The hypoxic microenvironment: A determinant of cancer stem cell evolution

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Tumors are often viewed as unique entities with specific behaviors. However, tumors are a mixture of differentially evolved subpopulations of cells in constant Darwinian evolution, selecting the fittest clone and allowing it to outgrow the rest. As in the natural environment, the niche defines the properties the fittest clones must possess. Therefore, there can be multiple fit clones because of the various microenvironments inside a single tumor. Hypoxia is considered to be a major feature of the tumor microenvironment and is a potential contributor to the cancer stem cell (CSC) phenotype and its enhanced tumorigenicity. The acidic microenvironment around hypoxic cells is accompanied by the activation of a subset of proteases that contribute to metastasis. Because of aberrant angiogenesis and the inaccessibility of their locations, hypoxic cells are less likely to accumulate therapeutic concentrations of chemotherapeutics that can lead to therapeutic resistance. Therefore, the targeting of the hypoxic CSC niche in combination with chemotherapy may provide a promising strategy for eradicating CSCs. In this review, we examine the cancer stem cell hypothesis and its relationship to the microenvironment, specifically to hypoxia and the subsequent metabolic switch and how they shape tumor behavior.

Keywords:
- cancer stem cell; hypoxia; microenvironment; tumor evolution.

Cancer stem cells

Two separate and mutually exclusive models have been developed to explain the development of tumors. The clonal evolution model postulates that all cells within a tumor contribute in varying degrees to the maintenance of the tumor. In this model, a number of genetic and epigenetic changes occur over time, with the result that the most aggressive cancer cells are ultimately responsible for driving tumor progression. Furthermore, through a series of genetic mutations, any cancer cell within the tumor can become invasive, lead to the development of metastases, and contribute to the resistance of therapies and ultimately to the recurrence of disease. The cancer stem cell model proposes that cancer stem cells, which form a subset of the tumor cells, are ultimately responsible for tumor initiation, progression and recurrence. It is thought that through self-renewal and differentiation, cancer stem cells are responsible for the production of the various tumor cell types and contribute to tumor heterogeneity. Furthermore, according to this hypothesis, tumor metastases and resistance to therapies arise directly from cancer stem cells.

As early as 1875, Julius Cohnheim elaborated a theory that tumors may arise from stem cells that remain after embryonic development [1]. However, the concept of cancer stem cells (CSCs) was first described in 1971 [2]; it was stated that the tumors were driven by a subpopulation of self-renewing CSCs that possess the ability to generate the diverse differentiated cell populations that comprise the tumor mass [3]. The theory was recovered in 1994 [4], and these cells were first isolated from leukemia samples in 1997 [5], demonstrating that only CD34+ CD38− cells derived from patients with acute myeloid leukemia (AML) could initiate hematopoietic malignancies in immunodeficient mice. Later, CSCs were also isolated from breast cancer solid tumors [6] and since then, from many other types, including brain tumors, melanomas, colon cancer tumors, lung cancer tumors and several types of sarcoma. CSCs possess strong tumorigenicity, metastaticity, radioresistance and chemoresistance and play critical roles in cancer progression and prognosis [7, 8].

Cancer stem cells are similar to normal stem cells in that they have the ability to self-renew and differentiate. They differ from normal stem cells in that the mechanisms that normally strictly regulate these processes are deregulated, such that there is a continuous expansion and production of differentiated progeny [9]. Furthermore, CSCs also differ from normal stem cells in that CSCs have tumorigenic activity that enables them to form tumors when transplanted into animals, something normal stem cells cannot do [10, 11].

Cancer stem cells comprise a small population of cells within a tumor. They are also known as ‘tumor-initiating cells’ or ‘tumorigenic cells’. CSCs and normal stem cells have similar cell

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surface markers [9], and like normal stem cells, CSCs have the capacity to self-renew, can give rise to different progeny and utilize common signaling pathways [12]. CSCs can be the source of all of the tumor cells present in a malignant tumor; (2) resistance to the chemotherapeutic agent used to treat the malignant tumor, thus making them responsible for recurrence; and (3) cells that give rise to distant metastases.

In general, the size of the CSC pool, measured by the stem cell markers and the transcriptional signatures specific to CSC populations, correlates with the aggressiveness of the tumor and is highly predictive of patient survival [13, 14]. However, the proportion of CSCs varies between tumors, even among tumors of the same type and origin [15].

Cancer stem cells have a great degree of heterogeneity. CSCs isolated from different grades or stages of the same type of tumors are distinct, as the CSCs isolated from the primary tumor are different from those found in the metastatic growths [16, 17]. Even within the same niche, different pools of CSCs usually coexist and may interconvert [18–22]. Recent research has put forward a new concept that CSCs are a cell 'status' but not a fixed 'category' of cells. Indeed, CSCs are not an immutable, frozen cell population. CSCs and non-CSCs coexist and can interconvert in a dynamic equilibrium. Non-CSCs can acquire CSC properties by reprogramming or through dedifferentiation [23–25]. The microenvironment plays a critical role in this plasticity. For example, myofibroblast-secreted factors restore CSC phenotypes in differentiated colon cancer cells in vitro and in vivo [26–28]. CSCs may develop de novo from differentiated cancer cells (i.e. by reprogramming) with the stimulus of the microenvironment. Therefore, the hierarchical model of mammalian CSCs should be considered to be bidirectional, switching between stem and nonstem cells within the tumor [23–25] (Fig. 1).

**Cellular plasticity**

The capability to move from one cellular compartment to another, or to interconvert between differentiated somatic and stem cell states, is called cellular plasticity [29]. Based on this principle, the dichotomy between the stochastic and hierarchical models is false because stochastic events would therefore be able to generate novel hierarchically organized populations. Therefore, depending on the genotype and the cross talk within the microenvironment, the CSC pools evolve to regain long-term repopulation capability [30]. This dedifferentiation capability may be inherited (hierarchical theory) or acquired via mutations that lead to a stem cell-like permissive epigenome (stochastic theory) [31]. For example, the inactivation of mutations in p53, the activation of telomerase by hTERT or the activation of stem-associated factors such as Myc, Notch or Wnt facilitates such phenotypic plasticity [32–36]. Similar traits are observed during the epithelial-mesenchymal transition (EMT) in differentiated cells, which has been clearly shown to increase the tumorigenicity of the CSC pool [37–44].

Therefore, symmetrical cell division may not be required as is suggested by the hierarchical model because the enlargement of the CSC pool may occur through the dedifferentiation of the asymmetrically dividing progenitor pools. This could explain why melanoma, lung, ovarian, breast and prostate tumor cells have altered gene expression, resembling cell lineages other than their original lineage [29, 31]. In fact, the inherent plasticity of stem cell pathways such as Wnt, Notch or Hedgehog, which are able to be modified by external stimuli, suggests that these pathways may be important for the regulation of cellular plasticity and more interestingly may be affected by signals from the microenvironment.

**Microenvironmental contributions to the CSC phenotype**

The host microenvironment in which the CSCs or the progenitor cells are located may alter the original tumorigenic potential of the cells. The specific microenvironment conditions may select for the most robust clone, allowing it to hierarchically evolve, generating a large tumor mass. Conversely, the same CSC or progenitor located in a nonpermissive environment may not contribute to tumor growth.

![Figure 1](image-url). Representative scheme of the hierarchical model of the cancer stem cells versus the stochastic model of tumorigenesis.
In this regard, a niche is an anatomical, molecular and distinct cellular microenvironment that can be present in a specific tumor. Therefore, multiple niches can coexist in one tumor, giving rise to multiple different CSCs and increasing the cellular diversity.

Cells within a niche produce factors that promote CSC self-renewal, stimulate angiogenesis and recruit other stromal cells, including cells from the immune system, to secrete additional factors to promote tumor cell invasion and metastasis. Two additional aspects of the niche must be highlighted: (1) the significant contribution of the senescent stromal cells in these niches through the senescence-associated secretory phenotype (SASP) (which has been reviewed elsewhere [45–47]), and (2) the epigenetic contribution of the niche to the dedifferentiation phenotype and increase in the CSC pool.

There is much evidence in the literature indicating that the cocultivation of normal fibroblast with tumor cells facilitates/increases the tumorigenicity of the tumor cells. The fact that senescent fibroblasts produce a myriad of secreted factors able to modify the growth properties of cells, known as SASP, provides a clear and plausible explanation for this effect [45–47]. However, more recent evidence suggests that the CSCs can modify the microenvironment by transforming neighbor fibroblasts into cancer-associated fibroblasts (CAFs) [48] that have increased proliferation rates and unique secretory factors compared with their normal counterparts [49].

CAFs stimulate stemness via the activation of the Wnt and Notch pathways [8,26,50], which have been implicated in stem cell maintenance and cell fate decisions [29]. Notch prevents cells from differentiation induced by signals produced by other cells in the niche [51]. Furthermore, the combination of these pathways with other pathways such as Hedgehog or Notch pathways [8,26,50], which have been implicated in stem cell proliferative deregulation and inflammation, among other tumor hallmarks. This means that hypoxia may contribute to cancer through several independent pathways that may be interconnected.

Hypoxia-inducible factor (HIF) transcription factors (HIF-1α and HIF-2α) hydroxylated in proline in the presence of oxygen. This interaction results in the ubiquitination and degradation of HIF proteins [63, 64], maintaining low levels of these transcription factors. Upon hypoxia, the hydroxylation is inhibited, and HIF-α

The hypoxic niche

In the primary tumor, hypoxia can develop within the tumor mass because of impaired vascularization (Fig. 2). Hypoxia, a characteristic feature of locally advanced solid tumors, has emerged as an essential factor of tumor physiology because it can promote tumor initiation, progression and resistance to therapy. Beyond its role in neovascularization as a mechanism for tumor adaptation to nutrient and oxygen deprivation, hypoxia has been related to elongated life span and immortality, changes in metabolism, stem cell proliferative deregulation and inflammation, among other tumor hallmarks. This means that hypoxia may contribute to cancer through several independent pathways that may be interconnected.

Hypoxia-inducible factor (HIF) transcription factors (HIF-1α and HIF-2α) hydroxylated in proline in the presence of oxygen interacts with VHL tumor suppressor protein. This interaction results in the ubiquitination and degradation of HIF proteins [63, 64], maintaining low levels of these transcription factors. Upon hypoxia, the hydroxylation is inhibited, and HIF-α
proteins rapidly accumulate, dimerize with HIF-1α and bind the promoter of target genes regulating their transcription. In mammals, genomic analyses and ChIP-seq experiments have described more than 1000 genes that are direct targets of HIF in response to hypoxia [65, 66]. However, the response to hypoxia only activates a small subset of these genes in any given cell [67], suggesting a rather specific response to hypoxia in different tissues. Recent work has determined that the HIF transcription factors also interact with the miRNA circuitry [68–72], engaging in cross talk with other signaling pathways such as Myc or Notch [73, 74]. These interactions initiate secondary signaling cascades, increasing the overall complexity of the system.

Hypoxia-inducible factors are the main effectors of oxygen homeostasis, allowing cellular adaptation to hypoxia by regulating the expression of more than a hundred genes involved in various biological processes such as angiogenesis, EMT, survival, invasion, metastasis and cellular energy metabolism (promoting anaerobic metabolism) [65, 66, 75–77]. Many studies on clinical samples have shown that solid tumors frequently contain highly hypoxic regions. Tumor hypoxia is associated with poor prognosis [reviewed in [65, 76, 79]]. This adverse association has been shown to be independent of the treatment modality and occurs in many tumor types [80, 81]. A clinical correlation between tumor hypoxia, HIF-1α and HIF-2α expression and patient mortality has also been demonstrated [78, 79]. HIF-1α and HIF-2α themselves, and transcriptional hypoxic signatures, have been correlated with aggressive tumor behavior, adverse prognosis and resistance to therapy [66]. Clinical evidence also suggests that patients with tumors that contain more extensive hypoxic and anoxic areas have higher rates of metastases [82]. However, hypoxia is not the only determinant of HIF-dependent signatures because oncogenes such as AKT are able to activate HIFs. Additionally, the loss of tumor suppressors such as PTEN, PML or TSC is able to activate the HIF response in normoxia by directly activating HIF-α protein translation [83–87]. Conversely, hypoxia activates a myriad of genes independently of HIF-1α (i.e. CEMP).

During the later stages of the tumor growth, the cells invade the surrounding stroma and the bloodstream and migrate to distant sites where they grow into secondary tumors or metastases. The process known as EMT has been recognized as the first step in this process [7, 25, 88]. Carcinoma cells that undergo EMT lose their epithelial phenotype and acquire mesenchymal characteristics via molecular and cellular changes, including the loss of adhesion molecules (such as E-cadherin), reorganization of the cytoskeleton and increased motility [89–92].

Hypoxia induces EMT by activating the EMT-associated signaling pathways via the upregulation of EMT-associated transcription factors or repressors [93, 94]. Hypoxia, through HIF-1α, induces EMT through the transcriptional control of Snail, ZEB1, TWIST and TCF3 [95, 96]. In breast cancer cells, EMT is activated under hypoxic stress through the upregulation of ZEB1 and down-regulation of MYB [97]. Under hypoxic conditions, signaling pathways directly involved in triggering EMT, such as NF-κB and Notch, are also activated. Recently, it was described that the hypoxia-induced upregulation of Jagged2, cyclooxygenase-2 and urokinase receptor also contributes to EMT changes and the invasive ability of breast cancer cells [98–100]. Hypoxia may also regulate EMT via regulating long noncoding RNAs, carbonic anhydrase and calcium signaling in cancer cells to promote invasion in hypoxic microenvironments [101–103].

Hypoxia-regulated EMT also has central role in cancer resistance to apoptosis and drugs as well as resistance to the immune response. EMT is one of the initiating factors of circulating tumor cells (CTCs) and is required for their capacity to migrate into blood vessels. Several studies have demonstrated the upregulation of EMT markers in CTCs, supporting this hypothesis [82, 104, 105]. CTCs expressing vimentin and Twist were identified in breast cancer patients, and the presence of CTCs was correlated with metastatic disease, confirming that EMT is involved in the metastatic potential of CTCs [106].

Hypoxia and HIFs have been shown to induce tumor cell dedifferentiation toward an immature phenotype and, similarly, to maintain the stem cell properties of tumor cells [107–109]. Several reports indicate a role for hypoxia and HIFs in promoting a stem-like phenotype through the expression of genes such as OCT4, SOX2 and NANO, which is required for the maintenance of self-renewal in stem cells or the activation of the Notch signaling pathway that regulates cell self-renewal and differentiation [108–110] (Fig. 3). However, both hypoxia and reactive oxygen species (ROS) upregulate the CSC stress signaling pathways to enhance cancer cell survival and maintain cancer cell stemness [27, 111–113] and occurs via the ROS-induced TGF-β and TNF-α signaling pathways [112–114].

Hypoxia induces stemness in differentiated progenitor and non-CSCs through the activation of stem genes and dedifferentiation [108, 109]. Hypoxia also induces CSCs to express HIFs, which are regulated and stabilized by TGF-β [53]. Furthermore, hypoxia increases the level of ROS, which promote cell survival and induce EMT via the TGF-β signaling pathway. The activation of TGF-β as well as WNT signaling pathways by hypoxia also facilitates stemness [115–117]. The stromal cells of the microenvironment promote self-renewal of CSCs by cell-cell contact or by nitric oxide production via the NOTCH signaling pathway [118], which can be directly activated by HIF-1α [119]. HIF-1α also antagonizes c-Myc proto-oncogene activation, thus slowing cell-cycle progression to protect CSCs from DNA damage, enhancing stemness [73].

Some of the effects of hypoxia on tumor cell differentiation are directly mediated by the HIFs. The targeting of HIF-1α or HIF-2α expression has been shown to reduce the expression of stemness-related genes in breast cancer cells [120]. In addition, hypoxia-induced expression of stemness-related genes has been shown to be mediated by the HIF-1α and HIF-2α signaling pathways [121].

Figure 3. Hypoxia favors the increase in the cancer stem cell pool via HIF-1α and HIF-2α activation. These factors activate stem cell pathways, amplifying the pool and providing additional dedifferentiation of tumor cells.
HIF-2α in CD133+ glioma stem cells decreased their survival and their tumorigenic and angiogenic capabilities [28, 120]. These studies also showed a preferential role for HIF-2α in the selective eradication of CSCs without adverse effects on normal progenitor cells. However, recent evidence in human leukemia showed selective activation of HIF-1α in CSCs under normoxic conditions due to VHL deficiency, and blocking of HIF-1α activity was necessary to sustain leukemia stem cell survival but not that of normal hematopoietic stem cells [82, 121].

The hypoxic conditions expand the CSC population through distinct molecular mechanisms [122]. In one way, the hypoxia-driven increase of CSC population can be due to the increase of limited CSC differentiation or to mature cell dedifferentiation. Changes in the hypoxia-driven CSC pool are not related to alterations in the proliferation nor apoptotic patterns, but rather to a dedifferentiation stage of cancer cells. Hypoxia also prevent the differentiation of CSC, believed to be due to the hypoxia-affected transition between epithelial-like and mesenchymal-like states, highlighting the role of hypoxia in tumor microenvironment-induced plasticity of CSC [122–125].

In primary breast carcinoma, hypoxia increases the CD44+/CD24− CSC pool. HIF-1α stabilization mediates the hypoxia-dependent effect in the increase of the ALDH+ CSCs [126] partly through the AKT/β-catenin pathway. Also, silencing PHD3 mimics hypoxia preventing CSC differentiation and inducing dedifferentiation of mature tumor cells. However, it seems that neither HIF-1α nor HIF-2α are involved in the hypoxia-induced expansion of CD44+/CD24− breast cancer stem pool in ER-negative breast cancer. It has been proposed that PHD3 leads to NF-κB activation in a HIF-independent manner [122].

Telomerase is an enzyme complex consisting of a telomerase reverse transcriptase catalytic subunit (TERT) and an RNA component necessary to maintain the length of the telomere. In normal tissue, telomerase is detectable in somatic and germ cells. Approximately 90% of tumors show increased telomerase activity, suggesting that it is an important factor in the maintenance of CSC properties [127]. HIF-1α mediates TERT transcription in hypoxic cancer cells [128], maintaining the immortal life span of the tumor mass.

Therefore, hypoxia, via the HIF factors or in HIF-independent PHD3-dependent way, can lead to the development of tumor cells with stem-cell-like features from nonstem cell-like tumor cells.

Cancer stem cells contribute to the metastatic and invasive processes that constitute the major causes of cancer mortality. Given that CTCs also contribute to metastasis, one could assume that the CTC population in the bloodstream is enriched in CSCs. Indeed, increasing evidence shows that CTCs exhibit CSC features. In castration-resistant prostate cancer, the vast majority of CTCs expressed the CD133 stem cell-like marker [129]. Additionally, CTCs with a CD45− ICAM-1+ phenotype, isolated from the bloodstream of hepatocarcinoma patients, showed sphere-forming capacities and tumorigenic ability in nude mice [130]. In breast carcinoma, the presence of CTCs was found to correlate with the stage and grade of the tumors as well as the expression of ALDH1 [131]. CTCs with a putative CSC-like phenotype [132], including the expression of NANOG, OCT3/4 and SOX2 genes, were also detected in the blood samples of cancer patients [133], confirming the CSC-like phenotype of these cells.

Because hypoxia and HIF factors can directly increase the expression of CSC markers and induce CSC properties, one would predict that hypoxia and HIFs might contribute to a CSC-like phenotype resistant to the immune system. The hypoxic environment also inhibits immunosurveillance by inhibiting NK and CD8+ T cells and cell cytotoxicity as well as phagocytosis by macrophages. Thus, initial CSCs may increase the pool and initiate the tumor. Hypoxic CSCs blocks CD8+ T cell proliferation and activation [134]. In the hypoxic regions of the tumor, both the stromal cells and CSCs activate their HIF genes, which are the primary factors that drive angiogenesis via the induction of VEGF. VEGF-A recruits monocytes and macrophages [56, 135], thus promoting angiogenesis. Tumor-associated macrophages become pro-angiogenic through their response to macrophage colony stimulating factor (M-CSF) [136], secreted by the tumor cells, which further induces VEGF-A production and suppresses antiangiogenic factor expression.

Importantly, chemotherapy triggers HIF-1α-dependent glutathione synthesis that induces the CSC phenotype. Paradoxically, recent studies have shown that CSCs and activated immune effector cells exhibit high HIF activity in normoxic environments and that this HIF activity is critical in the maintenance of CSCs as well as the differentiation and function of inflammatory cells. Given that inflammation and CSCs are two major barriers to effective cancer therapy, targeting HIF may provide a new approach to developing such treatments.

### Metabolic reprogramming and CSCs

As previously mentioned, HIF-1α is activated in response to hypoxia and is involved in the activation of numerous cellular processes, including the resistance to apoptosis, detoxification, angiogenesis and metastasis. Furthermore, HIF-1α induces the overexpression and increased activity of several glycolytic proteins that differ from those found in nonmalignant cells, including transporters (GLUT1, GLUT3) and enzymes of the glycolytic pathway (Fig. 4). The tumor-initiated glycolytic flux triggered by HIF-1α also changes that isoforms of key glycolytic enzymes are produced. The HIF-1α-induced isoforms provide cancer cells with reduced sensitivity to physiological inhibitors, lower affinities for the products of the reaction and a higher catalytic capacity in forward reactions. Some of the HIF-1α-induced glycolytic isoforms also participate in survival pathways, including the transcriptional activation of histone H2B (by LDH-A), inhibition of apoptosis (by hexokinase II) and promotion of cell migration (by enolase-α). HIF-1α action may also modulate mitochondrial function and oxygen consumption by inactivating the pyruvate dehydrogenase complex in some tumor types or by modulating the expression of the cytochrome c oxidase subunit 4 to increase oxidative phosphorylation in other cancer cell lines [137, 138].

Cellular metabolism has played a central role in cancer since the observation that cancer cells preferentially generate energy by metabolizing glucose to lactate [139]. Even in the presence of oxygen, cancer cells switch from generating ATP by the highly energy-efficient process of oxidative phosphorylation to the much less efficient process of glycolysis [140]. Undoubtedly, now we know that this metabolic switch is an important feature
glycolysis promoting enzymes PFKFB3 and PFK1 allows discrimination of CSCs [152], and when cultures under hypoxic conditions induced pluripotent stem cells, iPSCs cells, alter their expression to resemble those of CSCs, clearly supporting the causal role of the hypoxia-induced metabolic switch on the CSC properties [152]. It is possible that when CSCs leave quiescence to resume their growth, their dependence on glycolysis becomes crucial. Two classes of hexose transporters that transport glucose into the cell, sodium-dependent glucose transporter and facilitative glucose transporter (GLUT), are abundantly expressed in cancer cells and are essential for the maintenance of pancreatic, ovarian and glioblastoma CSCs [153]. In vivo, the administration of an inhibitor of GLUT1 in mice inhibited tumor formation after CSCs implantation, pointing to GLUT1 as a promising target for CSC-directed therapy [153]. Previously, it was thought that metabolic changes were a mere consequence of aberrant cancer cell growth. However, the metabolic reprogramming of CSCs induces cancer-causing activity and might not require preexisting mutations of well-established cancer genes. This metabolic reprogramming may be switched on by hypoxia through HIF transcription factors.

Targeting hypoxia

In the last years, an increasing number of chemicals have been shown to inhibit HIF-1α activity by different mechanisms, decreasing the levels of HIF-1α mRNA or protein, the HIF-1α-mediated DNA binding or its transactivation activity [154]. In many tumors, the constitutive activation of driver oncoproteins such as HER2, BCR-ABL, EGFR, Ras or PI3K/PTEN pathway leads to the activation of mTOR and the subsequent induction of HIF-1 activity [155, 156]. Because mTOR is a major determinant of HIF-1 protein levels, the inhibitors of these pathways result in a clear reduction of HIF-1 activity and the derived biological consequences [154]. However, mTOR inhibition affects more than only HIF-1α protein synthesis and, therefore, its biological effects cannot be ascribed only to HIF down-regulation [157].

Inhibitors of topoisomerases, CDKs, HSP90 histone deacetylases, microtubule dynamics and thioredoxin also inhibit HIF-1α protein expression; however, the mechanisms have not been fully elucidated [158]. The camptothecins also inhibit HIF-1α activity. Topotecan, a chemotherapy currently used in the treatment of SCLC and ovarian cancer inhibits hypoxia-induced accumulation of HIF-1α, altering angiogenesis and tumor growth in xenograft models [159]. Other HIF-1α inhibitors act preventing HIF-1 from activating the transcription of target genes. Such is the case of echinomycin, polyamides and the compound named DJ12 [160–162]. The proteasomal inhibitor bortezomib in one hand increase the levels of HIF-1α protein by inhibiting its degradation but at the same time blocks its function by interfering with the transactivation domain [163]. Amphotericin B, an antifungal drug, also inhibits HIF-1α-dependent transcription by promoting its interaction with FIH-1, leading to a decreased recruitment of p300 [164].

Given that none of the known regulators of HIF-1α appears to disrupt HIF pathway exclusively, the laboratories had made efforts in the identification of small molecules that can affect HIF-1α at protein level in a specific form [158]. EZN-2968 is a
third-generation oligonucleotide that specifically binds and inhibits the expression of HIF-1α mRNA. Its administration to xenografted mice triggers a potent HIF-1α down-regulation with a concomitant tumor volume reduction [165]. The compound 2ME2 was shown to inhibit HIF-1α translation and its nuclear translocation, downstream microtubule disruption, which was associated with antiangiogenic activity [166]. Furthermore, a series of synthetic derivatives were developed aimed to improve PK and PD properties and its efficacy in vivo [158].

Whatever the chemical outcome, it is foreseen that these inhibitors will be more valuable when used in therapeutic combinations [154, 167].

Conclusions

Hypoxia has been considered a major feature of the tumor microenvironment and a potential contributor to the CSC phenotype and its enhanced tumorigenicity [168]. The combination of the acidic microenvironment around hypoxic cells and the inaccessibility of their locations, hypoxic cells are less likely to accumulate functional concentrations of chemotherapeutics that lead to MDR. Therefore, targeting the hypoxic CSC niche in combination with chemotherapy may provide a promising strategy for eradicating CSCs.

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HIF-1α synthesis: novel mechanism for HIF-1-mediated vascular signaling increases the rate of hypoxia-inducible factor 1α expression pattern of PFKFB3 enzyme distinguishes between induced-pluripotent stem cells and cancer stem cells.

Identification of small molecule inhibitors of hypoxia-inducible factor (HIF)-1 inhibitors.

The expression pattern of PFKFB3 enzyme distinguishes between induced-pluripotent stem cells and cancer stem cells.

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