Published in final edited form as:

Adv Exp Med Biol. 2019 ; 1136: 43–55. doi:10.1007/978-3-030-12734-3_3.

Hypoxia-Induced Phenotypes that Mediate Tumor Heterogeneity

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Abstract

Intratumoral heterogeneity is an important factor contributing to metastasis and therapy resistance. The phenotypic diversity of cancer cells within the tumor microenvironment is strongly influenced by microenvironmental factors such as hypoxia. Clinically, hypoxia and the hypoxia inducible transcription factors HIF-1 and HIF-2 are associated with cancer stem cells, metastasis and drug resistance in multiple tumor types. Experimental models have demonstrated an important functional role for HIF signaling in driving CSC, metastatic and drug resistant phenotypes *in vitro* and *in vivo*. Here we will review recent studies that highlight novel mechanisms by which hypoxia promotes cancer stem cell, metastatic and drug resistant phenotypes.

Keywords

Hypoxia; Metastasis; Stemness; Cancer stem cell; HIF; Therapy resistance; Chemotherapy; Metabolism

3.1 Introduction

It is well established that tumors are composed of a heterogeneous population of tumor cells that are morphologically and phenotypically distinct. The phenotypic diversity of cancer cells within the tumor microenvironment is thought to be a major driver of metastasis and therapy resistance. There are multiple mechanisms driving tumor heterogeneity including genetic, epigenetic and microenvironmental factors [1]. Accumulating evidence suggests that hypoxia is an important microenvironmental factor that influences tumor heterogeneity by promoting cancer stem cell, metastatic and drug resistant phenotypes.

Areas of hypoxia, or low oxygen tensions, are commonly found in solid tumors. Hypoxia develops when there is an imbalance between oxygen delivery and oxygen consumption [2]. Within the tumor microenvironment, oxygen delivery is impaired due to the irregular vasculature characterized by distended capillaries with leaky and sluggish blood flow [2]. In addition, oxygen consumption rates are increased by proliferating tumor cells and infiltrating

immune cells. The hypoxia inducible transcription factors HIF-1 and HIF-2 coordinate the adaptive cellular response to low oxygen tensions by activating gene expression programs controlling glycolysis, angiogenesis, cell survival, invasion, immune suppression, the cancer stem cell phenotype, metastasis and resistance to conventional therapy [3]. In the presence of oxygen, the α -subunits of HIF-1 and HIF-2 are rapidly degraded through the cooperative actions of prolyl hydroxylase enzymes (PHD1, 2, and 3) and the E3 ubiquitin ligase substrate recognition component VHL [4]. Under hypoxia, HIF-1 and HIF-2 are stabilized and activate the expression of genes containing hypoxia response elements (HRE) [5]. Over 200 genes are activated in response to HIF-1 and HIF-2 that allow cells to survive and adapt to low oxygen tensions.

3.2 Hypoxia and Cancer Stem Cells

Cancer stem cells (CSCs) are a rare subpopulation of tumor cells within the tumor microenvironment that are thought to mediate tumor initiation, metastasis and therapy resistance. Cancer stem cells were first described in AML and have subsequently been identified in many solid tumor types including breast, colorectal, brain, pancreatic and ovarian cancers [6]. Functionally, CSC are defined by their unlimited self-renewal, clonal tumor initiation and clonal long-term repopulation capacity [7]. Recent evidence suggests that CSCs exist in a state of plasticity where they can transition between stem and non-stem cell states. Accumulating data suggests that cellular and molecular factors within the tumor microenvironment play a key role in determining CSC self-renewal, promote their metastatic potential as well as protect them from chemotherapy and radiotherapy [8]. Here we will review recent literature describing how hypoxia and HIF signaling within the tumor microenvironment influences the CSC phenotype.

Clinically, HIF-1 and HIF-2 are highly expressed in CSCs. Immunohistochemical analysis of HIF-1 and HIF-2 with CSC markers such as CD133 or CD44 revealed high levels of HIF-1 and HIF-2 expression within CSC pools in glioma, acute myeloid leukemia (AML), and breast cancer [9–11]. In experimental models, HIF signaling is required to maintain the CSC phenotype *in vitro* and *in vivo*. Samanta et al. observed that paclitaxel or gemcitabine treatment of triple negative breast cancer cell lines induced HIF expression and activity and enriched the breast CSC population [12]. In this model, coadministration of HIF inhibitors reduced the breast CSC resistance to paclitaxel and gemcitabine resulting in increased tumor control [12]. In the polyoma virus middle T antigen (PyMT) transgenic model of breast cancer, genetic inactivation of HIF-1 reduced the number of CD133+ breast CSCs and decreased the CSC pool in limiting dilution studies *in vivo* [13]. Moreover, Cecil et al. utilized an immunization approach to test the hypothesis that HIF-1 is immunogenic in triple negative breast cancer and active immunization against HIF-1 could impact tumor growth and CSCs in a basal model of breast cancer. Indeed, HIF-1 vaccination reduced the CSC population and growth of basal mammary tumors [14]. Knockdown of HIF-1 or HIF-2 in CSCs is also sufficient to reduce glioma CSC survival and suppress CSC-mediated tumor growth [11]. Similarly, genetic or therapeutic targeting of HIF-1 abrogated the colony-forming unit (cfu) ability of mouse lymphoma and human AML CSCs [10]. In chronic myeloid leukemia stem cells, deletion of HIF-1 inhibits CML progression by impairing cell-cycle progression and inducing apoptosis of LSCs [15]. Collectively, these studies

demonstrate that HIF-1 and HIF-2 are highly expressed within human and mouse CSCs where they play an important functional role in mediating CSC growth and survival. These findings suggest that therapeutic strategies targeting HIF-1/HIF-2 may be efficacious in reducing CSCs and promote therapeutic responses to conventional chemo radiation therapy.

3.3 Mechanisms of HIF-Mediated CSC Phenotype

There are multiple mechanisms by which hypoxia and HIF signaling promote CSC growth and survival including upregulation of CSC surface markers, induction of pluripotency factors, stemness pathways, metabolism, epithelial to mesenchymal transition (EMT), and mediating CSC crosstalk with their niche. Here we will review the recent literature identifying mechanisms by which HIF signaling promotes the CSC phenotype.

3.3.1 HIF Signaling and CSC Surface Marker Expression

There has been much interest in the identification of unique markers expressed by CSCs, as these markers have the potential to be exploited as both prognostic factors and therapeutic targets. Currently, there are approximately 30 CSC surface markers that have been identified across multiple tumor types [16]. Most of the CSC cell surface markers are glycoproteins including CD133, CD24 and CD44 [16]. More recently, there has been interest in the identification of metabolic CSC markers. For example, Li et al. identified an increase in lipid unsaturation levels as a marker of ovarian cancer stem cells (CSCs) compared to non-CSCs. Importantly, they found that lipid unsaturation, mediated by the lipid desaturase SCD1, is important to maintain CSC stemness and tumor formation indicating that lipid unsaturation levels may be an functional marker of ovarian CSCs [17]. Future studies are needed to determine if increased unsaturated lipid are found in additional CSC populations.

Given the important role of hypoxia and HIF signaling in maintaining CSCs, multiple CSC markers have been shown to be regulated by hypoxia. CD133 is an cell surface glycoprotein that is associated with CSCs in a variety of cancers including the brain, prostate, liver, pancreas and colon cancer [18]. Multiple studies observed that CD133 expression on CSCs is induced by hypoxia and HIF [19–22]. Molecular analysis of the CD133 (PROM1) promoter revealed that HIF-1 and HIF-2 bind to the PROM1 promoter at an ETS transcription factor binding site. PROM1 expression mediated by HIF likely occurs through an association with ETS-family transcription factors [22]. In support of this notion, immunoprecipitation studies identified a physical interaction between HIF-1 and ETS transcription factor ELK1 [22]. CD24 is another cell surface protein that is associated with a CSC phenotype and plays a particularly important role in the progression of hepatocellular carcinoma and male urothelial cancers [23, 24]. Combined analysis of HIF-1 and CD24 expression in a cohort of 101 human urothelial cancer specimens revealed a statistically significant association with reduced overall survival suggesting an coordinate regulation between HIF-1 and CD24 [25]. Functional studies demonstrated that HIF-1 is both necessary and sufficient for the hypoxic induction of CD24 in multiple cell lines. Moreover, a functional hypoxia-responsive element was identified in the promoter of CD24 demonstrating a direct regulation of CD24 by HIF-1 [25]. Importantly, genetic inactivation of HIF-1 reduced primary and metastatic growth that could be rescued in part by CD24

overexpression [25]. These suggest that the CSC membrane protein CD24 as an important effector of HIF-1 activity. In the glioma stem cell niche, CD44 expression correlates with hypoxia induced gene signatures. CD44 is a glycoprotein transmembrane receptor and CSC markers that binds to osteopontin. Osteopontin binding to CD44 results in cleavage of the CD44 intracellular domain (ICD) that promotes glioma growth and a stem cell-like phenotype in a HIF-2 dependent manner. Mechanistically, the CD44 ICD enhances the transcriptional activity of HIF-2 in a CBP/p300 dependent manner to promote stem cell marker gene expression and facilitate tumor growth [26]. Future studies are needed to elucidate the mechanisms by which the CD44 ICD promotes HIF-2 activity. A recent report suggests that CD44 ICD directly interacts with HIF-2, but not HIF-1, to promote stabilization of the HIF-2 CBP/p300 complex [27]. Collectively, these data identify a functional link between hypoxic signaling and CSC stem cell markers.

3.3.2 HIF Signaling and Pluripotency Transcription Factors

The core pluripotency transcription factors OCT4, SOX2, NANOG and KLF4 are required to maintain embryonic stem cell pluripotency and self-renewal [28, 29]. Similar to embryonic stem cells, CSCs express OCT4, SOX2, NANOG and KLF4 to maintain a stem cell phenotype and tumor growth [30–35].

Hypoxia and HIF signaling play an important role in stem cell proliferation and differentiation during development and cancer. During development, germline inactivation of *Arnt*, the common binding partner for Hif-1 α and Hif-2 α results in embryonic lethality at E9.5-E10.5 due to defective placental vascularization and trophoblast lineage differentiation [36]. Analysis of trophoblast stem cells revealed that HIF activity is required to induce the expression of Mash2, a bHLH protein that directs specific trophoblast lineages [36]. Similar results were observed in *Hif-1/Hif-2* double knockout mice demonstrating a critical role for HIF transcriptional activity in trophoblast lineage determination [36]. Covello et al. utilized an elegant genetic knock-in strategy to compare the relative contribution of Hif-1 and Hif-2 on stem cell function during development. Interestingly, *Hif-2* knock-in into the *Hif-1* locus resulted in embryonic lethality at an earlier timepoint (E6.5-E7.5) compared to the *Hif-1/Hif-2* deficient embryos. In this setting, embryonic lethality was associated with developmental patterning defects and increased expression of Oct-4 [37]. This observation led to the identification of Oct-4 as a novel Hif-2 target. Importantly, knockdown of Oct-4 was sufficient to rescue some of the hematopoietic defects in Hif-2 knock-in cells indicating that Hif-2 modulates stem cell function through Oct-4 during development [37].

In cancer, HIF activity has been shown to regulate the expression of multiple pluripotency factors. In prostate tumors, HIF-1, NANOG, OCT4 and SOX2 staining co-localize indicating a co-regulation of these factors in human tumors [38, 39]. Indeed, HIF signaling can promote the expression of the core pluripotency transcription factors in cancer cells through direct and indirect mechanisms [19, 38]. As described above, a direct role for HIF-2 in the regulation of OCT4 has been demonstrated through the identification of a functional hypoxia response element in the Oct-4 promoter [37]. Hypoxic regulation of Oct4 has been detected in multiple cancer cell lines [38, 40]. Data suggest that HIF-1 and HIF-2 indirectly promote SOX2 expression through the regulation of NOTCH1 signaling [39, 41]. NANOG

expression is regulated by HIF in cancer cells through transcriptional and posttranscriptional mechanisms. In breast cancer cells, HIF-1 can promote NANOG expression by upregulating the expression of adenosine receptor 2B (A2BR) resulting in an increase in adenosine signaling and activation of protein kinase C- δ leading to STAT3-mediated activation Nanog transcription [42]. In addition, HIF-mediated increase in the expression of the cystine transporter xCT and the regulatory subunit of glutamate-cysteine ligase (GCLM) results in an increase in intracellular glutathione levels that act to suppress mitogen activated protein kinase kinase (MEK) through copper chelation. MEK-ERK inhibition leads to the nuclear translocation of the FoxO3 transcription factor and activation of its target Nanog [43]. HIFs can also increase NANOG levels in cancer cells by controlling Nanog mRNA stability. HIF-1 transactivation of ALKBH5, an N⁶-methyladenosine demethylase, results in HIF dependent demethylation of N⁶-methyladenosine of Nanog mRNA [44]. While m⁶A modifications of mRNA can control protein binding, mRNA splicing, translation, stability and degradation; m⁶A modifications on pluripotency factors such as Nanog results in reduced mRNA stability [45, 46]. Indeed, HIF-mediated ALKBH5 promotes Nanog m⁶A demethylation and mRNA stability leading to increased Nanog and enhanced breast cancer stem cell enrichment [44]. Together these data illustrate multiple mechanisms by which HIF activity can promote the CSC phenotype through the activation of pluripotency factor gene expression.

3.3.3 HIF Signaling and CSC Signaling Pathways

Similar to embryonic or tissue stem cells, CSCs activate conserved signaling pathways that promote development and tissue homeostasis including Notch, Hedgehog and Wnt pathways (for a recent review [47]). Hypoxia and the activation of HIF signaling plays an important role in maintaining stem cell signaling in development and in cancer [48]. For example, Notch signaling is an evolutionary conserved pathway controlling cell fate in most metazoan species [49]. Gustafsson et al. discovered that hypoxia promotes an undifferentiated cell state through the activation of Notch signaling. They discovered that the Notch intracellular domain interacts with HIF-1 α and HIF-1 α is recruited to Notch-responsive promoters under hypoxic conditions to enhance the activation of downstream Notch target genes [50]. In human glioblastoma, hypoxia promotes the tumorigenic capacity of glioma stem cells through the activation of HIF and Notch signaling [11, 51]. HIF-1 may augment Notch signaling under hypoxic conditions by increasing the expression of vasorin, a protein that binds to Notch1 at the cell membrane to prevent its degradation [52]. In AML, HIF promotes CSC self-renewal by inhibiting the Notch negative feedback loop mediated by Hes1 [10]. Thus, there are multiple mechanisms by which HIF signaling controls Notch signaling to maintain a CSC phenotype in tumor cells. In addition to the Notch pathway, hypoxia has also been shown to promote the self-renewal properties of CSCs through activation of the Wnt/ β -catenin [53, 54], JAK/STAT3 [55], NF- κ B [56], PI3K/AKT and Hippo-YAP pathways [57].

3.3.4 HIF Signaling and CSC Metabolism

Altered metabolism is a hallmark of cancer [58]. There is growing interest in understanding the metabolic heterogeneity of CSCs and how metabolic stressors such as hypoxia affect the CSC phenotype. Samanta et al. observed that metabolic adaptation is required for the

hypoxic induction of breast cancer stem cells [59]. Under hypoxic conditions, BCSCs upregulate the expression of multiple genes controlling metabolic adaptation including phosphoglycerate dehydrogenase (PHGDH), an enzyme that shunts glucose metabolites into the serine synthesis pathway. Genetic inactivation of PHGDH resulted in increased oxidant levels, apoptosis, loss of BCSCs and reduced tumorigenic and metastatic potential of BCSCs [59]. Luo et al. recently observed that the transition of breast cancer stem cells (BCSCs) from a quiescent mesenchymal-like (M) state to a proliferative epithelial-like (E) state is regulated by the redox state within the cell. They found that ROS-induced BCSC transition from the M to E state is facilitated by activation of HIF-1 and Notch signaling [60]. This finding is consistent with another report demonstrating that MYC and MCL1 amplification in TNBC promotes CSC enrichment and tumor growth through ROS-mediated accumulation of HIF-1 [61]. These studies identify a molecular link between cellular redox status and CSC maintenance mediated by HIF-1.

3.3.5 HIF Signaling, EMT and CSC

The epithelial to mesenchymal (EMT) program is a key pathway driving the CSC phenotype. In human clinical samples, CSC populations express high levels of the EMT transcription factors (Slug, Snail, Zeb1) or mesenchymal markers (vimentin, N-cadherin and reduced E-cadherin) relative to non-CSC populations. Moreover, experimental manipulation of the EMT program through knockdown of EMT transcription factors such as Snail in CSC populations can significantly reduce the CSC phenotype in vitro and in vivo (for a recent review [1]). Conversely, overexpression of EMT transcription factors such as TWIST1 is sufficient to promote a CSC-like phenotype [62].

Hypoxia is an important microenvironmental factor promoting EMT. Hypoxic signaling promotes EMT in cancer cells through direct and indirect mechanisms. For example, the expression of the EMT transcription factors Twist1, Zeb1/2, and Snail are directly induced by hypoxia in a HIF-dependent manner through the activation of hypoxia response elements (HREs) within regulatory elements of these genes [63–65]. HIF signaling has also been shown to indirectly promote EMT through the activation of Notch, Wnt, TGF β , integrin-linked kinase (ILK), and the receptor tyrosine kinase AXL (for a recent review [66]). Thus, hypoxia and HIF signaling have the potential to influence CSC populations through the regulation of EMT. In support of this hypothesis, hypoxia induced CSC EMT and tumor formation through HIF-1 mediated activation of Snail in gastric cancer cell lines [67].

3.3.6 HIF Signaling and CSC Crosstalk in their Niche

Similar to normal stem cells, CSCs reside within specialized niches where they rely on stromal derived factors to maintain stemness, protect them from the immune system and facilitate their metastatic potential [8]. For example, cancer associated fibroblasts are an important cellular component of the CSC niche. They secrete factors that promote stem cell signaling pathways such as Wnt, Notch, and Hedgehog [8]. In colorectal cancer, CAFs secrete TGF- β 2, which activates the expression of GLI2, a transcription factor involved in the Hedgehog pathway. Interestingly, hypoxia cooperates with CAFs to further induce GLI2 expression in colorectal cancer cells resulting in robust resistance to chemotherapy [68].

Peritoneal metastases are commonly found in ovarian and gastric cancer patients, where CSC rich spheroids present in malignant ascites fluid are abundant sources for peritoneal dissemination, distant metastasis to peritoneal organs and therapy relapse [69]. A recent study suggests that the hypoxic peritoneal milky spots (PMS) at the omentum may create an ideal CSC niche for gastric cancer engraftments [70]. In animal models, gastric CSCs preferentially localized to hypoxic regions of PMS, and these engraftments expressed high levels of stem genes. Moreover, inhibition of HIF-1 in gastric cancer cells significantly reduced peritoneal dissemination and self-renewal [70]. Future studies are needed to understand how hypoxia is induced and maintained within milky spots and whether hypoxia influences other cell compartments of peritoneum surface (macrophages, adipocytes and mesenchymal cells, etc.) to support CSC stemness through paracrine signaling or cell-to-cell contact.

A growing area in CSC research is understanding the immunobiological properties of CSCs and how these properties are modulated by the tumor microenvironment. Cancer stem cells have been shown to inhibit both innate and adaptive immune responses through multiple mechanisms (for a recent review [71]). In addition, the hypoxic microenvironment promotes an immunosuppressive phenotype through inhibition of natural killer cell mediated antitumor responses, promoting resistance to T cell mediated killing, inhibition of macrophage phagocytosis, recruitment of regulatory T cells, and inhibition of T cell effector functions [66]. Recent studies have focused on understanding how hypoxia potentiates CSC immune suppression. In B16-F10 melanoma tumors, hypoxia-induced Nanog promotes a CSC phenotype, but also promotes the infiltration of regulatory T cells through the upregulation of TGF- β 1 providing a molecular link between the hypoxic induction of the CSC and immunosuppressive phenotype [72]. In glioblastoma, hypoxia enhanced glioblastoma CSC ability to inhibit T cell proliferation and activation as well as macrophage phagocytosis. The immunosuppressive effects of hypoxia in this model were mediated through the activation of STAT3 and HIF-1 [73]. Another mechanism by which HIF signaling couples immune suppression with a CSC phenotype is through the activation of CD47. CD47 is a cell surface protein that inhibits macrophage mediated phagocytosis by binding to signal regulatory protein SIRP α macrophages [74]. CD47 also regulates the breast cancer CSC phenotype through mechanisms that remain unclear [75]. Zhang and colleagues discovered that HIF-1 directly activates the expression of CD47 to promote a CSC phenotype and inhibit macrophage-mediated phagocytosis [75]. In addition, HIF signaling activates the expression of the immunosuppressive factors CD73 and PDL1 to promote chemotherapy-induced immune evasion in TNBCs [76]. Together, these studies suggest that HIF signaling is an important pathway coupling innate and adaptive immune suppression with a CSC phenotype.

3.4 HIF Signaling, CSC and Metastasis

Metastasis remains the leading cause of cancer related deaths. Metastatic disease is thought to arise from disseminated cancer cells, or cancer stem cells, that retain the ability to reinitiate tumor growth at a distant tissue site [3]. There are multiple proposed models to explain how metastatic stem cells accumulate at distant sites. For example, it is thought that in some cases such as colorectal cancer, the metastatic CSCs originate from the primary

tumor where they travel to distant tissue sites and resume their regenerative potential [3]. Another model is metastatic stem cells at distant sites arise through phenotypic plasticity and regain tumor initiating capacity [3]. Regardless of their origin, the tumor microenvironment at both the primary and distant metastatic sites plays an important role in promoting the metastatic phenotype of CSCs [3].

In addition to regulating the CSC phenotype, hypoxia plays an important role in metastasis. Hypoxia and the activation of HIF signaling is associated with metastasis and poor survival in variety of cancer patients [5, 66, 77, 78]. Moreover, preclinical studies have shown that hypoxia and HIF signaling promote multiple steps within the metastatic cascade and have been reviewed extensively elsewhere [66]. Here we will focus our review on recent literature that has identified a link functional link between hypoxic signaling, the CSC metastatic phenotype and metastasis.

As described above, Notch signaling plays an important role in stem cell self-renewal. This pathway is activated by HIF signaling through multiple mechanisms to promote a CSC phenotype. In breast cancer patients, the Notch ligand Jagged 2, correlates with poor overall and metastasis-free survival. Xing and colleagues discovered that breast cancer cells activate Notch signaling under hypoxic conditions to promote metastasis and a CSC phenotype. They found that Jagged 2 expression and activation of Notch are activated within the hypoxic invasive front of breast cancer specimens. Mechanistically, hypoxia increases the expression of Jagged 2 and Notch signaling to promote tumor cell EMT, survival and CSC-like phenotype, indicating that hypoxia may facilitate metastatic and CSC phenotype through the activation of Notch signaling [79]. Another mechanism by which hypoxia may coordinate the metastatic and CSC phenotype is through the activation of CD24 expression. In hepatocellular carcinoma, CD24 is an important functional CSC marker that is required to maintain self-renewal, differentiation and metastasis [24]. Thomas et al. discovered that CD24 is a direct HIF-1 target that promotes HIF-1 driven metastasis in preclinical models of bladder and prostate cancer [25]. Pang et al. recently linked hypoxia with the CSC phenotype and metastasis through the induction of integrin-like kinase (ILK). ILK is a kinase that transmits signals from the B1-integrin and the extracellular matrix to promote tumor cell migration, invasion, proliferation and angiogenesis [80]. Pang et al. found that hypoxia and stiff microenvironments upregulated the expression of B1-integrin and ILK to promote a CSC phenotype in invasive breast cancer cell lines suggesting an link between hypoxia, the ECM and the CSC metastatic phenotype [81]. A recent study utilized a genetic model of pancreatic ductal adenocarcinoma (PDAC) to identify a subpopulation of cancer cells with enhanced metastatic potential. Within this population, they found elevated levels of the transcription factor BLIMP1 and hypoxic gene signatures suggesting that BLIMP1 may be a hypoxia induced gene. Indeed BLIMP1 is a novel HIF-1 target that is required for the metastatic potential of PDAC metastatic stem cells. Interestingly, approximately 35% of hypoxia regulated genes within this metastatic population were regulated by BLIMP1 under hypoxic conditions suggesting that BLIMP1 is an hypoxia regulated factor that potentiates a subset of hypoxia inducible genes within metastatic cell populations. These data suggest that hypoxia promotes the metastatic behavior of PDAC cells through the activation of BLIMP1 [82]. Collectively, these studies identify an important role for hypoxia and HIF signaling in promoting metastatic stem cell phenotypes.

3.5 HIF, CSCs and Chemoresistance

Chemoresistance is a major clinical challenge in the treatment of cancer. Experimental evidence suggests that cancer stem cells are enriched in cancer populations that persist following chemotherapy and facilitate the regrowth of disease in the recurrent setting. There are multiple mechanisms by which CSCs can mediate chemoresistance including upregulation of drug efflux pumps, enhanced DNA-repair capacity, enhanced protection against reactive oxygen species and induction of an quiescent state (for a recent review [7]). Hypoxia is an important microenvironmental factor that facilitates chemoresistance. Clinically, hypoxia and HIF expression is associated with therapeutic resistance or decreased survival following ionizing radiation or chemotherapy [83–85]. There are multiple mechanisms by which hypoxia and HIF signaling promote chemoresistance including upregulation of drug efflux, induction of autophagy, hypoxia-driven selection of tumor cells with reduced apoptotic capacity and inhibition of DNA damage, metabolic reprogramming EMT and the CSC phenotype (for a recent review [86]). Here we will focus our discussion on recent studies that have identified a role of HIF in promoting chemoresistance associated with a CSC phenotype.

Advanced stage colorectal cancers are commonly resistant to chemotherapy. Cancer stem cells have been implicated in colorectal cancer relapse. Hypoxia is also known to promote chemoresistance in colorectal cancer cells although the mechanisms by which this occurs remain unclear. Tang et al. propose that one mechanism by which hypoxia facilitates chemoresistance in colorectal cancer occurs through an interaction with the CSC niche. Cancer associated fibroblasts are a key cellular component of the colorectal CSC niche and produce multiple factors including Wnts, HGF, and IL-11 to promote colorectal CSCs and metastasis. Under hypoxic conditions, CAFs secrete the growth factor TGF- β 2 leading to the induction of the hedgehog transcription factor GLI2 in colorectal cancer cells. Notably, GLI2 expression in colorectal cancer cells is required for chemoresistance induced by CAFs and hypoxia. Clinically, HIF-1/TGF- β 2/GLI2 expression are associated with relapse and poor patient survival suggesting that targeting these pathways in combination may be efficacious to sensitize resistant colorectal tumors to chemotherapy [68]. Future studies are needed to determine whether hypoxic signaling in additional stromal cell populations may enhance CSC and chemoresistance phenotypes.

Triple negative breast cancer (TNBC) is commonly treated with cytotoxic chemotherapeutic agents given the lack of known molecular targets. The development of recurrent chemoresistant disease is a leading cause of mortality in women diagnosed with TNBC. Cancer stem cells are thought to be a rare population of cells in TNBC patients that are resistant to chemotherapy and drive relapse following adjuvant chemotherapy [87]. Current research efforts are focused on elucidating the mechanisms by which breast cancer stem cells facilitate recurrence and metastasis. Multiple groups have observed that chemotherapy induces the normoxic expression of HIF-1 and/or HIF-2 to drive breast cancer stem cell enrichment and drug resistance [12, 43, 88, 89]. There are likely multiple mechanisms by which chemotherapy induces the normoxic accumulation of HIF. Cao et al. found that doxorubicin induced the expression of HIF in a STAT1-dependent manner. STAT1 activation by chemotherapy resulted in the activation of iNOS and the accumulation of intracellular

nitric oxide, which can impair the normoxic degradation of HIF-1 [88]. Another mechanism by which HIF-signaling is activated in TNBC following chemotherapy is mediated through the induction of MYC and MCL1. MYC and MCL1 are commonly coamplified in drug resistant TNBC following neoadjuvant chemotherapy. MYC and MCL1 cooperatively promote mitochondrial oxidative phosphorylation and the generation of ROS. In this setting, increased ROS enhances the normoxic stabilization of HIF-1 to promote CSC enrichment in chemotherapy resistant TNBC [61]. Once stabilized, HIF promotes the BCSC phenotype and chemoresistance through multiple mechanisms including increased IL-6 and IL-8 signaling, increased expression of multidrug resistance 1 gene expression and the activation of pluripotency factors [12, 43, 89].

3.6 Conclusion

Together the findings presented in this review highlight the central role for hypoxia and HIF signaling in coordinating CSC, chemoresistant and metastatic phenotypes in cancer (Fig. 3.1). With the clinical development of HIF inhibitors, it will be important to investigate the combination of these agents with chemotherapy and/or targeted therapies aimed at the inhibition of CSC signaling pathways.

Acknowledgements

This work was supported by the Office of the Assistant Secretary of Defense for Health Affairs through the Department of Defense Ovarian Cancer Research Program under Award No. W81XWH-15-1-0097 (EBR). We apologize to those colleagues whose work we could not cite due to space constraints.

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Fig. 3.1. Mechanisms of hypoxia induced tumor heterogeneity.

The hypoxic tumor microenvironment facilitates cancer stem cell, metastatic and therapy resistant phenotypes through multiple mechanisms. Both HIF-dependent and independent mechanisms drive the selection of cancer stem cells within the hypoxic tumor microenvironment that contribute to metastatic disease and therapy relapse. Additionally, the selection of chemoresistant clones can stabilize HIF under normoxic conditions to further promote stemness and metastatic phenotypes