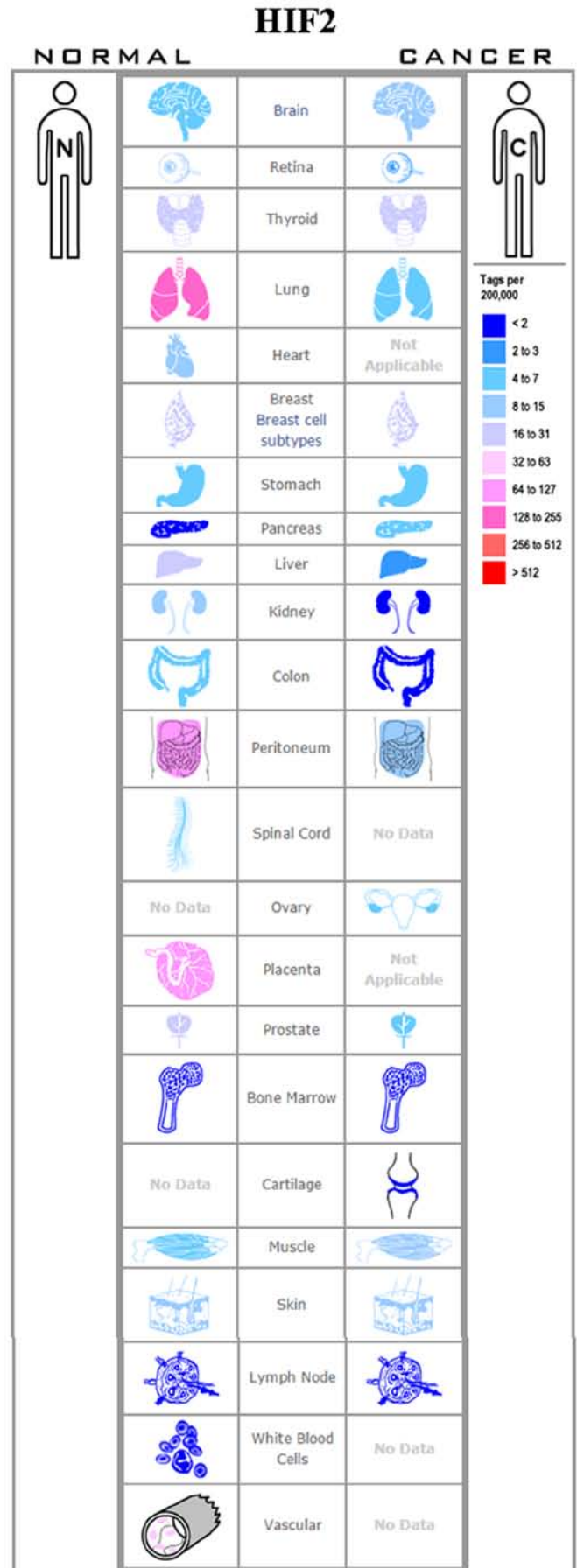
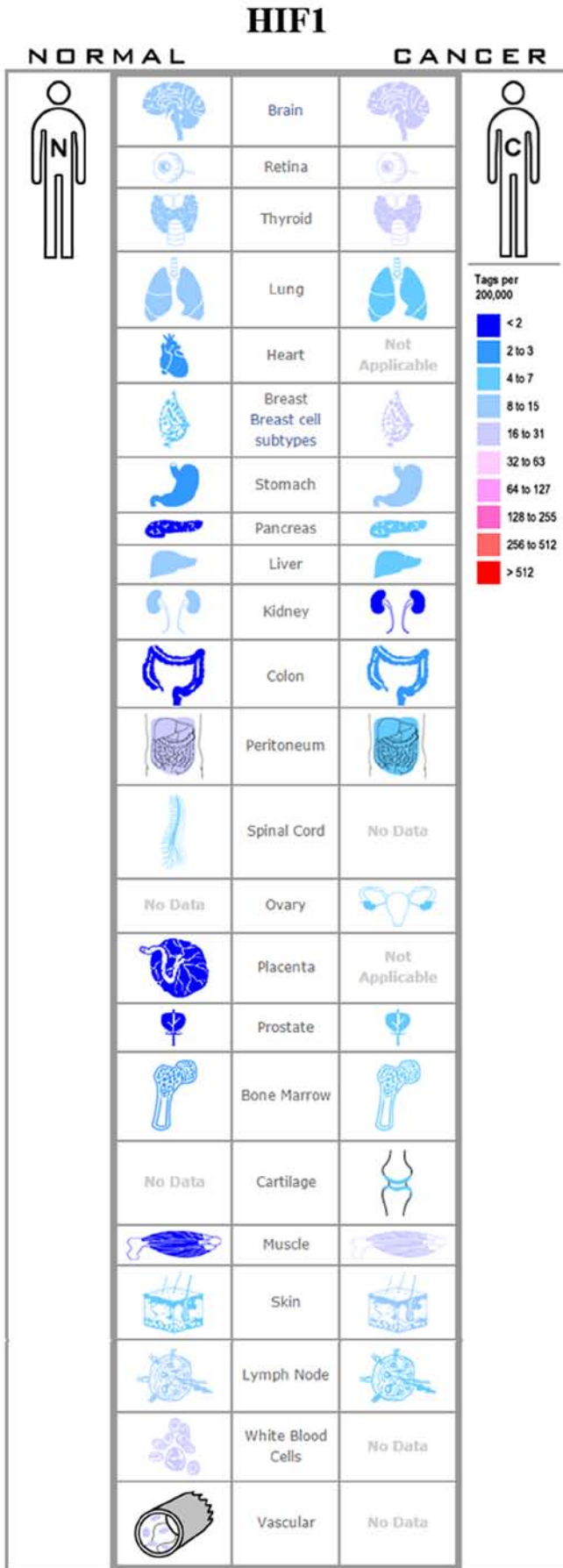


Figure 4. Expression profile for HIF-1 α in human cancers found by the SAGE DGED (<http://www.ncbi.nlm.nih.gov/SAGE/>). HIF-1 is expressed highly in the brain, thyroid, breast, pancreas, stomach and in prostate cancer. HIF, hypoxia inducible factor.

Figure 5. Expression profile for HIF-2 α in human cancers found by the SAGE DGED (<http://www.ncbi.nlm.nih.gov/SAGE/>). HIF-2 expressed highly in brain, pancreas, and stomach cancer. HIF, hypoxia inducible factor.

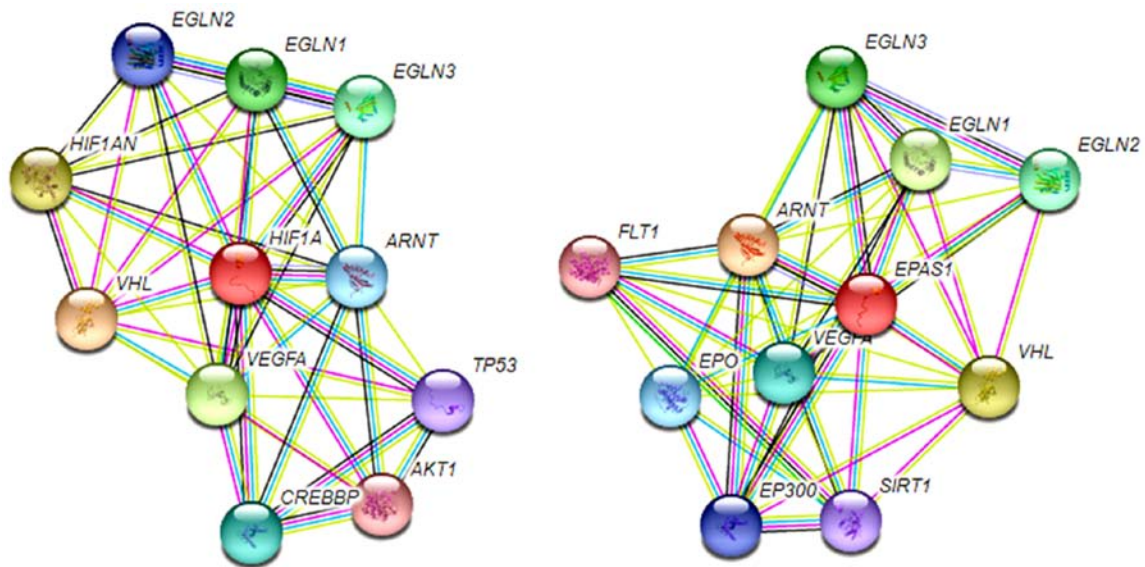


Figure 6. Interaction genes of HIF-1 α and HIF-2 α were analyzed using STRING (<https://string-db.org/cgi/input.pl>). HIF-1 α could interact with EGLN1-3, AKT1, TP53, and VHL. HIF-2 α could interact with EGLN1-3, EPO, SIRT1, and VHL. HIF, hypoxia inducible factor.

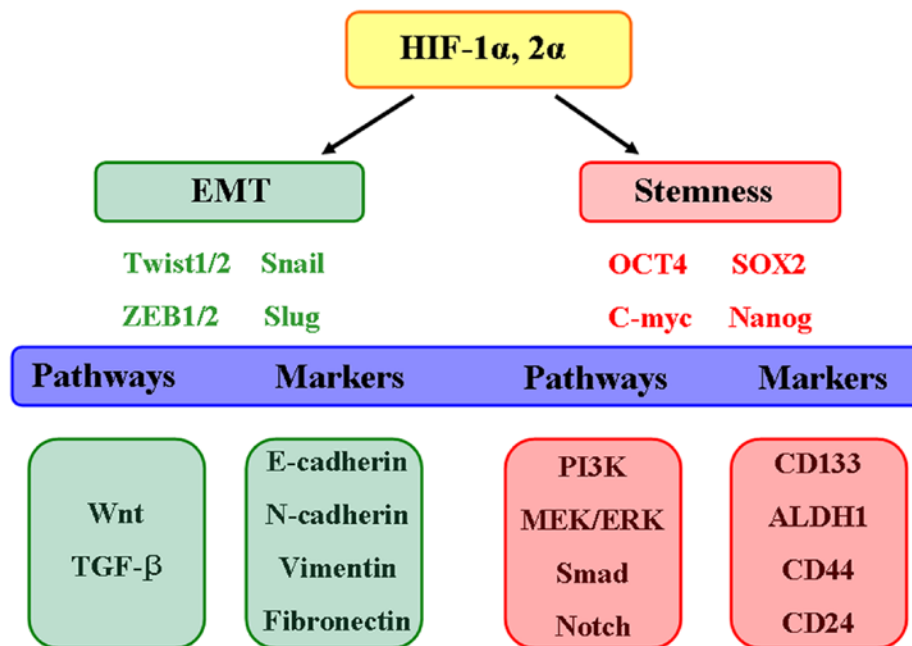


Figure 7. HIF-1 α and HIF-2 α in the driver seat of tumorigenesis. HIF, hypoxia inducible factor.

nucleus and bind to their target genes, such as BNIP3, PGK1, HK1 and TP11 (Fig. 6) (65). The target genes participate in the proliferation, apoptosis, metabolism and invasion, as well in the and resistance of cancer cells to therapy (66-68).

Genetic polymorphisms of HIF-1 α or HIF-2 α in human cancers have been found in previous studies (69-74). The single nucleotide polymorphisms 1772C/T (rs11549465) and 1790 G/A (rs11549467) have been shown to be significantly associated with the overall risk of developing lung, breast, oral, prostate, cervical and renal cancers (69). Frank *et al* (70) demonstrated a significant association between rs2057482 in HIF-1 α with the risk of rectal cancer. Guo *et al* (71) found that rs2057482 was associated with worse clinical outcomes

of patients with hepatocellular carcinoma. Han *et al* (72) observed that rs9679290, rs4953346 and rs12617313 of HIF-2 α were associated with the risk of developing renal cell carcinoma. Yamamoto *et al* (73) reported that HIF-2 α rs13419896 was associated with a decreased risk of developing lung cancer. HIF2A rs1125070 and rs4953352 are associated with the disease-free and overall survival of patients with colorectal cancer (74).

A non-coding RNA (ncRNA) is a functional RNA molecule that is not translated into a protein (75). miRNAs are the perfect candidates for controlling HIF expression during hypoxia (76). These so-called hypoxamiRs contribute to HIF-1 accumulation and the maintenance of HIF-2 and

HIF-3 (77,78). For example, the hypoxic induction of miR-18a may allow HIF-1 α level decreases and thus contribute to the HIF switch (79). miR-17, miR-20a and miR-20b have been reported to be involved in the HIF-related response during hypoxia in cancer cells (80). lncRNA HIF-1A-AS2 negatively regulates HIF-1 α and is upregulated in non-papillary clear cell renal carcinomas (81). lncRNA sONE or NOS3AS regulates the expression of endothelial nitric oxide synthase (eNOS), under normal oxygen conditions and hypoxic conditions (82).

5. The roles of HIFs in CSCs

Increasing evidence indicates that HIFs regulate the subpopulations of CSCs (83,84). The activation of HIF-1 α not only increases the number of cluster of differentiation (CD)133-positive glioma stem cells, but also enhances the stem-like phenotype of cell lines (85). CSCs within several brain tumors are preferentially located in hypoxic niches (86). HIFs induce the self-renewal capacity and inhibit the differentiation of glioblastoma CSCs (87). The impact of hypoxia is mediated by HIF-1 α , but not by HIF-2 α , and is associated with the induction of the Hippo signaling pathway in breast CSCs (88). One key regulator of BCSC activity is a Hippo pathway effector, TAZ, which is a direct target of HIF-1 α (89). HIF activity can promote a stem-like phenotype and increase the number of leukemia stem cells (90). In AML, HIF-1 α is overexpressed and selectively activated in CD34⁺CD38⁻ subsets (91). In lung cancer, hypoxia-induced CD133 expression is associated with the binding of OCT4 and SOX2 to the PROM1 promoter (92). The targeting of HIF-1 α or HIF-2 α by short hairpin RNA in CD133⁺ cells from a patient with glioblastoma inhibited their neurosphere-forming ability and proliferation, induced the caspase-dependent apoptotic effect *in vitro* and attenuated their tumor-initiating potential *in vivo* (93). The expression of CD44 and Oct4 stem cell markers is decreased in colorectal cancer cells in response to HIF-1 α knockdown (94). CD24 expression is strongly induced by hypoxia in a human bladder cancer cell line (95). In addition, combined HIF-1 α and CD24 immunostaining in human urothelial cancer samples showed a statistically significant association (95). HIF-2 α expression stimulates Oct-4 expression and promotes c-Myc activity, which powerfully impact cancer stem cell formation (96). HIF-2 α mRNA is significantly transcriptionally upregulated under normoxia and hypoxia in glioma stem cells (GSCs) (97).

6. Future perspectives

Hypoxia has notable potential to exert significant effects on the maintenance and evolution of CSCs. Both HIF-1 α and HIF-2 α contribute to the regulation of cellular adaptation to hypoxia and the resistance to cancer therapies (Fig. 7). The simultaneous targeting of the HIF-1 α and HIF-2 α pathways may improve clinical responses within the hypoxic tumor microenvironment. Therefore, the concept of personalized medicine should be applied in designing clinical trials for HIF inhibitors.

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Availability of data and materials

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Authors' contributions

WWT and YL conceived the study. WWT and GHT collected the data and wrote the manuscript; WWT prepared the figures and revised the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

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