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Hypoxia and free radicals: role in tumor progression and the use of engineering-based platforms to address these relationships

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Abstract

Hypoxia is a feature of all solid tumors, contributing to tumor progression and therapy resistance. Through stabilization of the hypoxia-inducible factor 1 alpha (HIF-1α), hypoxia activates the transcription of a number of genes which sustain tumor progression. Since the seminal discovery of HIF-1α as a hypoxia-responsive master regulator of numerous genes and transcription factors, several groups have reported a novel mechanism whereby hypoxia mediates stabilization HIF-1α. This process occurs as a result of hypoxia generated reactive oxygen species (ROS), which in turn, stabilize the expression of HIF-1α. As a result, a number of genes regulating tumor growth are expressed, fueling ongoing tumor progression. In this review, we outline a role for hypoxia in generating ROS and additionally define the mechanisms contributing to ROS-induced stabilization of HIF-1α.We further explore how ROS-induced HIF-1α stabilization contribute to tumor growth, angiogenesis, metastasis and therapy response. We discuss a future outlook, describing novel therapeutic approaches for attenuating ROS production while considering how these strategies should be carefully selected when combining with chemotherapeutic agents. As engineering-based approaches have been more frequently utilized to address biological questions, we discuss opportunities whereby engineering techniques may be employed to better understand the physical and biochemical factors controlling ROS expression. It is anticipated that an improved understanding of the mechanisms responsible for the hypoxia/ROS/HIF-1α axis in tumor progression will yield the development of better targeted therapies.

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Introduction

Energy is indispensable for life and is produced as ATP in the mitochondria, a process referred to as oxidative phosphorylation. During metabolism, fuels such as glucose, are broken down into pyruvate which pass into the mitochondrial matrix. Here, pyruvate is further metabolized and oxidized to carbon dioxide and high energy electrons in the form of the electron carriers NADH and FADH2. These electrons enter the electron transport chain in the inner mitochondrial membrane, which is comprised of 5 (I–V) respiratory complexes. Here, electrons are transferred between complexes, generating a proton gradient across the inner mitochondrial membrane. The energy from this electrochemical gradient is used to make ATP from ADP and inorganic phosphate. At the end, oxygen serves as the final electron acceptor, which is subsequently reduced to water. In cases where oxygen is incompletely reduced, reactive oxygen species (ROS) are formed.

ROS is estimated to occur in 1–2% of the oxygen utilized by mitochondria during oxidative phosphorylation and is therefore considered to be a normal byproduct of energy metabolism [1]. Some common forms of ROS produced during oxidative phosphorylation include superoxide anion ($O_2^{\cdot -}$), which itself acts as a ROS or assists in the formation of other ROS such as hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\cdot -}$). Together, these ROS act as oxidants, stealing electrons from other macromolecules such as nucleic acids, proteins and lipids. Under homeostatic conditions, cells are well equipped to respond to elevated levels of ROS by activating apoptotic pathways if the level of DNA damage is high [2]. Other mechanisms used by cells to inactivate ROS are endogenous scavenger enzymes (e.g. antioxidants) which convert the ROS into non-toxic products [2]. Increasingly high ROS levels overwhelm the cell's ability to adequately respond. As such, uncontrolled production of ROS poses a significant threat to nucleic acids, proteins, and lipids and as a result, can lead to a favorable scenario in which oncogenic transformation takes place. As a result, it's not surprising that ROS levels are greater in tumor cells as opposed to non-tumor cells [3]. In particular, regions of low oxygen tension, referred to as hypoxia, have been reported to elevate ROS levels associated with tumorigenesis. Given the implications of hypoxia-induced ROS on tumorigenesis, the goal of this review is to elucidate the mechanisms wherein hypoxia induces ROS formation and to further examine how this pathway activates downstream hypoxia targets to promote tumor growth, angiogenesis, metastasis and therapy responses. We will end by discussing novel engineering-based approaches, which may be utilized to address hypoxia effects on ROS production and tumorigenesis in a platform that better recapitulates the *in-vivo* environment. Overall, a better understanding of the mechanisms responsible for ROS directed tumorigenesis is anticipated to yield the development of novel chemotherapeutic agents designed at interrupting this process.

ROS and Tumorigenesis

Several groups have pointed to a role for ROS in tumor progression. For instance, ROS generation was shown to be necessary for anchorage independent growth of tumors in Kras transgenic mice, a process dependent on the presence of mitochondria [4]. In addition, it was reported that constitutive expression of Rac1, a small GTPase and member of the Rho family, in transgenic mice, promoted the development of Kaposi Sarcoma-like tumors, a process in part dependent on ROS-mediated cell proliferation [5]. With regard to metastasis, Ishikawa et al [6] isolated mitochondria from a mouse tumor cell line that was highly metastatic and transplanted it into a mouse cell line that was poorly metastatic. The authors demonstrated that the mitochondrial DNA from the metastatic tumor cell line induced metastasis and ROS formation from an otherwise non-metastatic cell line, providing a direct link between mitochondrial dysfunction and ROS production to tumor aggressiveness [6].

Elucidating the origins of ROS in tumors has thus been the subject of extensive research. Although elevated ROS may result from a number of factors including mitochondrial mutations, incomplete reduction of oxygen during respiration, chemical or biological compounds and poisoning or irradiation [7–9], numerous groups have recently identified a role for hypoxia in ROS production. Moreover, these groups have further demonstrated a role for ROS in stabilization of the hypoxia-responsive subunit, hypoxia inducible factor 1 alpha (HIF-1α), work which is discussed in greater detail below.

Hypoxia and HIF Activation

Hypoxia participates in the transcriptional activation of a number of genes, many of which play a prominent role in growth, development, homeostasis and tumorigenesis. Hypoxia primarily accomplishes these divergent roles through hypoxia inducible factor 1 (HIF-1), a heterodimeric transcription factor which is comprised of the oxygen responsive HIF-1α subunit and the constitutively active HIF-1β subunit [10]. In the presence of oxygen, HIF-1α is hydroxylated at several prolyl residues by prolyl 4 hydroxylase domain proteins (PHDs) which allow binding of von Hippel-Lindau proteins (pVHLs) [11–13]. VHLs, in turn, recruit E3 ubiquitin ligase which subsequently targets HIF-1α for proteasomal degradation [14, 15]. In the presence of low oxygen, however, HIF-1α escapes the above degradation mechanism, translocates to the nucleus and dimerizes with HIF -1β [10, 16, 17]. Here, this complex binds to hypoxia response elements in the promoter regions of several target genes, recruiting co-activator proteins and activating gene expression [10, 18]. Many of these targets are transcription factors which have been linked to tumor progression, metastasis and chemotherapy resistance [19].

Given the established roles of ROS, hypoxia and HIF-1α in tumorigenesis, this review will explore the interconnectedness of the local tumor environment (e.g. hypoxia) and intracellular redox dysfunction and HIF-1α stabilization on tumor progression, angiogenesis, metastasis and therapy responses. It’s important to note that in addition to HIF-1α, ROS-induced HIF-2α stabilization has also been reported. Although an important mechanism potentially regulating tumorigenesis, this review will primarily focus on ROS-regulated HIF-1α pathways in tumor progression given the wide body of available literature on this

topic. This review will further explore how engineering-based strategies may be utilized as novel cell culture platforms to address hypoxia-ROS-HIF-1α pathways in the context of tumorigenesis.

Hypoxia-Induced ROS

The cellular response to hypoxia is an adaptive mechanism wherein cells activate numerous genes which enable them to survive in an otherwise inhospitable climate. While PHDs have been regarded as the primary oxygen sensor mediating cellular responses to hypoxia, mitochondria have also been reported to respond to low oxygen tension, generating ROS which then activates intracellular pathways which govern the expression of several pro-survival genes. One of the earliest reports of hypoxia-induced ROS expression came from the work performed by Chandel et al [20] who reported that Hep3B hepatoma cells produced increased levels of ROS when challenged with hypoxia (1.5% O_2). When the cells were depleted of mitochondrial DNA, they failed to produce ROS following treatment with hypoxia, an observation the authors attribute to a role for an intact mitochondria in hypoxia-stimulated ROS production [20]. Interestingly, the authors reported decreased expression of HIF-1 target genes in mitochondria depleted cells subjected to hypoxia, pointing to a potential role for ROS in the stabilization of HIF-1α [20]. In a later study, hypoxia was shown to up-regulate the expression of ROS in rat aortic smooth muscle cells, a phenomenon which was attenuated through administration of antioxidants [21]. The authors further demonstrated that rotenone-mediated inhibition of cellular mitochondrial respiration resulted in reduced levels of ROS, supporting the work by Chandel et al [20] that functional mitochondria are needed in order for cells to respond to hypoxic insult [21]. Similar work conducted by Lluis et al [22] documented an increase in ROS expression from HepG2 rat hepatocytes exposed to 5% O_2, observations which were diminished via disruption of mitochondrial complexes I and II. Interestingly, the authors demonstrated that specific inhibition of mitochondrial glutathione (GSH), a protein which plays a protective role against oxidant-induced cell death, resulted in cellular death following hypoxia generated ROS [22]. Results from this work not only substantiated the findings by others, but elucidated a mechanism wherein cell responses to hypoxia-induced ROS are guarded.

In addition to the above studies linking hypoxia to ROS production *in-vitro*, analogous findings for hypoxia-induced ROS have been reported in animal models. For instance, Peng et al [23] demonstrated up-regulated levels of ROS in mice treated with chronic intermittent hypoxia, a phenomenon which was reversed when animals were given antioxidants against $O_2^{\cdot-}$. In an effort to understand the molecular mechanisms driving neurocognitive impairments associated with obstructive sleep apnea, Xu et al [24] discovered that, upon exposure to chronic intermittent hypoxia, mice exhibited a markedly higher expression of ROS in cortical neurons. The increase in ROS expression resulted in some cortical apoptosis, which was attenuated when animals were given the antioxidant Cu, Zn-superoxide dismutase [24]. Similar work showed that ROS levels were up-regulated in carotid bodies, a collection of oxygen-sensitive chemoreceptors in the internal carotid artery, of neonatal rats treated with chronic intermittent hypoxia [25]. Subsequent administration of antioxidants was shown to reverse the increased ROS levels observed in the animals [25]. Overall, these studies point to a role for hypoxia in ROS generation and have established an

important framework from which others have delineated mechanisms whereby hypoxia-induced ROS regulate cell responses. In future work, it would be interesting to examine the influence of chronic versus intermittent hypoxia on ROS production as such studies, to the extent of our knowledge, have not yet been examined side by side.

Hypoxia-Generated ROS and its Role in Stabilization of HIF-α

Cells sense changes in low oxygen through an intricate series of intracellular events ultimately resulting in HIF-α stabilization and subsequent activation of gene expression. While several mechanisms likely play overlapping roles in HIF-stabilization, one mechanism receiving widespread interest is mitochondria generated ROS. In particular, mitochondrial complex III, has received extensive interest for playing a central role in the production of ROS in response to hypoxia. It's important to note that while other cellular sources including the NOX family of NADPH oxidases, produced at the plasma membrane, have been reported to stabilize HIF-α [26], mitochondrial-derived ROS will be the focus this report.

As a follow up study to a prior report documenting the role of mitochondria in hypoxia-induced ROS production, Chandel et al [27] reported that hypoxia-generated ROS production in Hep3B cells resulted in accumulation of HIF-1α. The role of mitochondria-generated ROS in HIF-1α stabilization was validated as cells depleted of mitochondria were incapable of stabilizing HIF-1α in the presence of both hypoxic and normoxic stimuli [27]. The authors subsequently confirmed that mitochondrial complex III was the primary oxygen sensor and is thus, the primary driver for ROS expression during hypoxia [27]. In a similar study, Guzy et al [28] demonstrated the indispensable role for mitochondria-generated ROS in both HIF-1α and HIF-2α stabilization. Here, the authors utilized RNAi to disrupt mitochondrial function, demonstrating that Rieske iron sulfur protein (RISP), a catalytic subunit of mitochondrial complex III, was necessary for hypoxia-mediated stabilization of HIF-1α and HIF-2α proteins [28]. These results point to the specific role of RISP in ROS production as the authors reported that exogenous administration of ROS in RISP RNAi treated cells restored HIF-1α and HIF-2α stabilization in HEK 293 and Hep3B cells [28]. These results were confirmed by Brunelle et al [29] who reported that treatment of HEK 293 cells with RNAi against RISP abolished ROS induced HIF-1α stabilization during hypoxia. Similarly, Bell et al [30] found that RNAi of RISP abolished ROS induced HIF-1α stabilization in cytochrome b deficient osteosarcoma cells cultured in the presence of hypoxia. These studies clearly point to a role for RISP in ROS mediated HIF-1α stabilization; however, the absence or presence of other respiratory subunits of mitochondrial complex III has been shown to participate in ROS production in response to hypoxia. For instance, ROS was increased in hypoxia-exposed 143B osteosarcoma cells lacking cytochrome b, which functions as an electron acceptor in the respiratory chain of complex III [30]. As expected, the increased ROS production from cytochrome b deficient cells resulted in HIF-1α accumulation [30]. In a separate study, it was reported that cytochrome c, which oxidizes cytochrome c1, keeping RISP oxidized and thus able to form superoxide radicals [20, 27], was essential for hypoxia-induced stabilization of HIF-1α and HIF-2α [31]. The absence of functional cytochrome c in murine embryonic cells resulted in decreased ROS production, which in turn, led to the degradation of HIF-1α and HIF-2α

[31]. The authors further report that reintroduction of cytochrome c into cytochrome c null cells restored hypoxia-induced ROS levels and HIF-α accumulation, providing evidence for the indispensable role of cytochrome c as an oxygen sensor [31]. Illustrating the important role of HIF-1α in hypoxia-induced ROS signaling in cells, Papandreou et al [32] demonstrated that murine embryonic fibroblasts lacking HIF-1α and pyruvate dehydrogenase kinase 1 (PDK 1) succumbed to cell death following treatment with hypoxia. These results are presumably due to excessive buildup of ROS, which is otherwise lethal in cells lacking adaptive mechanisms in the form of HIF-1α. Combined, these studies clearly define a role for intact, functional mitochondria, especially complex III in the inner mitochondrial membrane, as a critical factor mediating cellular adaptations to hypoxia via stabilization of HIF-α proteins. Subsequent sections will explore how hypoxia-generated ROS influences tumor growth, angiogenesis and metastasis through stabilization of HIF-1α (Figure 1). In addition, the role of hypoxia and ROS-induced stabilization of HIF-1α on tumor therapy response will be discussed.

Influence of the Hypoxia-ROS-HIF-1α Pathway on Tumorigenesis

Tumor Growth

Aberrant cellular activities leading to tumor initiation and growth have been attributed to hypoxia-induced oxidative stress. In an attempt to understand the role of hypoxia-induced ROS on tumor cell behavior, Lluis et al [33] characterized ROS and HIF-1α accumulation in several tumor cell lines following exposure to hypoxia. The authors reported that hypoxia generated ROS led to HIF-1α accumulation and expression of HIF-1α target genes including the pro-angiogenic transcription factor vascular endothelial growth factor (VEGF) and the pro-proliferative and pro-cell survival gene PDK1 [33, 34]. In addition, the authors also documented that mitochondrial ROS induced the expression of NFκB [33], a transcription factor which has been shown to play a prominent role in cell proliferation [35] and invasion and metastasis [36], and of which promoted survival of the tumor cells in the present study. Although the authors did not provide a direct link between HIF-1α and NFκB, rather demonstrating the involvement of cSrc in NFκB activation, it is possible that indirect mechanisms involving HIF-1α stabilization may have played a role in activation of the cSrc-NFκB pathway. Indeed, previous work has shown that VEGF, a downstream target of HIF-1α, activated cSrc [37]. Given that HIF-1α promoted the activation of VEGF in the report by Lluis et al [33], it is possible that the up-regulated expression of VEGF may have been responsible for activation of cSrc and thus, NFκB. More recent work, however, illustrates that HIF-1α accumulation in pulmonary artery smooth muscle cells is dependent on ROS-induced activation of NFκB rather than the reverse [26]. Regardless of the mechanism, it appears that ROS-induced HIF-1α and NFκB are intimately associated and may play an important role in cell behavior. In future work, it will be important to dissect the involvement of these interconnected pathways in oncogenic activities.

In addition to ROS-induced NFκB in tumor cell survival, another mechanism reported to play a role in hypoxia-ROS mediated cell survival is microRNA-210 (miR-210). miR-210 specifically targets the iron sulfur scaffolding protein ISCU, necessary for the proper assembly of iron complexes [38], which, in turn, regulate electron transport in the inner

mitochondrial membrane [39]. In cancer cell lines where ISCU expression was down-regulated as a result of miR-210 activity, ROS levels were reported to be increased in normoxic conditions and exacerbated by hypoxic conditions [39]. Additionally, the authors observed increased cell survival in miR-210-expressing breast MCF7 and colon HCT116 cancer cell lines following challenge with hypoxia but not normoxia and additionally found increased expression of HIF-1α and its target VEGF in xenograft glioblastoma tumors expressing higher levels of miR-210 [39]. Results from this study not only implicate that a dysfunctional mitochondrial electron transport chain combined with hypoxia aggravate ROS expression and elicit abnormal cell behavior, but potentially influence tumor growth through stabilization of HIF-1α.

In an effort to characterize tumor cell behavior following ROS stabilization of HIF-1α, Jing et al [40] assessed *in-vitro* colony formation and *in-vivo* tumor formation from lung epithelial cells treated with cadmium. The authors discovered increased colony formation, indicative of anchorage-independent growth and thus malignant capabilities, in cadmium treated lung epithelial cells and formation of larger tumors in animals transplanted with cadmium treated lung epithelial cells [40]. Metal ions, such as cobalt chloride are known to act as hypoxia mimetics [41–44]. In the above work, cadmium may have served as a hypoxia-mimetic agent as it was shown to exert its tumor-promoting effects via ROS-dependent activation of ERK/AKT which in turn, promoted the stabilization of HIF-1α [40]. In a similar report, tumor growth was also enhanced through hypoxia-induced oxidative stress. In this study, the authors discovered that thymidine phosphorylase activated haem oxygenase-1 (HO-1), a marker of oxidative stress which correlates with formation of ROS [45], in transitional bladder carcinoma cells [46]. Thymidine phosphorylase-induced HO-1 was shown to stabilize HIF-1α, a mechanism which was enhanced when cells were exposed to hypoxia [46]. In-vivo, thymidine phosphorylase transfected bladder carcinoma cells generated significantly more tumors following implantation into the bladder wall of rats in comparison to non-transfected, control bladder carcinoma cells, observations which were hampered following administration of antioxidants [46]. Together, these reports define a role for hypoxia/hypoxia-mimetic agents in activation of ROS and the concomitant role of ROS in cellular acquisition of oncogenic behaviors and enhanced tumor-forming capabilities, observations linked to ROS-mediated stabilization of HIF-1α.

In addition to hypoxia, up-regulated ROS has also been reported to occur in conditions of normoxia. For instance, RNA interference of the tumor suppressor succinate-ubiquinone oxidorectase (SdhB), a component of mitochondrial complex II, resulted in ROS formation and ROS dependent HIF-1α stabilization in hepatoma, lung carcinoma and osteosarcoma cell lines under normoxic conditions [47]. Suppression of SdhB gene activity resulted in increased tumor cell proliferation *in-vitro* and greater tumor formation and tumor cell expression of HIF-1α, HIF-2α and VEGF *in-vivo*, observations the authors attribute to ROS-mediated stabilization of HIF-1α [47]. In gastric carcinomas, Griffiths et al [48] summarized that increased HIF-1α expression in patient tumor sections was associated with tumor formation and disease progression. In particular, the authors speculated a role for ROS-induced stabilization of HIF-1α in tumor cell proliferation and resistance to apoptosis in tumors expressing *H-pylori* [48].

Tumor Angiogenesis

Angiogenesis, the process wherein new blood vessels are formed from pre-existing blood vessels, is a critical determinant of cancer growth and is also regulated by ROS-HIF-1α pathways. In bone marrow mast cells, for example, hypoxia, as assessed through cell treatment with the hypoxic mimetic agent cobalt chloride, was demonstrated to increase VEGF production through a mechanism dependent on ROS formation [49]. Similarly, Brown et al [46] reported HIF-1α-dependent secretion of VEGF from bladder carcinoma cells exposed to hypoxia during treatment with thymidine phosphorylase, an enzyme which up-regulates the expression of markers of oxidative stress. Using an animal model, the authors further demonstrated that mice orthotopically injected with thymidine phosphorylase-overexpressing bladder carcinoma cells developed fewer tumors following treatment with anti-VEGF than untreated controls while animals treated with both antioxidants and anti-VEGF did not form tumors [46]. In addition to aberrant patterns of angiogenesis and angiogenic factor expression in tumor cell lines and xenografts, it was recently found that endothelial cells (ECs), isolated from hypoxic regions of human xenograft tumors in mice, expressed higher levels of VEGF mRNA than non-tumor ECs [50]. This observation was most likely due to elevated levels of hypoxia-induced ROS in tumor ECs as the authors reported increased ROS levels, along with VEGF, in hypoxia-exposed human microvascular endothelial cells (HMVECs) [50]. Interestingly, the authors found that inhibition of VEGF receptor signaling in hypoxia-exposed HMVECs led to a concomitant decrease in ROS production [50]. These results would suggest that ROS production not only arises as a result of VEGF signaling but point to the involvement of multiple inter-related pathways which ensure a constant feed forward loop for ROS production under hypoxic conditions.

Heavy metals such as arsenic, chromium and cadmium have been reported to activate tumor angiogenesis via ROS-HIF-1α pathways [40, 51, 52]. For example, He et al [51] reported that hexavalent chromium [Cr (VI)] treatment of human lung epithelial BEAS-2B cells resulted in down-regulation of the miRNA-143 (miR-123). Cr (VI) induced repression of miR-123 in BEAS-2B cells led to aberrant cell activities including increased proliferation, anchorage-independent growth, tumor formation in mice and elevated angiogenesis observed in *in-vitro* tube formation assays and *in-vivo* chick chorioallantoic membrane (CAM) assays [51]. With regard to angiogenesis, the authors discovered elevated IL-8 expression in BEAS-2B cells treated with Cr (VI), a result which was likely dependent on HIF-1α expression as IL-8 was reduced following knockdown of HIF-1α [51]. Although the authors did not investigate a role for Cr (VI) induced ROS in HIF-1α expression and angiogenesis, others have reported that Cr (VI) treated cells exhibit increased ROS expression [53]. It's likely that Cr (VI) may have exerted its effects on HIF-1α induced IL-8 via elevated ROS. In a later study, the authors similarly reported that arsenic down-regulated the expression of the miRNA-199 (miR-199) in BEAS-2B cells, a phenomenon which resulted in highly vascularized tumors from arsenic exposed BEAS-2B xenograft tumors [52]. Elucidating a mechanism for these observations, the authors reported elevated expression levels of HIF-1α in arsenic-exposed BEAS-2B cells which in turn, led to increased expression of the pro-angiogenic factor COX-2 [52]. The observations were reported to be a result of arsenic-induced ROS expression, which was responsible for down-

regulating miR-199 [52]. In a similar study, exposure of immortalized lung epithelial cells and BEAS-2B cells to cadmium was reported to result in ROS-dependent ERK, AKT and p70S6K1 activation, important mediators of angiogenesis, which in turn, stabilized HIF-1α [40]. Subsequently, the authors showed that cadmium increased VEGFA expression and transcription in lung epithelial cells via ROS-induced HIF-1α stabilization [40]. Assessing the angiogenic potential of cadmium-treated cells, Jing et al [40] observed increased tube formation on Matrigel from ECs exposed to conditioned media from cadmium-treated cells. In-vivo, the authors utilized the CAM assay to demonstrate that tumors resulting from cadmium treated lung epithelial cells expressed more blood vessels than non-treated cells [40]. The authors attribute these findings to ROS-mediated activation of HIF-1α and VEGF expression [40].

Hypoxia independent pathways have also been demonstrated to increase ROS and ROS-induced accumulation of HIF-1α in angiogenic processes. For example, angiotensin II was reported to elevate ROS production and ROS-dependent stabilization of HIF-1α and expression of VEGF in vascular smooth muscle cells [54]. In support of the role of ROS in activating HIF-1α dependent VEGF expression, pre-incubation of cells with ROS inhibitors prior to stimulation with angiotensin II attenuated VEGF mRNA expression in vSMCs [54]. In a separate study, it was reported that the introduction of p53 mutations in HCT116 colon carcinoma cells resulted in increased cellular production of ROS and ROS-mediated stabilization of HIF-1α [55]. As a result of ROS-induced HIF-1α accumulation, VEGFA mRNA expression was increased in *in-vitro* cell cultures and tumor angiogenesis and growth was enhanced in xenograft tumors containing p53 mutations [55]. Confirming the role of ROS-mediated stabilization of HIF-1α and its downstream activation of angiogenic pathways, animals whose diets were supplemented with antioxidants presented with decreased ROS levels in addition to declines in tumor angiogenesis and growth [55]. In an effort to assess the effects of mutant mitochondrial DNA on tumor growth, Ishikawa et al [6] transplanted mitochondrial DNA from a highly metastatic mouse tumor cell line into a poorly metastatic cell line. The authors not only discovered that the introduction of mitochondrial DNA from the metastatic cell line to the non-metastatic cell line conferred a tumor growth pattern similar to that of the parental cells, but additionally found that the resulting tumors exhibited higher ROS production and elevated HIF-1α and VEGF expression [6]. In addition to growth factors and genetic mutations, GTPases have also been implicated for their participation in ROS-dependent stabilization of HIF-1α. For instance, transgenic mice designed to over-express the small GTPase Rac1 under control of the alpha smooth muscle actin promoter, developed Kaposi's tumors that exhibited greater VEGF secretion and angiogenesis, a consequence of ROS-induced HIF-1α accumulation [5]. The role of ROS in HIF-1α dependent activation of tumor angiogenesis was confirmed following treatment of explanted tumor cells with ROS scavenging antioxidants, which reduced HIF-1α expression and lowered VEGF secretion to basal levels [5]. Finally, endogenous expression of ROS in tumor cells, elevated independently of an identified molecular mechanism, has also been reported to induce tumor growth and angiogenesis through HIF-1α-dependent VEGF expression [56]. Similar to previous work, tumor growth and angiogenesis were subsequently reduced following addition of antioxidants to Matrigel-mixed ovarian carcinoma cells prior to transplantation in the CAM assay [56]. Work in our

lab has shown that tubulogenesis of ECs in collagen gels is governed by increased ROS production, a result of differential oxygen concentrations in the gels [57]. Surprisingly, these observations were not due to HIF-α stabilization, potentially suggesting that additional HIF-α independent mechanisms were responsible [57].

Tumor Metastasis

Metastasis is the greatest determinant of cancer-associated morbidity. An early event associated with tumor metastasis is the acquisition of morphological changes in tumor cells, collectively referred to as epithelial-mesenchymal transition (EMT), which endow cells with genotypic and phenotypic changes necessary for migration to new organ sites. For instance, Shimojo et al [58] found that pancreatic tumor cell lines underwent EMT following exposure to hypoxia [58]. Specifically, the authors found that hypoxia exposure resulted in ROS-dependent changes in cell invasiveness and morphology [58]. These observed changes included loss of cell-cell contact, acquisition of a fibroblast-like appearance, and expression of SNAIL, SLUG and TWIST, genes associated with EMT [58]. These results may have been due to ROS-dependent activation of HIF-1α as administration of antioxidants reduced SLUG expression in both hypoxic and normoxic regions of tumors and further attenuated the expression of HIF-1α and EMT-associated markers in xenograft tumors [58]. In a similar report, Cannito et al [59] utilized several tumor cell lines to demonstrate that hypoxia induced EMT, evident in cellular acquisition of a fibroblast phenotype, up-regulated nuclear translocation of β-catenin and SNAIL and increased cell invasiveness and migration, was dependent on cellular ROS production. Interestingly, the authors attributed early events in EMT, such as loss of SNAIL nuclear translocation, to hypoxia-induced ROS expression while late EMT events, such as invasiveness and migration, were dependent on HIF-1α induced VEGF secretion [59]. These results illustrate dual mechanisms whereby hypoxia controls early and late metastatic cell behaviors through ROS and HIF-1α. Although ROS-regulated HIF-1α pathways were not explored in this study, it's possible that this pathway played a role in late metastatic cell behaviors as down-regulation of HIF-1α would have limited intracellular signaling via ROS. Indeed, Ma et al [60] discovered that ROS-regulated HIF-1α accumulation was responsible for increased cell mobility and invasion, assessed through use of Transwell chambers with and without Matrigel, respectively, in breast cancer subclones depleted of mitochondrial respiratory activity. The authors furthered demonstrated that these breast cancer subclones exhibited increased expression of HIF-1α under both hypoxic and normoxic conditions, with administration of antioxidants decreasing the observed HIF-1α levels [60]. While the results clearly point to a role for ROS in the regulation of HIF-1α induced cell migration and invasion, these observations were not due to hypoxia-induced changes in ROS levels, but were rather a result of endogenous deregulated ROS activity. It's likely that hypoxia acts as a catalyst to increase ROS production, but does not contribute to higher ROS levels in cells exhibiting endogenously high levels of ROS as a result of disruptions to mitochondrial respiratory activity.

Regarding hypoxia-independent mechanisms regulating ROS, it was shown that conditioned media from patient-derived prostate carcinoma associated fibroblasts (CAFs), tumor-resident fibroblasts which play an active role in tumor progression [61], induced an EMT signature in prostate carcinoma cells [62]. The authors reported that this response was

mediated via CAF-induced expression of HIF-1α, COX2, and NFκB in prostate carcinoma cells as RNA silencing of these genes abrogated cell invasiveness and expression of EMT-associated genes [62]. In an attempt to elucidate a mechanism responsible for the observed aberrant gene expression, the authors discovered that ROS was not only up-regulated in prostate carcinoma cells exposed to CAF conditioned media, but dictated the expression of HIF-1α and NFκB and directed the EMT signature in the tumor cells [62]. While ROS expression in tumor cells was reported to be under the control of COX2, rather than hypoxia, it is interesting to speculate that hypoxic-induced patterns of gene expression in CAFs may have been responsible for COX2 activation in tumor cells. Indeed, CAFs have been reported to be associated with hypoxic regions of tumors [63]. In this manner, it's possible that CAF-induced activation of COX2 and ROS in the tumor cells may be a result of activation of hypoxia-mediated signaling in CAFs.

Therapy Response

Tumor cell responses to chemotherapy or radiotherapy are governed by a number of extracellular events, including hypoxia, which modulate the efficacy of the therapeutic agent. In an attempt to better understand the mechanisms regulating tumor cell resistance to chemotherapeutic agents, Calvani et al [64] discovered that hypoxia enhanced survival of the metastatic Hs29-4T human melanoma cell line to etoposide. Specifically, hypoxia reduced etoposide-induced apoptosis of HS29-4T cells through activation of VEGF-A, a downstream target of HIF-1α [64]. The pro-survival effect of VEGF-A in this study was attributed to hypoxia-induced expression of ROS and ROS-mediated stabilization of HIF-1α [64]. Interestingly, the authors demonstrated that VEGF-A is regulated in an autocrine manner [64]. Its expression stimulated continued production of ROS, which then stabilized HIF-1α and thus expression of VEGF-A, a mechanism likely to result in continued melanoma cell resistance to etoposide [64]. In a similar study, it was shown that glioblastoma cells exhibited the greatest degree of radioresistance *in-vitro* and *in-vivo* following exposure to cycling (e.g. intermittent) hypoxia as opposed to continuous hypoxia [65]. The mechanism responsible for enhanced tumor growth and higher numbers of surviving cells following radiotherapy treatment under cycling hypoxia conditions was attributed to hypoxia-induced expression of ROS and ROS-mediated stabilization of HIF-1α [65]. Although the authors didn't explore how genes induced by HIF-1α facilitated cell survival and tumor growth, they nonetheless reported increased mRNA expression of VEGF in cells subjected to hypoxic conditions [65]. Similar to the report by Calvani et al [64], it's possible that VEGF may be in part responsible for glioblastoma cell radioresistance under hypoxia.

Taking into account the above studies which point to increased hypoxia-induced expression of VEGF and its implications in cell survival in response to therapy, it would follow that therapy resistance may be accomplished through vascular-related mechanisms. Indeed, it has been suggested that a major obstacle to effective anti-tumor therapy is resistance of the tumor vasculature to radiotherapy [66]. For example, Moeller et al [67] treated tumor-bearing animals with radiation and discovered that tumor re-oxygenation led to ROS-dependent HIF-1 accumulation in tumors. This pathway-specific activation of HIF-1 was shown to have a radio-protective effect on ECs, an observation the authors point to increased

expression of EC-derived cytokines [67]. When the authors combined radiotherapy with a HIF-1 targeting drug, significant delays in tumor growth were observed, a result attributed to therapy-induced vascular destruction [67]. The results of this study confirm a role for ROS-dependent HIF-1-activation in vascular sensitivity to radiation therapy, pointing to a novel mechanism wherein tumors become sensitized to therapeutic agents. In a more recent report, it was shown that treatment of tumor cells with the commonly utilized chemotherapeutic agent doxorubicin, up-regulated HIF-1α expression which concomitantly increased VEGF secretion by murine breast tumor cells *in-vitro* and accelerated tumor angiogenesis *in-vivo* [68]. Doxorubicin-induced HIF-1α expression was specifically regulated by the synthesis of nitric oxide [68], a type of free radical known to induce oxidative stress and cellular damage [69–71]. Although chemotherapy resistance was not explored in this study, the observed increases in tumor angiogenesis as a result of doxorubicin treatment would suggest a novel mechanism wherein a chemotherapeutic agent may induce therapy resistance. Given the results of this study as the preceding studies, it is interesting to speculate that chemotherapy resistance may proceed via mechanisms related to improved vascular stability.

Targeting ROS to Improve Cancer Therapy

ROS activation of HIF-1α and its downstream targets may be a barrier for effective cancer therapy. In order to improve the efficacy of therapeutic agents, strategies are needed which reduce and/or eliminate cellular sources of ROS. Xia et al [56] utilized rotenone, an inhibitor of electron transport function, and diphenyleneiodonium (DPI), a NAPDH oxidase inhibitor, to reduce ROS in the human ovarian carcinoma cell line, OVCAR-3. The authors reported that pre-treatment of OVCAR-3 cells with either rotenone or DPI prior to implantation in the CAM resulted in decreased tumor angiogenesis and tumor growth, a result which was likely due to decreased ROS stabilization of HIF-1α [56]. While rotenone and DPI reduced ROS formation in this study, these agents are not likely to be utilized as a form of chemotherapy given their reported toxicity to healthy cells [72,73]. In another study, administration of the antioxidant resveratrol, a plant-based product with cardio-protective and anti-cancer properties, was reported to limit glycolytic metabolism in several cancer cell lines as a result of its down-regulation of intracellular ROS, which resulted in a concomitant decrease in HIF-1α expression [74]. Evaluating the use of antioxidants to limit metastasis, Shimojo et al [58] discovered that administration of the antioxidant n-acetylcysteine (NAC) suppressed markers of EMT in human pancreatic cancer cell lines exposed to hypoxia *in-vitro* and *in-vivo* and reduced metastatic spread in xenograft pancreatic cancer-bearing mice. These results were attributed to NAC inhibition of ROS which in turn, resulted in reduction of HIF-1α and NFκB expression [58].

Utilizing a novel strategy to attenuate ROS production, Jung et al [75] treated tumor cells and xenograft tumors with terpestacin, a small microbial-derived molecule previously reported to have anti-angiogenic capabilities [75]. The authors discovered that terpestacin specifically interacted with UQCRB, a component of mitochondria complex III, reducing ROS production and thereby inhibiting HIF-1α stabilization under hypoxic conditions [75]. Using a xenograft animal model of human breast cancer, the authors further demonstrated that terpestacin reduced tumor angiogenesis, a result of decreased ROS production and thus reduced HIF-1α accumulation and VEGF expression [75]. The novelty of the study is

terpestacin's mechanism of action, which was reported to be unrelated to mitochondrial respiration, suggesting that oxygen consumption was not affected [75]. This feature is desirable in terms of limiting cytotoxicity to normal, healthy cells.

While inhibition of ROS appears to be favorable for limiting tumor growth, it is important to recognize that chemotherapy-generated ROS is also necessary for its mechanism of action. For example, certain chemotherapeutic agents, such as cisplatin, doxorubicin, or 2-methoxyestradiol, directly or indirectly modulate higher tumoral ROS production [76–79], a process which is necessary for tumor death. Indeed, while tumors utilize ROS to their growth advantage, very high levels of ROS are cytotoxic and result in tumor cell death [80]. As a result, administration of antioxidants or suppressors of ROS activity would negate the effects of the therapy. In this manner, it is important to consider the metabolic effects of chemotherapeutic agents prior to the inclusion of ROS inhibitors into a therapeutic regime.

Future Outlook: Engineering-Centered Approaches for Investigating ROS Formation

The preceding sections have discussed the role of hypoxia in ROS-induced HIF-1α in tumor progression and have highlighted several excellent studies that have identified mechanisms linking this pathway to tumor growth, metastasis and therapy response. While these studies have undoubtedly shed new light on the interplay of hypoxia and ROS in tumorigenesis, they do not completely capture the complexity of the environment in which tumors resides. In this manner, this final section will explore the use of novel engineering-based strategies, which not only provide the investigator with platforms from which to better mimic the *in-vivo* environment, but additionally allow one to exert control over the cellular microenvironment. These strategies have already been incorporated in a number of studies seeking to better understand the mechanisms driving tumorigenesis and angiogenesis and it is foreseeable that they may also be employed to address the role of hypoxia and ROS in tumor progression.

Three-Dimensional Culture

Traditionally, investigations of cell behaviors and responses to exogenous factors have been conducted in 2 dimensional (2D) tissue culture dishes, conditions which fail to recapitulate the complexity of the *in-vivo* environment. To overcome the limitations imposed by 2D culture, several groups have moved toward the use of 3D hydrogels to elucidate cell responses in a relevant culture setup [81–84]. The hydrogels used for 3D culture are inspired by the extracellular matrix (ECM), an a-cellular collection of macromolecules which provide cells with a structural scaffold necessary for supporting their growth, survival, and differentiation [85]. The ECM regulates these diverse cell behaviors as a result of its rich protein landscape (e.g. collagens, fibronectin, glycosaminoglycans, etc) in addition to its physicochemical properties [85]. Due to their structural similarity to the natural ECM, hydrogels are increasingly being explored as platforms to study and control cellular behaviors. As summarized in a recent review, Herrmann et al [86] discuss the work of several groups who have utilized 3D ECM-mimetic hydrogels to assess tumor cell behaviors and therapy responsiveness, describing the similarities of these results to *in-vivo* tumors,

works which illustrate the necessity for using 3D hydrogels to investigate tumor cell activities.

To evaluate dissolved oxygen concentrations in 3D collagen gels encapsulated with ECs, we reported that gradients of oxygen concentration were unique in different regions of collagen gels cultured under atmospheric conditions [57]. Here, lower oxygen tension occurred at the bottom of the gel while higher oxygen tension occurred near the top of the gel [57]. Interestingly, the decrease in oxygen tension in collagen gels was associated with ROS-induced vascular tubulogenesis, a phenomenon that was independent of HIF-α stabilization [57]. Although we did not examine ROS expression at it related to gradients of dissolved oxygen, it is foreseeable that such studies may be conducted. For instance, one could utilize a number of small molecule probes which emit a fluorescent signal in response to cellular ROS [87], allowing the investigator to observe oxidative stress in cells positioned at various regions/thicknesses of the hydrogel. Moreover, one could section the gel at pre-determined depths and assess for HIF-α protein expression in encapsulated cells. While we did not observe a role for ROS in HIF-α expression, it is possible that this finding was limited to a bulk measurement of all encapsulated cells, limiting subtle changes which may have taken place in cells found at various depths of the gel, or to the cell population evaluated. Finally, this culture setup may be further utilized to investigate the influence of dissolved oxygen on patterns of ROS/HIF-α expression and the relationship of this pathway to acquisition of oncogenic behaviors and/or invasive activities in non-malignant and malignant cells, respectively.

A unique feature of hypoxia in the tumor microenvironment is its dynamic nature. For instance, tumors have been reported to possess fluctuating levels of hypoxia [88], a result which is most likely due to the aberrant architecture of the tumor vasculature. In order to achieve cycling hypoxic conditions characteristics of tumors, one could further incubate cellencapsulated 3D hydrogels in controlled oxygen environments representing various degrees of hypoxia for set periods of time. Recently, we have engineered a hypoxia-inducible hydrogel, where oxygen levels and gradients were tightly controlled in 3D gelatin matrices through incorporation of oxygen consuming functional groups [89]. Results from this work demonstrated that these hydrogels readily promoted neovascularization *in-vitro* via HIF-mediated pathways and *in-vivo* where increased numbers of vessels along with larger vessels were observed in animals subcutaneously injected with hypoxic as opposed to non-hypoxic gels [89]. Although this work is an important step wherein a hypoxic environment can be induced in a hydrogel, additional efforts are necessary to more fully capture the dynamic nature of hypoxia within the tumor microenvironment. It's anticipated that such studies would provide important information regarding the influence of cycling hypoxia on cellular oxidative stress, HIF-α expression and oncogenic activities including growth factor release, invasive properties and acquisition of a metastatic phenotype in encapsulated tumor cells. Moreover, since chemotherapeutic agents are delivered via way of the tumor vasculature, setting up conditions of cycling hypoxia would be important for mimicking the microenvironmental conditions tumor cells reside in upon first encounter with a therapeutic agent. In this manner, this culture setup would allow the investigator to

test tumor cell responses to therapies in the context of potential hypoxia-induced effects on ROS.

Micropatterning

While 3D cultures offer numerous advantages for investigating cell behaviors in a biologically relevant system, others have developed sophisticated technologies that enable fine control over the cellular presentation of ECM instructive cues. One form of technology which allows investigators to de-couple certain features of the ECM is micropatterning.

Micropatterning is the fabrication of a culture surface that features microscopic instructive cues, allowing the investigator to evaluate how differences in the geometry (e.g. width and alignment of ECM fibers), topography (e.g. height and texture of ECM proteins), elasticity (e.g. matrix stiffness) and composition of the ECM, features which are reported to be abnormal in the tumor environment [90], direct cell behavior. In our lab, we have utilized this technique to address vascular network assembly from endothelial progenitor cells on micropatterned fibronectin and fibrin gel strips [91] and the adhesion of different breast cancer cell lines on micropatterned hyaluronan [92], discovering that substrate geometry and topography impact cellular responses. This approach also allows the investigation of single-cell interactions between ECs and cancer cells at different stages of tumorigenesis [92]. Others have utilized micropatterning to investigate the role of matrix geometry and stiffness on mammary epithelial cell transformation [93] and tumor spheroid generation [94].

To our knowledge, none have investigated patterns of oxidative stress following micropatterning of cell populations on ECM substrata despite the reported implications for matrix proteins on ROS expression. For example, it has been shown that early cell interactions with a fibronectin matrix, a protein which is associated with cancer progression [95–99], support an initial burst of mitochondrial ROS release from NIH3T3 fibroblasts [100]. In addition, collagen I, a hypoxia-sensitive ECM protein [101–103], has also been implicated in redox pathway activation in human monocytes [104]. As a new approach for evaluating oxidative stress, it may be important to first delineate how matrix properties including the geometric organization, topography, and mechanical stiffness of select ECM proteins elicit ROS/HIF-α expression from malignant and/or non-malignant cells on micropatterned substrata. Prior to analysis, one may wish to narrow the selection of a particular ECM protein based on the tissue of origin, aberrant expression of the protein in the selected tumor environment or some predetermined role in oxidative stress. For example, the investigator may utilize micropatterned collagen or fibronectin to investigate matrix effects on breast cancer ROS production given the aberrant expression of these proteins in the breast tumor environment [97–99].

Upon distinguishing the selected contributions of the physical features of the ECM toward ROS-HIF-α expression, the investigator may further elucidate the contribution of hypoxia on cellular oxidative stress, HIF-α stabilization and resulting oncogenic behaviors. Here, the investigator could examine either chronic hypoxia, representative of conditions in the tumor interior, and intermittent, cycling hypoxia, representative of conditions at the tumor periphery, on ROS-HIF-α expression. Although there's no distinct frame of time which constitutes chronic hypoxia, such conditions are frequently selected based on experimental

endpoints. For instance, an investigator who wishes to model chronic hypoxia may opt to culture cells in hypoxic conditions for the total desired culture period. Intermittent hypoxia, on the other hand, may be achieved by alternating culture in hypoxic and atmospheric conditions for set periods of time based on total time in culture. To add complexity to the system, one could micropattern additional tumor microenvironment associated cells along with tumor cells. For example, one could micropattern fibroblasts, ECs or smooth muscle cells along with tumor cells to assess the coordinated interplay of hypoxia and cell secreted factors on the ROS/HIF-α axis. Finally, it's foreseeable that micropatterning of 3D cultures could be to achieve a more biologically relevant system from which to investigate hypoxia effects on ROS/HIF-α.

Microfluidic Devices

In addition to micropatterning, the use of microfluidic devices, an apparatus which consists of one or more nano or micro-sized channels, have been employed to address cellular responses in a miniaturized environment. Using a microfluidic device, an investigator may assess how cells respond to the introduction of physical and biochemical stimuli, such as dissolved oxygen, shear stress, a force tumor cells encounter upon intravasation into blood vessels; and bioactive factors, autocrine or paracrine cytokines or growth factors which participate in tumor progression. Evaluating EC responses to environmental stimuli, Chin et al [105] utilized a microfluidic device to quantify ROS expression from ECs exposed to shear stress and glucose, concluding that glucose generated higher ROS levels from ECs under both static and pulsatile shear stress than shear stress alone [105]. Regarding ROS expression from tumor cells, Bischel et al [106] co-cultured prostate cancer cells with bone marrow stromal cells in a microfluidic device and discovered that the bone marrow stromal cells facilitated invasive prostate cancer cell behaviors as a result of increased tumoral ROS expression. Lo et al [107] seeded lung cancer cells in a microfluidic device and examined ROS expression in response to shear stress, reporting that high levels of shear stress resulted in proportionately higher levels of cellular ROS expression.

Indeed, the use of microfluidic devices is advantageous for examining ROS production in response to physical and chemical stimuli, work which may be extended to investigations using hypoxia. Work from our lab and others has shown that the spatial distribution of oxygen may be controlled using a microfluidic device [108, 109]. Given the fluctuating oxygen concentrations associated with tumors [88], microfluidic devices would be aptly suited for setting up gradients of oxygen concentrations to evaluate mitochondrial responses, ROS expression and HIF-α expression in both malignant and non-malignant cells. One could further investigate how dissolved oxygen gradients and corresponding ROS-HIF-α expression patterns facilitate tumor cell migration and cellular acquisition of phenotypic and genotypic markers of metastasis. In order to better recapitulate the complexity of the *in-vivo* environment, 3D encapsulated cell populations may be introduced into microfluidic devices. For example, Chung et al [110] utilized a microfluidic device to assess angiogenic properties of 3D encapsulated ECs cultured in the presence of tumor cells. In this manner, it is possible that tumor cells may be seeded along with other tumor microenvironment cells for studies investigating the reciprocity of hypoxia and cell-cell connections and/or their secreted factors on tumoral ROS and HIF-α expression. Finally, since perfusion of biological factors

can be readily controlled using microfluidic devices, it is conceivable that one may use this platform to assess the utility of antioxidants and/or other redox-sensitive drugs on ROS inhibition.

Taken together, the use of engineering-based approaches for the study of ROS expression may afford a wealth of information which may be utilized for the future development of novel therapeutic agents directed against the exogenous or endogenous mediators of ROS.

Conclusions

Overall, the results from the above studies have elucidated a role for mitochondrial generated ROS in stabilization of HIF-1α in tumors. In addition, these studies point to a role for ROS-mediated HIF-1α stabilization in oncogenic behaviors. While these studies eloquently illustrate a novel pathway wherein cells sense changes in oxygen tension via mitochondria, activating downstream pathways such as HIF-1α, additional studies addressing the mechanisms associated with ROS-mediated stabilization of HIF-1α are needed in order to provide novel therapeutic targets for disruption of this pathway in tumors. It is foreseeable that great advances in this field may be achieved through the use of engineering-based techniques which enable fine control over spatiotemporal cues and permit a more accurate representation of the native host environment.

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Abbreviations

CAF	Carcinoma Associated Fibroblasts
CAM	Chick Chorioallantoic Membrane
DPI	Diphenylene
EC	Endothelial Cell
ECM	Extracellular Matrix
EMT	Epithelial-Mesenchymal Transition
HIF	Hypoxia Inducible Factor
HMVEC	Human Microvascular Endothelial Cells
ISCU	Iron Sulfur Scaffolding Protein
PDK1	Pyruvate Dehydrogenase Kinase 1
PHDs	Prolyl 4 Hydroxylase Domain
VEGF	Vascular Endothelial Growth Factor
VHL	von Hippel-Lindau

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Highlights

- Hypoxia elevates ROS expression.
- ROS expression stabilizes HIFα.
- ROS-HIF-1α facilitates tumor progression, angiogenesis, and metastasis.
- ROS-HIF1α modulates tumor cell responses to therapy.
- Engineering based approaches may be utilized to address ROS expression.

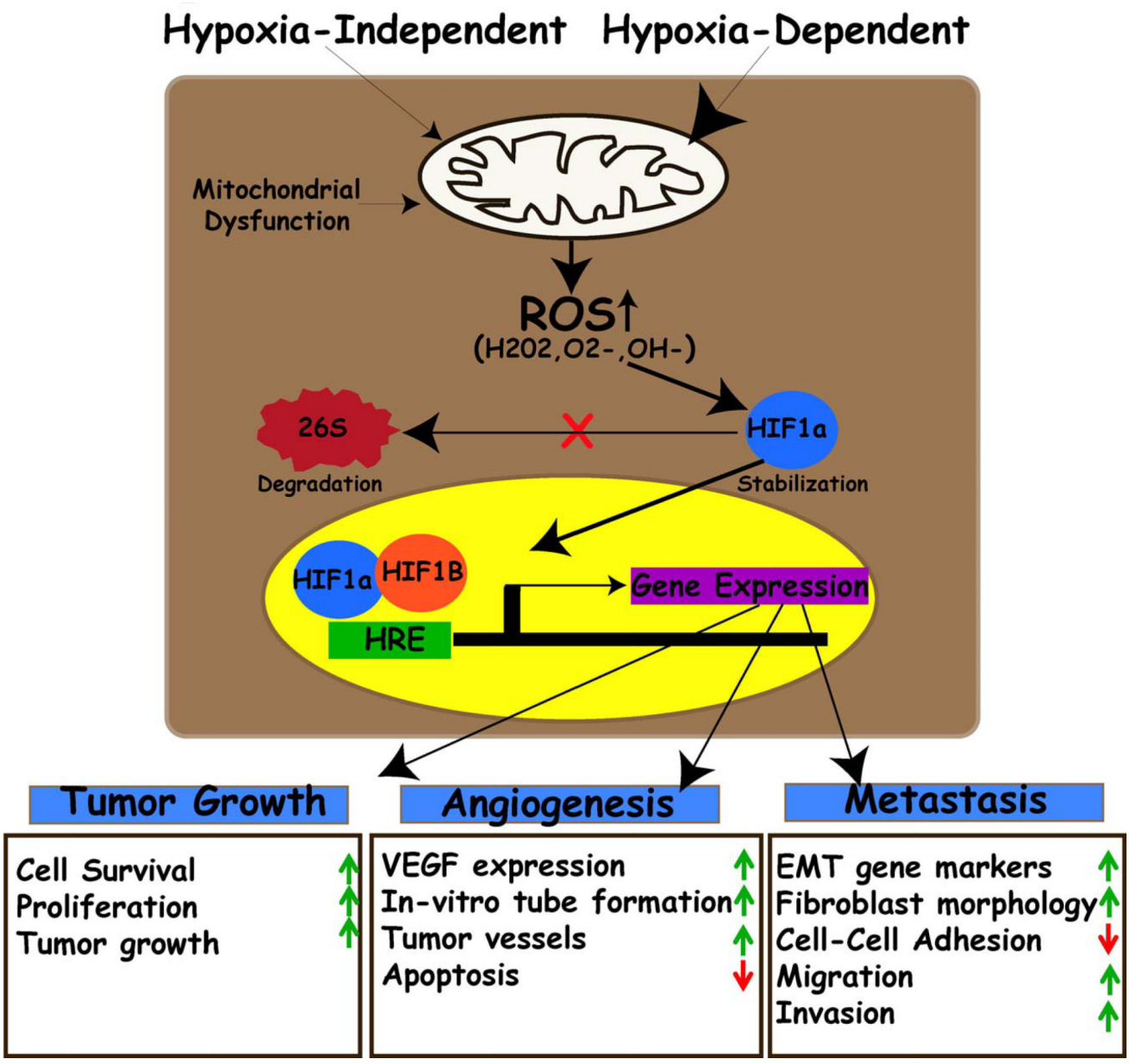

Figure 1. Overview of the effects of ROS-induced HIF-1α on tumorigenesis

Exogenous factors, primarily related to extracellular hypoxia, induce the formation of ROS from mitochondria. ROS may also be increased as a result of hypoxia-independent activities in addition to mitochondrial dysfunction. Increased expression of ROS stabilizes HIF-1α, preventing its degradation in the 26S proteasome. HIF-1α translocates to the nucleus, associates with constitutively expressed HIF-1β and together with co-activators, bind to HRE sequences in the promoter regions of genes. As a result, target gene transcription is up-regulated leading to tumor growth, angiogenesis and metastasis.