Hypoxia-Regulated Gene Expression and Metastasis

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Abstract

Mammalian cells have adopted mechanisms to regulate cellular oxygen levels for the maintenance of the cellular homeostatic balance. "Hypoxia" states the condition of insufficient oxygen supply at the tissue level. Commonly hypoxia is known as a characteristic feature of solid tumors playing an important role in supporting tumor progression and metastasis by promoting various processes such as angiogenesis or epithelial to mesenchymal transition (EMT). Hypoxia-inducible factors (HIFs) are the major transcription factors activated under hypoxic conditions and implicated in transcriptional activation and regulation of metastatic processes. In particular, HIF1α enables tumors to gain invasive and metastatic properties by regulating the major transcription factors leading to EMT. In this chapter, we reviewed the recent knowledge on hypoxia-regulated gene expressions including transcriptional factors, enzymes, extracellular matrix elements, and signaling molecules that are involved in the process of cancer metastasis.

Keywords

Hypoxia · EMT · HIFs · Metastasis · Gene expression

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6.1 Introduction

Eukaryotic organisms have evolved various mechanisms regulating oxygen tension to maintain cellular, tissue, and organ homeostatic balances. Mammalian cells require a physiological oxygen level (physioxia) of 4–9% to retain the necessary energy and aerobic metabolism. However, under various conditions, the oxygen level of human tissues or cells drops to the lower levels than physioxia, which is called "hypoxia." Hypoxia has an impact on the physiological process of embryogenesis or erythropoiesis, but also implicated in various pathological conditions such as heart attack, inflammatory diseases, and cancer (Shi et al. 2021; Wilson et al. 2020; Nakayama and Kataoka 2019).

Hypoxia is a characteristic of solid tumors, yet even hematological cancer types are affected by hypoxia (Chee et al. 2019). Each tumor body consists of a heterogeneous population of cells in its microenvironment (Tam et al. 2020). In a heterogeneous tumor population, the degree of hypoxia varies with the distance of the tumor from the tumor microvessels, with some areas being anoxic (0% oxygen) or even severely hypoxic (approximately 0.2% oxygen) (Shi et al. 2021).

Biological responses to hypoxia are quite extensive; adaptations of cancer cells to hypoxia involve a transition to anaerobic energy production via changes in gene expression. Hypoxia modulates processes such as lipid and glucose metabolism by increasing oxygen transport and delivery as well as glycolysis and glucose uptake, regulates apoptosis, and mediates a series of cellular responses, including invasion and metastasis of cancer cells. Consequently, readjustment of biological processes in hypoxia enables cancer cells to survive and adopt the hypoxic environment (Tam et al. 2020; Filippopoulou et al. 2020).

Metastasis is a multistep biological process starting with local invasion into the tumor-associated stroma and subsequently results in intravasation into the hematopoietic, lymphatic systems, or peritoneum. Cancer cells survive in the blood stream throughout this entire process and extravasate for its next step to pre-metastasis. Ultimately, all steps are accomplished by colonizing in distant organs to form metastatic niches and metastases. Cancer cells generally experience metabolic problems in terms of oxidative stress as well as nutrient and oxygen availability in the regions where they colonize by metastasizing (Wei et al. 2020).

For tumor cells proliferation, it is crucial to transport nutrients and oxygen to the cells creating the microenvironment. Thus, the vascularization around the tumor provides the cell required nutrients and the oxygen. This vascularization is generally irregular, does not have adequate function, and has a leaky structure, leading to insufficient vascular perfusion. Hypoxia-inducible factor (HIF) is the major transcription factor activated under hypoxia that provides transcriptional activation of various genes that are implicated in adaptation of the cellular responses to hypoxia in tumor microenvironment (Akanji et al. 2019; Zhang et al. 2021a).

6.2 Hypoxia-Inducible Factors

Hypoxia-inducible factor (HIF-1) is the main transcription factor activated under hypoxic conditions and also involved in the tumor microenvironment to mediate adaptive cellular responses (Lv et al. 2017). The HIF-1 protein consists of two subunits; while the α subunit is regulated by oxygen and stabilized only under hypoxia, the β subunit (HIF-1β or ARNT) is constitutively expressed (Pilevneli and Kilic-Eren 2021). Among the three types of HIF-α, HIF-1α is the most intensively studied hypoxia-inducible factor. HIF-1α and HIF-2α are sensitive to varied levels of hypoxia as they harbor different prolyl hydroxylase domains. In severe hypoxia (0–2%), HIF-1α is accumulated, while under moderate hypoxia (2–5%) HIF-2α exhibits more persistent expression. Functions of the other HIF-α variant HIF-3α are based on the layout of other HIF complexes (Tao et al. 2021).

In normoxic conditions, HIF1-α, which contains an oxygen-sensitive domain, namely prolyl hydroxylase domain protein 2 (PHD2), becomes hydroxylated by prolyl hydroxylases and thereby recognized by E3 ubiquitin ligase complex involving the von Hippel–Lindau protein for degradation. In hypoxia, however, the HIF-1α protein dimerizes with the HIF-1β subunit and becomes stabilized and allows to translocate to the nucleus to induce expression of various genes, implicated in angiogenesis and glycolysis, to promote cell survival and metastasis (Reczek and Chandel 2017).

HIFs and tumor hypoxia are involved in numerous hallmarks of cancer, such as genomic instability, immune evasion, cell proliferation, metabolism, apoptosis, invasion, vascularization, and metastasis. Implication of HIFs in regulation of cellular processes in cancer cells hypoxia also endorses resistance to chemotherapy and radiotherapy. HIFs' expressions have been clinically associated with the relapse and poor prognosis in cancer therapy as well. Furthermore, HIFs are recognized among the molecular targets that can be employed in the clinical field in order to improve the treatment of metastatic and treatment-resistant tumors (Wigerup et al. 2016).

6.3 Hypoxia-Induced Regulators of Metastasis

6.3.1 Epithelial to Mesenchymal Transition

Metastasis is the most important process complicating the treatment modalities and ultimately leading to death in cancers. Hypoxia has an important role in terms of the induction and regulation of the various steps of metastasis. The metastatic process in tumor cells consists of multiple steps starting with the epithelial to mesenchymal transition (EMT) and results in colonization (Tsai and Wu 2012). EMT plays a substantial role in diverse physiological processes, including embryo implantation, wound healing, and inflammation, but also it enables the transformation of epithelial cancer cells into mesenchymal cells, which mainly provides tumors' acquisition of invasive and metastatic properties. EMT is a reversible process in which epithelial

features of malignant tumors are lost for supporting the mesenchymal properties, including improved migratory potential and invasiveness. The process of transition comprises the downregulation of epithelial cell markers, such as E-cadherin, and subsequent upregulation of mesenchymal factors such as N-cadherin, vimentin, fibronectin, several matrix metalloproteases (MMPs), and integrins (β1 and β3) (Tsai and Wu 2012).

EMT causes cancer cells to acquire invasive and metastatic features mainly by activation of the specific transcriptional factors, cell surface proteins, and extracellular matrix (ECM) enzymes (Tam et al. 2020). Hypoxia is one of the major regulators of EMT implicated in the promotion of metastasis. Hypoxia impacts the process of EMT through several ways, including direction of signaling pathways implicated in EMT, control of the expression of EMT-associated TFs, and coordination of EMT-associated miRNA and lncRNA interplays. Hypoxia activates a number of signaling pathways implicated in induction of EMT, including TGF- β, NF-κB, Notch, and Wnt.

6.3.2 Signal Mediators of Hypoxia-Regulated EMT

As mentioned earlier, hypoxia-regulated EMT, metastasis, and invasion of tumor cells are governed by signaling pathways such as TGF-β, Wnt, Jagged/Notch19, PI3K/Akt, and AMPK (Tirpe et al. 2019; Saxena et al. 2018). Activation of AMPK under hypoxic conditions has a supporting role in EMT and metastasis (Saxena et al. 2018), whereas TGF-β signaling executes EMT-mediated progression, tumor immunity, and organ fibrosis (Lin et al. 2020). The TGFβ signaling pathway is activated in a variety of important developmental processes such as cell proliferation, cell differentiation, morphogenesis, tissue homeostasis, and regeneration. In developing cancers, TGFβ functions as a tumor suppressor in early steps of the disease. In later stages, TGFβ lose the growth inhibitory functions and may transform to an oncogenic protein that is recognized as an initial inducer of EMT (Hapke and Haake 2020). **TGF-β** increases cancer invasion and metastasis by inducing EMT. Under hypoxic conditions, HIF-1α expression in renal cell carcinoma increases TGF-β expression, and HIF-TGF-β interaction activates the EMT pathway (Mallikarjuna et al. 2019).

Notch signaling displays different functions in processes such as cell development and differentiation, cell proliferation, and cell death (Hori et al. 2013). Deregulation of Notch pathway is associated with different types of cancers, with either oncogenic or tumor suppressor properties in a context-dependent manner (Moon et al. 2021). Upon **Notch** receptors binding to its ligand, the activated Notch intracellular domain (ICD) is fragmented and translocates to the nucleus, where it transactivates a variety of target genes. In cancer cells, Notch1 activation was found to induce EMT (Zhang et al. 2017). Notch1's role in EMT is complicated, but mainly depends on its interactions with EMT transcription factors (TFs) SNAIL1 and SNAIL2, accompanied by TGFβ pathway activation (Sahlgren et al. 2008). Hypoxia signaling shares similarities with Notch signaling and suggests a functional

cooperation or interaction between the two of them. Hypoxia activates Notch signaling by alleviating Notch1 ICD and enhancing the expression of Notch ligand, Jagged2. Conversely, Notch ICD may function as a competitive inhibitor of FIH-1, which is likely to be responsible for the increase in HIF activity upon Notch activation. Hypoxia-induced Notch activation stimulates EMT in human cancer (Chen et al. 2010). In addition, hypoxic tumor cells entail Notch signaling for induction of EMT and implies that Notch is essential for hypoxia-induced EMT under different circumstances (Sahlgren et al. 2008). It stimulates HIF-1α-mediated proliferation, migration, and invasion via TLR4/MyD88 (Toll-like receptor 4/myeloid differentiation primary response 88) pathway in hypoxia-induced hepatocellular carcinoma (HCC). The TLR4/MyD88/NF-kB pathway participates the process by facilitating the proliferation, invasion, and migration of HCC cells, along with the stabilization of TLR4 by hypoxia-induced ubiquitin-specific peptidase 13 (USP13) (Gao et al. 2020). NOTCH leads to hypoxia-induced EMT with tumor invasion and migration via SLUG and SNAIL (Tian et al. 2015; Liu et al. 2018). NOTCH ligand Jagged2, urokinase-type plasminogen activator receptor (Dudonne et al. 2011), and cyclooxygenase-2 (COX-2), which is one of the hypoxia-induced biomolecules, contributes to the invasion and EMT of breast cancer cells (Liu et al. 2015).

NF-κB and **β-catenin** pathways mediate the hypoxia-induced EMT as well as the invasive and metastatic properties of the tumors in hepatocellular carcinoma cells (HCCs) (Guo et al. 2020). HIF-lα-induced activation of **histone lysine-specific demethylase 4B** (KDM4B, JMJD2B) is involved in epigenetic regulation and increase of invasion and metastasis in colorectal cancers (CRC) (Glaser et al. 2020). JMJD2B is also associated with breast cancer and lung metastasis (Luo et al. 2012). **Myc** is known to regulate the expression of several genes that control the variety of cellular processes, including cell cycle, cell growth, cell death, and differentiation (Dang 2013). In tumors, Myc is often constitutively expressed and promotes cell cycle progression; however, in adaptation to low levels of oxygen, Myc is negatively regulated by HIF-1α (Li et al. 2020).

uPAR is a GPI-anchored cell membrane receptor that mediates hypoxia-induced invasion (Nishi et al. 2016). uPAR is involved in invasion and metastasis as a member of the protease system. Hypoxia promotes cell invasion and EMT by upregulating uPAR expression and activating AKT and RAC1 downstream signaling pathways. uPAR is activated upon binding to its ligand urokinase plasminogen activator (uPA), thereby providing plasminogen activity. Plasminogen activator inhibitor-1 (PAI-1), one of the well-characterized endogenous inhibitors of uPA urokinase, also supports angiogenesis and tumor metastasis (Mahmood et al. 2018; Peterle et al. 2018). A 67-kDa laminin receptor (67LR)-mediated increase in uPA, MMP-9 (matrix metalloproteinase-9), and TIMP-1 (tissue inhibitor of matrix metalloproteinase protein) expressions in hypoxic conditions induces gastric cancer metastasis (Liu et al. 2010). Another receptor that plays a role in invasion and metastasis via HIF-1α is the RON tyrosine kinase receptor that affects the invasion and migration of tumor cells in the ECM and blood vessels (Kato et al. 2021). A different signal player controlled by hypoxia through a regulation of HIF1 or HIF2 is the expression level of IRS2. Studies on metastatic breast cancer cell lines have

shown that IRS2 plays a role in tumor survival and invasion in hypoxic conditions (Tsai and Wu 2012).

Ion channels and transporters are among those that play a significant role in the processes of proliferation, migration, invasion, and apoptosis of human tumor cells in hypoxia. **Chloride intracellular channel 1 (CLIC)** is an intracellular channel associated with p64 and participates in the invasion and metastasis of gastric cancer cells by regulating hypoxia and reoxygenation-induced intracellular ROS. **Sodium hydrogen antiporter 1 (NHE1)** is important in various biological processes of the cell, such as regulation of cell volume and intracellular pH. NHE1 expression level, responsible for regulating the pH, is found to be higher in gastric mucosa. In gastric cancers, NHE1 activation and upregulation under hypoxic conditions are implicated in tumor cell migration and invasion (Chen et al. 2021).

6.3.3 Transcription Factors of Hypoxia-Induced EMT

Hypoxia-induced EMT promotes the invasive and metastatic potential of cancer cells in particular by decreasing the epithelial-associated gene expression and increasing the mesenchymal-associated gene expression (Muz et al. 2015). Hence, HIF-1α plays a significant role in hypoxia-mediated promotion of metastatic potential by activating EMT-associated transcription factors (TF), including **SNAIL1**, **SNAIL2** (SLUG)**, TWIST1, E12/E47 and zinc finger E-box binding homeobox 1 (ZEB1),** and **2 (ZEB2)** (Peng et al. 2021). Among those, SNAIL1, SLUG, TWIST1, and ZEB1 contain HRE sequences in their promoters, suggesting a direct interaction with HIF-1α.

SNAIL1 is an indispensable factor for development and is involved in mesoderm formation. SNAIL1 upregulates the expression of crucial mesenchymal factors such as fibronectin (FN1) and matrix metalloproteinase 9 and downregulates the E-cadherin expression, thereby inducing EMT. SNAIL1 expression was found to correlate with HIF-1α expression and increased the invasiveness of liver and ovarian cancer cells (Hapke and Haake 2020).SNAIL1 and SLUG's joint action repressing the transcription of E-cadherin contributes to the induction of EMT. Expression of SLUG was found to be correlated with HIF-1α expression in head and neck squamous carcinoma, lung, and pancreatic cancer cells (Tam et al. 2020). TWIST1 is a basic helix–loop–helix transcription factor and also plays a crucial role in metastases. Previously, it was shown that hypoxia-induced EMT is associated with increased expressions of HIF-1α and Twist1. HIF-1α-induced increased expression of TWIST1 promotes invasion and metastasis in various types of cancers, including stomach, pancreas, breast, non-small-cell lung, nasopharyngeal, prostate, and uterine cancers under hypoxic conditions (Yang and Wu 2008). HIF-lα-induced TWIST expression has been shown to be responsible for promoting EMT in thyroid cancer cells as well (Yang et al. 2015)**. ZEB1** is another transcription factor that is known to support tumor invasion and metastasis by promoting EMT in cancer cells. HIF-1α and ZEB1 are both accepted as tumor-initiating factors, but studies also reported that ZEB1 is a downstream target of HIF-1α and upregulated in various cancers such as

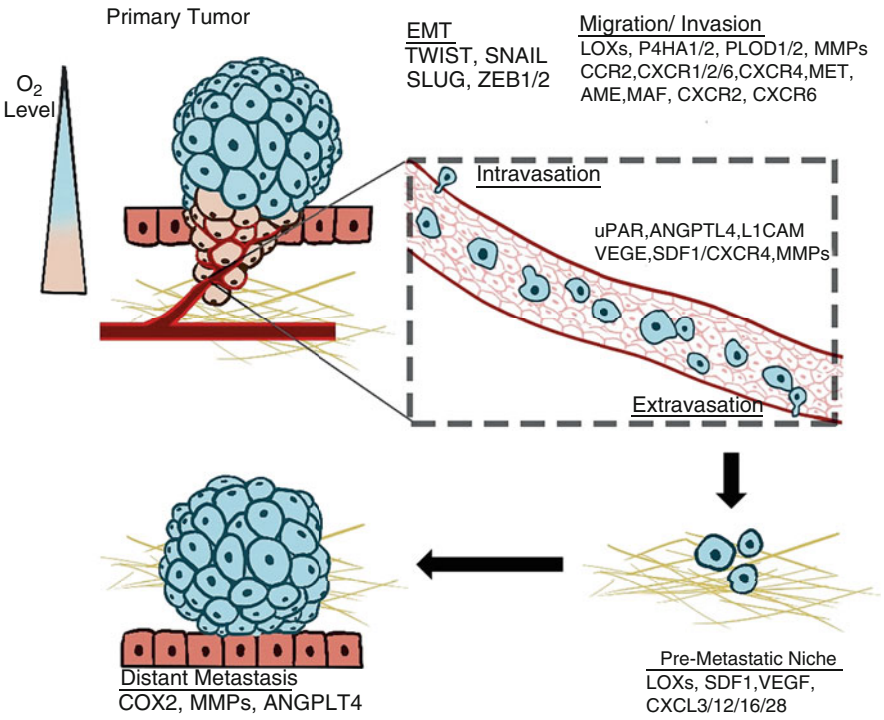

Fig. 6.1 Hypoxia controls multiple steps in cancer metastasis. Hypoxia-induced stages of tumor metastasis and associated metastatic players including epithelial–mesenchymal transition (EMT), invasion, intravasation, circulation, extravasation, pre-metastatic niche, and distant metastasis are shown

colorectal (Zhang et al. 2015), bladder cancer (Zhu et al. 2018), or ameloblastic carcinoma (AC) (Yoshimoto et al. 2019). In AC cells, in hypoxia ZEB1 and HIF-1α persuades the induction of TGF-β, which results in increased EMT (Yoshimoto et al. 2019). Inhibitor of differentiation/DNA binding 2 (ID2) is another factor regulated by HIF-lα, which causes progression of neuroblastoma cells (Lofstedt et al. 2004) (Fig. 6.1).

Zinc finger E-box binding homeobox 2 (ZEB2) is a transcriptional regulator that downregulates E-cadherin and other epithelial genes. HIF-1α and transforming growth factor ß (TGFß) regulate EMT-associated ZEB2 expression in tumors, and overexpression of ZEB2 promotes cancer metastasis (Fardi et al. 2019). In renal cell carcinoma (RCC), HIF-1α activates ZEB2 transcription that binds to the promoter of the E-cadherin-encoding gene and activated ZEB2 inhibits E-cadherin transcription, thereby activating EMT in cancer (Krishnamachary et al. 2006).

6.3.4 Hypoxia-Regulated Enzymes in Cancer Invasion and Metastasis

Lysyl Oxidase (LOX) is an HIF1α-dependent and hypoxia-induced extracellular matrix (ECM) remodeler protein secreted by tumor cells and creates a "pre-metastatic niche" in hypoxic tumors (Chan and Giaccia 2007). Breast cancer cells often secrete LOX enzyme into the circulation. After localization of LOX in the metastatic tissue in bone marrow-derived cells, a premetastatic niche is formed as a result of the collagen cross-linking of LOX enzymes in metastatic tissues (Liu et al. 2015). Hypoxia-induced carbonic anhydrase IX (**CAIX)** is an enzyme commonly found in tumors and activated in acidic conditions. CAIX promotes the invasion and metastasis ability of tumor cells by disrupting the basal membrane structure. In addition, CAIX inhibition has been shown to reduce tumor metastasis in breast tumors (Ward et al. 2015). **Hypoxia-induced migration inhibitor factor** (MIF) is another enzyme involved in COX-2 and PGE2 upregulation, in angiogenesis and tumor proliferation, as well as invasion and metastasis (Conroy et al. 2010). Hypoxia-induced **Supervillin** also promotes metastasis and invasion by activation of RhoA/ROCK and ERK/p38 signaling pathway. In addition, hypoxia-induced supervillin accelerates HCC metastasis by regulating the expression of EMT genes. The increase in hypoxia-induced supervillin expression causes an increase in HCC metastasis, leading to poor survival in patients (Chen et al. 2018). **Glutaminase 1** (GLS1) is known to be an enzyme that ensures the rapid proliferation of cancer cells. In hypoxic conditions, HIF1α increases mRNA and protein expression of GLS1. Hypoxia-induced GLS1 expression in colorectal cancer cells causes tumor growth, invasion, and metastasis (Xiang et al. 2019). **Growth and differentiation factor 15** (GDF15) is a member of the TGF-β superfamily. Circulating levels of GDF15 have been reported to be elevated in a variety of cancers, including pancreatic, colorectal, endometrial, and prostate cancers. Hypoxia-mediated ER stress and PERK-eIF2a activation in CRC cells promotes metastasis by regulating GDF15 expression, which is thought to be involved in EMT (Zheng et al. 2020). Increased HIF1α expression in breast cancers causes an increased risk of metastasis and cancer-related deaths. Recently, the contribution of HIF1α-induced disintegrin and metalloproteinase-12 (**ADAM12)** in increasing breast cancer metastasis was demonstrated. Activation of HIF1α-dependent ADAM12-mediated EGFR-FAK signaling leads to cell migration, invasion, and distant metastasis. HIF-1α-dependent ADAM12 signaling induces cell motility and invasion through the extracellular matrix under hypoxic conditions (Wang et al. 2021)**.**

It has been also shown that there is a significant correlation between HIF-1α and matrix metalloproteinases (**MMPs)** in various cancers. In ovarian cancers, a correlation between HIF-1α **MMP13** expression in metastatic lesions has been reported. In addition, the expression of MMP13 was found to be significantly higher in in A2780 ovarian cancer cells in hypoxic conditions than in the normoxic conditions. Of note, suppression of HIF-1α expression by siRNA suppresses the expression of MMP13 despite the hypoxia suggesting MMP13 as a HIF-1 α-dependent enzyme. Thus, HIF-1α is an important factor affecting the invasion and metastasis of ovarian cancer

by modulating MMP13 expression under hypoxic conditions (Zhang et al. 2019). Other studies have also reported that HIF-1α upregulates the expression of MMP-2 and MMP-9 (Liu et al. 2015).

COX-2 has been also associated with cancer invasion and distant metastasis. COX-2 participates in EMT regulation together with HIF-1α and plays an important role in metastasis. HIF-1α-mediated COX2 increases proliferation and metastasis of ovarian cancer (Ding et al. 2021)**.** HIF-1α increases COX-2 expression and promotes EMT in hepatocellular tumors, resulting in enhanced invasion of HepG2 cells under hypoxic conditions (Huang et al. 2016). Expressions of pro-collagen prolyl (P4HA1, P4HA2) and lysyl (PLOD1 and PLOD2) are known to be increased in breast cancer metastases. HIF-1α was found to be responsible for the increased expression levels of **PLOD1**, **PLOD2, P4HA1,** and **P4HA2** in breast cancer cells (Liu et al. 2015).

6.3.5 Hypoxia-Regulated Chemokines in Cancer Invasion and Metastasis

Chemokines are divided into four groups depending on the positioning and the number of the first two conserved cysteine motif: C, CC, CXC, and CX3C61. Hypoxia-regulated chemokines are associated with metastasis and cancer progression (Semenza 2016). Hypoxic condition in ovarian cancer was shown to induce the expression of the chemokine ligands **(CCL2/CXCL12)** and receptors **(CCR2/CXCR1-2-4)**. In prostate cancer cells, hypoxia mediates the expression of **CX3CR1** and **CXCR6**, which are involved in invasion and migration61. CX3CR1 expression is regulated by HIF-1α in primary and metastatic ovarian cancer cells. High levels of CX3CR1 expression responsive to CX3CL1 (Fractalkin/chemokine ligand 1) lead to progression and metastasis of ovarian cancer63. Chronic hypoxia affects the expression of **CXCL12**; however, the level of expression is different among various cancer types (Wong and Tran 2020; Fujikuni et al. 2014; Gilkes 2016; Xin et al. 2016; Theys et al. 2011; Chang et al. 2021). It has been reported that in hypoxia the expression of CXCL12 is decreased in melanoma and ileal carcinoids but increased in cervical cancer cells and is not changed in breast cancer69. Furthermore, chronic hypoxia increases the expression of **CXCR4** via HIF-1α in multiple myelomas. CXCR4 stimulates the migration of myeloma cancer cells via the CXCL12→CXCR4 pathway and allows them to metastasize by colonization in the bone marrow. Upregulation of CXCL12, primarily controlled by HIF-2 and partly by HIF-1, causes angiogenesis and osteoclastic bone resorption in the bone marrow. Increase in **CCR1** expression, together with the hypoxia-mediated increase in HIF-2, results in the emergence of multiple myeloma cells (Hao and Li 2020). **CCL3** (CC-motif chemokine ligand 3), a CCR1 ligand, reduces the response of multiple myeloma to CXCL12 and consequently leads to the migration of these cells from bone marrow. Increased expression of CXCR4 in chronic hypoxia promotes proliferation, invasion, and migration of cancer cells (Chang et al. 2021). Hypoxia is one of the mechanisms responsible for the high metastatic potential of diffuse-type gastric cancer to the peritoneum by controlling upregulation of CXCR4 via the

CXCL12/CXCR4 axis71. CXCR4, a chemokine receptor regulated by HIF, directs metastasis in breast cancer through binding to stromal-derived factor-1 (SDF-1α) in primary and metastatic tumor cells14. Increased expression of CXCL12 mediated by HIF signaling not only stimulates bone metastasis in osteoblast-lineage cells, but also stimulates breast cancer growth and spreads to other secondary organs72. Another chemokine ligand controlled by hypoxia and HIF-1 is **CXCL16**. Regulation of CXCL16 expression in chronic hypoxia is tightly controlled in tumor metastasis. Increased expression of CXCL16 in breast cancer cells in chronic hypoxia affects the migration of cancer cells by increasing the expression level of **CXCR6** due to HIF-1α. Various studies have shown that upregulation of CXCR6 increases migration in renal cell carcinoma, prostate cancer, and breast cancer. In addition, expression level of CXCR6 is found to be higher in metastatic tumors, including cervical cancer, Ewing's sarcoma, nasopharyngeal carcinoma, ovarian carcinoma, melanomas, and papillary thyroid cancer in comparison to human primary tumors (Nath et al. 2013) (Fig. 6.1).

6.3.6 Hypoxia-Regulated Adhesion Molecules in Cancer Invasion and Metastasis

Changes in the expression levels of various membrane proteins through hypoxia or HIF play major roles in cancer metastasis. In hypoxic breast cancer cells, HIF1-dependent upregulation of **L1 cell adhesion molecule (L1CAM/CD171)** and **angiopoietin-like 4 (ANGPTL4)** lead to vascularization-mediated metastasis of cells to the lungs. ANGPTL4 stimulates extravasation of breast cancer cells by binding to receptors on endothelial cells (EC) and thereby inhibits EC–EC interactions. In vivo studies have shown that inhibition of L1CAM, which is responsible for binding to vascular endothelial cells, prevents lung metastasis in breast cancer (Semenza 2016). ANGPTL4 and L1CAM adhesion molecules modulate intra- and extravasation (Wigerup et al. 2016). Expression levels of various integrin proteins are increased under hypoxic conditions in an HIF-dependent manner as in increased expression of α5β1-integrin, related to lymph nodes and lung metastasis of breast cancer cells by HIF-1 and HIF-2 (Yousefi et al. 2021). Cluster of differentiation membrane proteins **CD151**, **CD24,** and **CD147** are also involved in hypoxia-mediated cancer metastasis (Tsai and Wu 2012). Hypoxic conditions were shown to regulate cell adhesion molecules and metastasis via regulation of CD151 expression. In hypoxia, CD151 expression was downregulated in colorectal cells by HIF-1α activity (Wong and Tran 2020). The studies investigating P-selectin ligand CD24 showed that shRNA-mediated suppression of CD24 reduction in metastasis of breast cancer cells. CD24 also functions as the L1CAM interacting protein, and its overexpression in breast cancer has a potential role in HIF-1 regulation (Fujikuni et al. 2014; Gilkes 2016). **CD147/EMMPRIN**, a member of immunoglobulin family, mediates the secretion of MMP from cancer cells, endometrial cells, and fibroblasts, causing degradation of ECM and basement

membrane and promoting tumor proliferation, invasion, and metastasis (Xin et al. 2016).

E-cadherin is an important transmembrane protein that ensures epithelial cell–cell adhesion. E-cadherin loss has been found to play a major role in metastasis and invasion in renal cancers (Theys et al. 2011). In hepatocellular cancer cells (HCC) cells, HIF-1α binds to the SNAL1 promoter-activating EMT and decreasing expression of E cadherin, increasing expression of N-cadherin and vimentin, which results in instigation of metastasis (Guo et al. 2020). Overexpression of **desmoglein2** (DSG2), a desmosome-mediated intercellular adhesion molecule, has been found to promote tumor growth and promote metastasis. Hypoxia-mediated downregulation of DSG2 leads to an increase in the expression of epithelial–mesenchymal transition (EMT) genes that further encourage cells of the primary tumor to undergo intravasation (Chang et al. 2021)**. EFNA1** (Ephrin A1), a membrane protein anchored to the cell surface by GPI linkage expression, is induced by hypoxia through HIF-1α and HIF-2α, triggering HIF-dependent angiogenesis in tumors. EFNA1 also contributes to the regulation of HIF-2α by binding to the HRE on the HIF-2α promoter and thereby upregulating its expression level (Hao and Li 2020).

Hypoxia-mediated increased **galectin-3** expression also plays a role in breast cancer progression and metastasis. Overexpression of galectin-3 has been reported in hypoxic regions of primary tumors that is associated with metastasis and further plays a role in increasing tumor aggressiveness in vivo (de Oliveira et al. 2015). **MUC1** is an O-glycoprotein membrane-bound mucin that is upregulated directly by HIF-1α in hypoxic conditions and was shown to be responsible for initiating metastasis and angiogenesis. *MUC1* gene overexpression in hypoxia is associated with metastasis and poor prognosis in patients with pancreatic, colon, and breast cancer (Khodabakhsh et al. 2021; Nath et al. 2013; Thompson et al. 2006).

Semaphorins in transmembrane and GPI-linked glycoproteins (Sema3A, Sema3B, Sema3C, Sema3E, Sema3F, Sema4B, and Sema4D proteins) are involved in tumor growth, metastasis, and vascularity in relation to hypoxia. Investigations on prostate cancer delineated the importance of Sema3 family proteins *Sema3A, B, C, D, E,* and *F* genes comprise HRE sequences at their promoters. While Sema3C upregulation induces metastasis downregulation of Sema3A and 3E inhibits metastatic spread, suggesting that Sema3 family proteins play important roles in the progression of prostate cancer in hypoxia. A recent study showing that hypoxia induces Sema4B overexpression and inhibition of Sema4B causes increased invasion in lung carcinoma suggests that Sema4B is also important in suppression of growth and metastasis of NSCLC. Inhibited *Sema4* gene leads to increased invasion in lung carcinoma, whereas induction of Sema 4B by hypoxia causes overexpression and thus suppresses invasion in cancer cells. Sema4D has been shown to play a role in tumor growth and vascularization in an HIF-dependent manner in head and neck squamous cancer and epithelial ovarian cancer (Valentini et al. 2021; Lontos et al. 2018). HIF-1α-mediated expression of Sema4D under hypoxic conditions inhibits osteoblast differentiation in osteolytic bone metastasis of lung cancer, along with ADAM17 (metalloproteinase 17) upregulation (Chen et al. 2019a). **Caveolin-1**

(CAV-1), a scaffold protein, is known to exert both metastasis-promoting and tumor-suppressing activities (Sanhueza et al. 2020). Hypoxia promotes invasion and metastasis by inducing HIF-1α-mediated CAV1 expression in metastatic cancer cells (Castillo Bennett et al. 2018). In hepatocellular carcinoma, hypoxia-induced CAV1 mediates tumorigenesis and metastasis (Chen et al. 2019b). The increased expression of CAV1 calcium-binding protein supports S100P-mediated metastasis (Mao et al. 2016).

NEDD9 (neural precursor cell expressed developmentally downregulated protein 9) and HEF1 is overexpressed in gastric cancer under hypoxic conditions. It regulates cell migration and metastasis depending on MICAL1 (microtubule-associated monooxygenase, calponin, and LIM domain containing 1) and RAC1 (Zhao et al. 2019). NEDD9 is also overexpressed in hypoxic regions of human colorectal cancers. NEDD9 is among the targets of HIF-1α downstream genes and causes cancer cell migration together with SOX2(Wang et al. 2019) (Fig. 6.1).

6.3.7 Hypoxia-Regulated Other Players Associated with Cancer Metastasis

Hypoxia and HIFs-regulated secondary signal players involved in cancer metastasis comprise small GTPases, chemokines, kinases, cell membrane receptors, ion channels, and trasporters (Tsai and Wu 2012). **Ras-related C3 botulinum toxin substrate 1 (RAC1)** and **cell division control protein 42 homolog (CDC42)**, plasma membrane-associated small GTPases promote tumor migration, metastasis, and progression in hypoxic conditions (Yang and Wu 2008). Loss of RAC1 has been shown to reduce HIF1α expression (Maldonado and Dharmawardhane 2018; Tatrai et al. 2017). Transcription levels of another small GTPase **RhoA** and its main upstream effector serine/threonine kinase Rho-associated helix-helix kinase (ROCK) are increased in breast cancer by hypoxia-inducible factor in the hypoxic microenvironment (Wei et al. 2016). Both RHOA and ROCK1 are associated with migration, invasion, and microvesicle formation in breast cancer cells. RHOA and ROCK1 mRNA levels were found to be increased in the metastatic breast cancer cell lines compared to the nonmetastatic cell lines. In addition, elevated RhoA and ROCK 1 protein levels were observed in metastatic biopsy specimens and connected with breast cancer progression. **RIOK3 (RIO3 kinase)** is a serine/threonine kinase regulated in an HIF-1α-dependent manner and promotes cancer cell migration, invasion, and metastasis by rearranging the actin cytoskeleton within breast cancer cells (Wigerup et al. 2016). Functional isoform 1 of the **FAM13A (Sequence Similarity Family 13 Member A)** protein that contains Rho GTPase-activating protein (GAP) domain is implicated in non-small-cell lung cancer (NSCLC) proliferation, migration, invasion, and apoptosis under hypoxic conditions. Knockdown of FAM13A in NSCLC cells negatively regulates proliferation, migration, and invasion by altering actin cytoskeleton under both normoxic and hypoxic conditions (Ziolkowska-Suchanek et al. 2021). Besides hypoxia-mediated induction of

metastasis, there is also an HIF-dependent control of metastasis that is interceded by various proteins such as HACE1, Parkin, p53, and GLS2.

HACE1 (Ankyrin repeat-containing E3 ubiquitin-protein ligase and HECT domain) acts as a tumor suppressor and decreases HIF1α accumulation by mediating degradation of RAC1 GTPase (Turgu et al. 2021). **Parkin**, the E3 ubiquitin ligase, plays a role in the inhibition of metastasis by directly binding and thereby leading to the degradation of HIF-1α in breast cancer (Liu et al. 2017). **p53** plays a significant role in suppressing cancer metastasis and invasion (Zhang et al. 2020). As a consequence of hypoxia and ROS, the mitochondrial glutaminase GLS2 enzyme is activated in a p53-dependent manner. Regardless of its enzymatic activity, GLS2 inhibits RAC1 by directly binding to the molecule and thereby leads to inhibition of metastasis (Zhang et al. 2021b). Molecular hallmarks of hypoxia and HIFs-induced tumor metastasis are shown in Fig. 6.2.

6.4 Conclusion

Adaptation of cancer cells to hypoxia involves the understanding of a number of biological processes, namely the transition to anaerobic energy production through changes in gene expressions. In hypoxia, biological processes are readjusted and cancer cells require an adaptation to survive. Hypoxia and HIFs contribute to the metastatic process in different levels. In particular, hypoxia-induced EMT plays a key role in cancer metastasis and entails diverse signaling pathways and transcriptional factors. Additionally, hypoxia-induced enzymes, small GTPases, chemokines, kinases, cell membrane receptors, signal mediators, ion channels, and transporters are implicated in metastasis. Currently, hypoxia-associated metastatic players and their individual relationship to the various stages of metastasis are known. Further investigations are needed for a better understanding of the interrelationships of these factors in order to identify their potential as a therapeutic target and develop new treatment strategies.

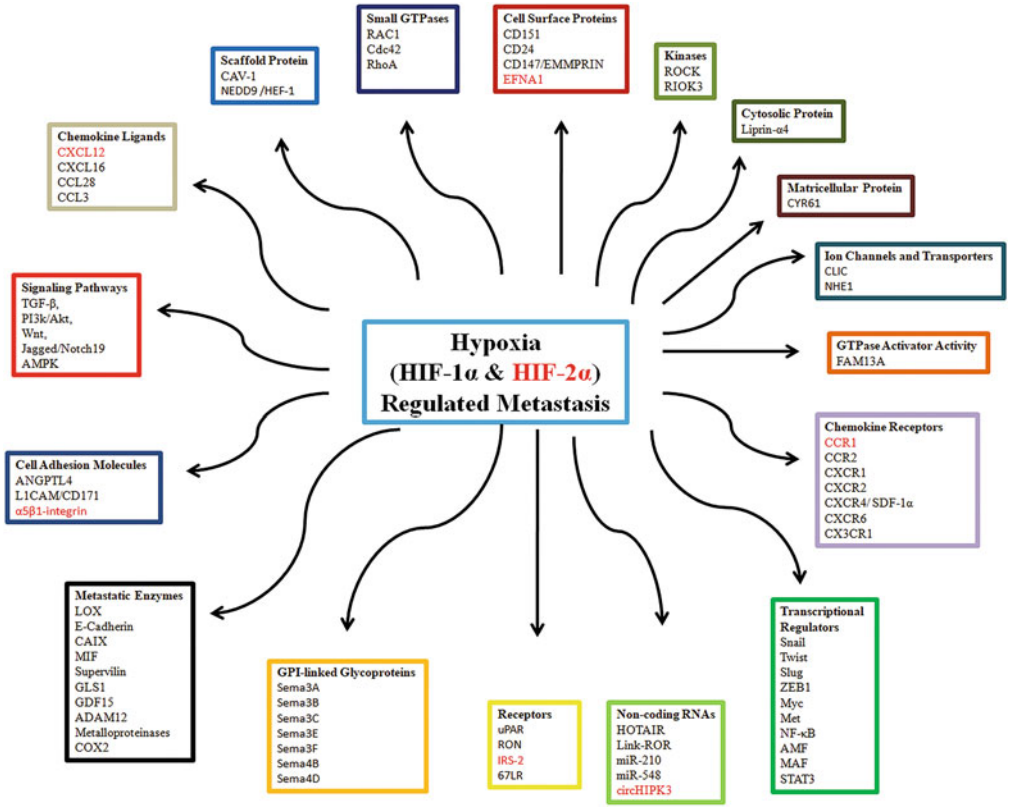

Fig. 6.2 Molecular hallmarks of hypoxia and HIFs-induced tumor metastasis. Hypoxia and HIFs regulate metastasis including transcriptional regulators, signaling pathways, metastatic enzymes, noncoding RNAs, etc.

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