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Hypoxia-inducible factors: mediators of cancer progression and targets for cancer therapy

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Abstract

Hypoxia-inducible factors (HIFs) mediate adaptive physiological responses to hypoxia. In human cancers that are accessible for O_2 electrode measurements, intratumoral hypoxia is common and is associated with increased risk of mortality. HIF activity in regions of intratumoral hypoxia mediates angiogenesis, epithelial-mesenchymal transition, stem cell maintenance, invasion, metastasis, and resistance to radiation therapy and chemotherapy. A growing number of drugs have been identified that inhibit HIF activity by a variety of molecular mechanisms. Because many of these drugs are already FDA-approved for other indications, clinical trials can (and should) be initiated to test the hypothesis that incorporation of HIF inhibitors into current standard-of-care therapy will increase the survival of cancer patients.

Hypoxia and cancer

Human cells require adequate supplies of O_2 on a continuous basis for use as the terminal electron acceptor in the process of mitochondrial respiration that generates ATP, which is used to power most biochemical reactions. Both the delivery and consumption of O_2 are precisely regulated through the activity of hypoxia-inducible factors (HIFs) [1]. As cells proliferate, increased O_2 consumption results in hypoxia (reduced O_2 levels), which activates HIFs, leading to transcription of the *VEGF* gene, which encodes vascular endothelial growth factor, a secreted protein that stimulates angiogenesis and thereby increases O_2 delivery. Cancer cells are characterized by dysregulated cell proliferation, and the blood vessels that form within solid tumors are often structurally and functionally abnormal, resulting in severe hypoxia. To adapt to the hypoxic microenvironment, cancer cells co-opt physiological responses to hypoxia that are mediated by HIFs. In the process of doing so, hypoxic cancer cells acquire invasive and metastatic properties as well as resistance to chemotherapy and radiation therapy, which together constitute the lethal cancer phenotype. Despite ample data to support this model, there are few drugs in the cancer armamentarium that target hypoxic cancer cells. Not coincidentally, the options for treatment of advanced metastatic disease (and their efficacy) are extremely limited, and this year over 570,000 Americans will die of cancer [2]. Given the magnitude of this unmet clinical need, novel therapeutic strategies that are not limited to those few approaches employed by the pharmaceutical industry must be considered. This review will summarize

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the molecular mechanisms by which HIF activity is regulated in an O_2-dependent manner, the roles of HIFs in cancer progression, the chemical compounds that have been shown to inhibit HIF activity, and their potential use as anti-cancer agents.

Molecular biology of HIFs

The nucleated cells of all metazoan species analyzed to date express HIF-1, which is a heterodimer that is composed of HIF-1α and HIF-1β subunits [1]. Certain cell types of vertebrate organisms also express HIF-2, which is composed of HIF-2α and HIF-1β subunits. A principal mechanism by which O_2 regulates HIF activity is through proline and asparagine hydroxylation [3, 4]. The hydroxylation of two proline residues in HIF-1α and HIF-2α (Pro^{402} and Pro^{564} in human HIF-1α) by prolyl hydroxylase domain protein 2 (PHD2) is required for the binding of the von Hippel-Lindau protein (VHL), which leads to HIF-α ubiquitination and proteasomal degradation. Hydroxylation of an asparagine residue (Asn^{803} in human HIF-1α) by factor inhibiting HIF-1 (FIH-1) blocks the recruitment of the coactivator p300. These hydroxylation reactions use O_2 and α-ketoglutarate as substrates and enzyme activity is inhibited under hypoxic conditions, leading to increased HIF-α stability and transcriptional activity. HIFs bind to hypoxia response elements that contain the consensus sequence 5'-RCGTG-3' [5]. Based on genome-wide chromatin immunoprecipitation combined with DNA sequencing or mRNA microarrays (ChIP-seq and ChIP-chip, respectively), the number of direct HIF target genes is currently greater than 800, (i.e. at least 1 out of every 30 human genes)[6, 7]. HIFs also indirectly regulate gene expression by transactivating genes encoding microRNAs [8] and chromatin modifying enzymes [6, 9].

HIFs in cancer progression

HIFs play key roles in many critical aspects of cancer biology including angiogenesis [10–12], stem cell maintenance [13–15], metabolic reprogramming [16, 17]; autocrine growth factor signaling [18, 19]; epithelial-mesenchymal transition [9, 20–22], invasion [23, 24], metastasis [25–27], and resistance to radiation therapy [28] and chemotherapy [29]. An extensive body of experimental and clinical data has validated HIFs as targets for cancer therapy: first, in addition to intratumoral hypoxia, loss-of-function for tumor suppressor genes (most notably, *VHL*) and gain-of-function for oncogenes and viral transforming genes increase HIF activity (Figure 1); and second, levels of HIF-1α or HIF-2α are correlated with tumor growth, vascularization, and metastasis both in animal models and in clinical studies [30]. In different cancers, the HIF-dependent expression of these genes may be increased either by genetic alterations or by intratumoral hypoxia. Several specific HIF-regulated genes that play key roles in critical aspects of cancer biology are discussed in greater detail below.

Increased cell proliferation and survival

Among the first changes that distinguish neoplastic from normal cells are an increased rate of cell proliferation and a decreased rate of cell death, due to increased expression of secreted growth/survival factors. Often, the same cells express the cognate membrane receptors for these factors, resulting in autocrine signaling. Among the growth/survival factors that are encoded by HIF-regulated genes (italicized and in parentheses below and in Figure 2A) and participate in autocrine signaling are: transforming growth factor-α (*TGFA*) in clear-cell renal carcinoma [31]; insulin-like growth factor-2 (*IGF2*) in colorectal carcinoma [32, 33]; vascular endothelial growth factor (*VEGF*) in colorectal, gastric, and pancreatic cancer [34, 35]; endothelin 1 (*EDN1*) in breast, prostate, and ovarian cancer [36, 37]; adrenomedullin in pancreatic and prostate cancer [38, 39], and erythropoietin (*EPO*) in breast, prostate, and renal cancer and melanoma [40]. Immortalization of cancer stem cells

also requires the expression of telomerase (*TERT*), pluripotency factors such as NANOG and OCT4, and factors that block cellular senescence such as the glycolytic enzymes glucosephosphate isomerase (*GPI*) and phosphoglycerate mutase (*PGM*) [41–43].

Metabolic reprogramming

The uptake of glucose by metastatic cancer cells is so reliably and markedly increased relative to normal cells that it serves as the basis for the clinical test that is used to screen cancer patients for occult metastases, in which ^{18}F-fluorodeoxyglucose is imaged by positron emission tomography (FDG-PET). HIF-1 mediates expression of genes encoding glucose transporters (*GLUT1, GLUT3*) and glycolytic enzymes (ALDOA, ENO1, GAPDH, HK1, HK2, PFKL, PGK1, PKM2, LDHA) that convert glucose to lactate (Figure 2B). HIF-1 also actively suppresses mitochondrial oxidative metabolism by increasing the expression of pyruvate dehydrogenase kinase 1 (PDK1), which phosphorylates and inactivates pyruvate dehydrogenase (PDH), the enzyme that converts pyruvate to acetyl coA for entry into the TCA cycle [44]. HIF-1 also downregulates oxidative metabolism by activating the expression of BNIP3 and BNIP3L, which mediate mitochondrial-selective autophagy [45, 46]. Recent data suggest that in some cell types HIF-1 may also mediate expression of transketolase enzymes (*TKT, TKTL2*) of the pentose phosphate pathway (PPP), which non-oxidatively catalyze the production of NADPH and nucleotides that are required for lipid and nucleic acid synthesis [47]. *Angiogenesis*. HIF-1 controls the expression of multiple genes encoding angiogenic growth factors, including vascular endothelial growth factor (*VEGF*), stromal-derived factor 1 (*SDF1*), placental growth factor (*PGF*), platelet-derived growth factor B (*PDGFB*), and angiopoietin (*ANGPT*) 1 and 2 [48] (Figure 2C). In mouse models, inhibition of HIF-1 activity dramatically inhibits tumor vascularization [11, 12].

Epithelial-mesenchymal transition

HIF-1 activates the transcription of genes encoding repressors (*ID2, SNAI1, SNAI2, TCF3, ZEB1, ZEB2*) that block the expression of E-cadherin and other proteins that contribute to the rigid cytoskeleton, cell-cell adhesion, and other differentiated characteristics of epithelial cells [9, 20, 21]. HIF-1 also mediates expression of genes (*TGFA, VIM*) that promote the flexible cytoskeleton and other characteristics of the mesenchymal phenotype [31] (Figure 2D).

Invasion and metastasis

HIF-1 activates transcription of genes encoding: proteases that degrade (*CTSC, MMP2, MMP9, MMP14, PLAUR*) or remodel (*LOX, LOXL2, LOXL4*) the extracellular matrix within the primary tissue and at distant sites of metastasis [25, 26]; motility factors (*AMF, MET*); permeability factors (*VEGF, ANGPT2*) that promote the intravasation of cancer cells into blood vessels; and cell surface (*L1CAM*) and secreted (*ANGPTL4*) proteins that promote extravasation of cancer cells into the parenchyma at metastatic sites such as the lung [27] (Figure 2E).

HIF-1 inhibitors for cancer therapy

A growing number of chemical compounds have been shown to block tumor xenograft growth and inhibit HIF activity through a wide variety of molecular mechanisms, including decreased HIF-1α mRNA levels, decreased HIF-1α protein synthesis, increased HIF-1α degradation, decreased HIF subunit heterodimerization, decreased HIF binding to DNA, and decreased HIF transcriptional activity (Figure 3), as described below. Many of these are drugs that are in clinical cancer trials or are already approved for the treatment of cancer or other diseases. The list of drugs discussed below is meant to be illustrative rather than comprehensive.

HIF-1α mRNA expression

Aminoflavone, the active component of the prodrug AFP-464, which is currently in phase I cancer trials, partially inhibits HIF-1α mRNA expression but almost completely blocks HIF-1α protein expression [49], suggesting that it decreases both the stability and translation of HIF-1α mRNA.

HIF-1α synthesis

Drugs that inhibit the translation of HIF-1α mRNA into protein include: (i) mTOR inhibitors, such as rapamycin, temsirolimus (CCI-779), and everolimus (RAD-001) [50]; (ii) cardiac glycosides, such as digoxin [51], which have been used for decades to treat heart disease; (iii) microtubule targeting agents, such as 2-methoxyestradiol and taxotere [52]; (iv) topoisomerase I and II inhibitors, such as topotecan [53] and NSC-644221 [54], respectively; and (v) synthetic oligonucleotides, such as EZN-2968, a locked nucleic acid oligonucleotide that binds to HIF-1α mRNA and blocks its translation, which is currently in phase I clinical trials [55]. This category also includes anti-cancer drugs that inhibit receptor tyrosine kinases, such as BCR/ABL, EGFR, and HER2, and thereby indirectly inhibit mTOR activity. YC-1 [3-(5'-hydroxy methyl-2'-furyl)-1-benzylindazole] may also block HIF-1α protein expression by inhibiting mTOR [56]. PX-478 (*S*-2-amino-3-[4′-*N*,*N*,-bis(2-chloroethyl)amino]phenyl propionic acid *N*-oxide dihydrochloride) inhibits HIF-1α translation by an undetermined mechanism and sensitizes tumor xenografts to radiation therapy [57]. Many of the drugs in this category show preferential activity against HIF-1α, with less or no effect on HIF-2α.

HIF-1α stability

Drugs that induce the degradation of HIF-1α protein include: (i) HSP90 inhibitors, such as 17-allylamino-17-demethoxygeldanamycin, which cause VHL-independent, RACK1-dependent ubiquitination and proteasomal degradation of HIF-1α [58]; (ii) antioxidants, such as ascorbate (vitamin C) and N-acetyl cysteine, which block tumor xenograft growth by promoting HIF-1α degradation through the PHD2-VHL-proteasome pathway [59]; (iii) the thioredoxin inhibitor PX-12, which induces HIF-1α degradation that may be due in part to increased expression of SSAT1, a protein that binds to HIF-1α and promotes RACK1-dependent ubiquitination and proteasomal degradation [60, 61]; (iv) Class II histone deacetylase (HDAC) inhibitors, such as LAQ824, which stimulate ubiquitination of HIF-1α by an undetermined mechanism [62]; (v) G-rich oligonucleotides, which represent another nucleic acid-based strategy; but in this case, the target is HIF-1α protein rather than mRNA [63]; (vi) berberine, a natural product that induces HIF-1α degradation, has anti-angiogenic effects both in cancer cells and in endothelial cells [64]; (vii) Se-methylselenocysteine, which induces HIF-1α degradation and sensitizes hypoxic cancer cells to the effects of the chemotherapy drug irinotecan [65]; and (viii) YC-1, a guanylate cyclase activator that induces HIF-1α degradation by an unknown mechanism [66]. YC-1 and deguelin, an HSP90 inhibitor, sensitize tumor xenografts to radiation therapy [66, 67]. Most of the drugs in this category show similar activity against HIF-1α and HIF-2α.

HIF heterodimerization

Acriflavine, a drug that was used clinically as an antibacterial agent prior to the discovery of penicillin, binds directly to the PAS-B subdomain of HIF-1α and HIF-2α and blocks their interaction with HIF-1β, thereby blocking HIF-dependent gene transcription, leading to impaired tumor growth and vascularization [12].

HIF DNA-binding activity

Anthracyclines, such as doxorubicin (Adriamycin) and daunorubicin, bind to DNA and block the binding of HIF-1 and HIF-2 in cultured cells and block HIF-1-dependent expression of angiogenic growth factors leading to impaired tumor growth and vascularization [11]. Echinomycin, another DNA intercalating agent that inhibits HIF-1 activity, has been shown to block lymphoma and acute myelogenous leukemia growth by eradicating cancer stem cells [15].

HIF-1α-dependent transactivation

The proteasome inhibitor bortezomib, which is approved by the FDA for treatment of mantle cell lymphoma and multiple myeloma [68], inhibits HIF-1 transcriptional activity by targeting the carboxyl-terminal transactivation domain of HIF-1α that interacts with the coactivator p300, although drug treatment does not disrupt the interaction [69]. In prostate cancer cells, bortezomib blocks HIF-1α protein expression by inhibiting phosphatidylinositol-3-kinase/AKT/mTOR and ERK signaling [70].

Pursuing “failed” drugs?

Several known chemotherapeutic agents, such as topotecan, have been shown to inhibit HIF-1. One of the criticisms of proposed trials involving compounds such as topotecan is that these are drugs that have already failed to show significant anti-cancer effects in clinical trials. However, the key distinction that must be made between prior and current trials is that these drugs have previously been administered episodically at maximum tolerated dose as cytotoxic agents, whereas their current utilization as HIF inhibitors involves frequent administration at lower doses in an effort to maintain continuous inhibition of HIF activity. In a recently completed pilot pharmacodynamic study involving 16 patients with advanced cancer and biopsy-proven HIF-1α overexpression, the administration of topotecan orally at 1.6 mg/m^2/day × 5 days/week × 2 weeks/28-day cycle resulted in decreased tumor blood flow by DCE-MRI in 7 of 10 patients and loss of HIF-1α expression on repeat biopsy in 4 of 7 patients, suggesting that the drug hit the target *in vivo* [53]. During the trial, the topotecan dose was reduced to 1.2 mg/m^2/day due to myelosuppression, although it is not known whether this side effect reflected cytotoxicity due to DNA damage or was a direct consequence of HIF inhibition, independent of DNA damage. In either case, it remains to be determined (i) whether 2-week-on/2-week-off inhibition of HIF will be an effective anti-cancer strategy; and (ii) whether there is a therapeutic window that will allow chronic use of topotecan as a HIF inhibitor. Digoxin is an appealing candidate in this regard because it has been used for decades in the treatment of heart disease and blood levels of the drug that are safe and therapeutic (in the context of heart disease) are well established.

No drug has a single effect

Another means of disparaging the use of existing (and off-patent) drugs is the claim that novel anti-cancer agents are “specific” whereas repurposed drugs are “non-specific”. It does not require much understanding of pharmacology to appreciate that this claim represents a triumph of hope over data and marketing over science; i.e. the older the drug, the more likely it is that additional targets have been identified, which is a reflection of time, not inherent drug specificity. In the case of digoxin, it is not clear whether inhibition of HIF-1α synthesis is dependent on its known activity as a Na^+/K^+ ATPase inhibitor. However, mouse xenograft studies have demonstrated that the arrest of tumor growth that occurred when mice bearing prostate cancer xenografts were treated with digoxin was lost when the prostate cancer cells were engineered to express HIF-1α from a vector that was not inhibited by digoxin, demonstrating that the ability of digoxin to block tumor growth was dependent upon its ability to inhibit HIF-1α expression [51]. Hence, the activity of digoxin as an anti-

cancer agent can be directly attributed to its activity as a HIF inhibitor. Combining several HIF inhibitors may allow lower doses of each individual drug, thereby reducing the likelihood of off-target effects. Combination therapy may also reduce the probability of selecting for drug resistant cancer cells.

Can HIF inhibitors improve current therapies?

In addition to extensive data indicating that HIFs mediate resistance to radiation therapy [28] and chemotherapy [29], there is mounting evidence that HIF-1 activity may contribute to the development of resistance to novel targeted therapies, such as imatinib treatment of chronic myeloid leukemia [47]. HIF-1 appears to mediate resistance to imatinib through metabolic reprogramming, by activating expression of transketolase and thereby increasing glucose flux through the non-oxidative arm of the pentose phosphate pathway [47]. The switch from oxidative to reductive metabolism that is mediated by HIF-1 [44] has the effect of reducing cellular ROS levels [16, 44], which may increase resistance to cytotoxic chemotherapy [71].

In the case of VEGF receptor inhibitors, data from several mouse models indicate that treatment, either with the anti-VEGFR2 antibody DC-101 or the small molecule tyrosine kinase inhibitor sunitinib, reduced primary tumor growth and vascularization but increased metastasis, probably because impaired angiogenesis led to increased intratumoral hypoxia and increased HIF activity [72–74]. The failure of the anti-VEGF antibody bevacizumab to affect breast cancer progression, which led to revocation of approval by the FDA [75], may involve HIF-1-dependent expression of other angiogenic growth factors. In contrast, HIF inhibitors dramatically decreased the spontaneous metastasis of human breast cancer cells to the lungs in mouse orthotopic transplantation models by affecting multiple steps in the metastatic process [26, 27]. Taken together, these results suggest that combination treatment with HIF inhibitors may improve the efficacy of anti-angiogenic agents, a conclusion that is supported by data from mouse models [76].

Traditional chemotherapy may also be more effective when administered with a HIF inhibitor and many different molecular mechanisms underlie this effect in a cell-type and chemotherapy-specific manner. First, HIFs have been shown to regulate the expression of genes encoding ATP-binding cassette multidrug transporters, including MDR1 (*ABCB1*) and BCRP (*ABCG2*), which efflux chemotherapy drugs from cancer cells [77, 78]. Second, HIF-1 inhibits chemotherapy-induced cancer cell senescence [79]. Third, HIF-1 inhibits expression of pro-apoptotic mitochondrial proteins (*BAX, BID*) and caspases (*CASP3, CASP8, CASP10*) and induces expression of anti-apoptotic proteins (*BCL2*, *BIRC5*) [80–84]. Fourth, HIF-1 prevents chemotherapy-induced DNA damage by inhibiting the expression of topoisomerase IIα protein [85] or the DNA-dependent protein kinase complex [86]. Fifth, HIF-1-dependent metabolic reprogramming [16, 17, 30, 44, 45, 87, 88] may decrease ROS levels and thereby inhibit chemotherapy-induced cell death [89].

Caveats

There are important caveats regarding the safety and efficacy of HIF inhibitors as anti-cancer agents. A major safety issue is that patients with severe ischemic cardiovascular disease may experience exacerbation of their condition, and HIF inhibitors are contraindicated in such cases. With respect to efficacy, many of the inhibitors described have been found to inhibit HIF activity in some but not all cancer cell lines tested. This may be due to several potential mechanisms. First, drug levels in resistant cell lines may not be high enough to inhibit HIF activity. Second, the targeted pathway may not be contributing to HIF activity in the resistant cell line. Third, an alternative pathway may be activated in response to treatment. Strategies for monitoring HIF activity *in vivo* to test for drug response early in the treatment course would be valuable as a means of demonstrating that the drug is

hitting its target. Although indirect and highly expensive, imaging techniques that monitor tumor perfusion (e.g. DCE MRI) or glucose uptake (FDG-PET) may be useful for this purpose.

Concluding remarks

Preclinical data provide compelling evidence that HIFs play important roles in many critical aspects of cancer biology and that inhibition of HIFs, both in cancer and stromal cells, inhibits tumor growth, vascularization, metabolic reprogramming, invasion, metastasis, and resistance to radiation therapy and chemotherapy. Clinical data indicate that HIF-1α overexpression is associated with increased risk of patient mortality in many cancers. The addition of HIF inhibitors to existing therapeutic regimens is likely to improve their efficacy, particularly in cancers in which HIF-1α overexpression is documented in the diagnostic tumor biopsy and in those cases in which the existing therapy induces HIF activity, as in the case of anti-angiogenic and vascular targeting agents. Preclinical data from relevant mouse models is particularly important in providing scientific justification to support clinical trials. Finally, the HIF inhibitor acriflavine, which potently inhibits prostate cancer xenograft growth [12] and breast cancer metastasis to the lungs [90] in mouse models, was used as an antimicrobial agent in the 1930s [91] but is not currently available in a pharmaceutical preparation suitable for administration to patients. Since pharmaceutical companies are unlikely to manufacture such off-patent drugs, the NCI has an obligation to the oncology community to perform this function.

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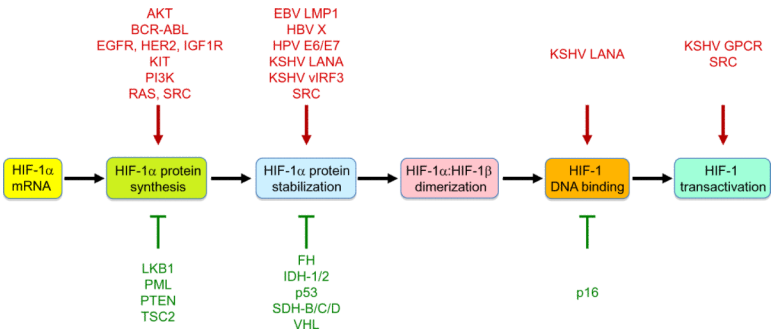

Figure 1.

Regulation of HIF-1 activity by oncoproteins and tumor suppressors. HIF-1 activity is stimulated by oncoprotein (red) gain-of-function. HIF-1 activity is inhibited by tumor suppressors (green) and their loss-of-function therefore stimulates HIF-1 activity.

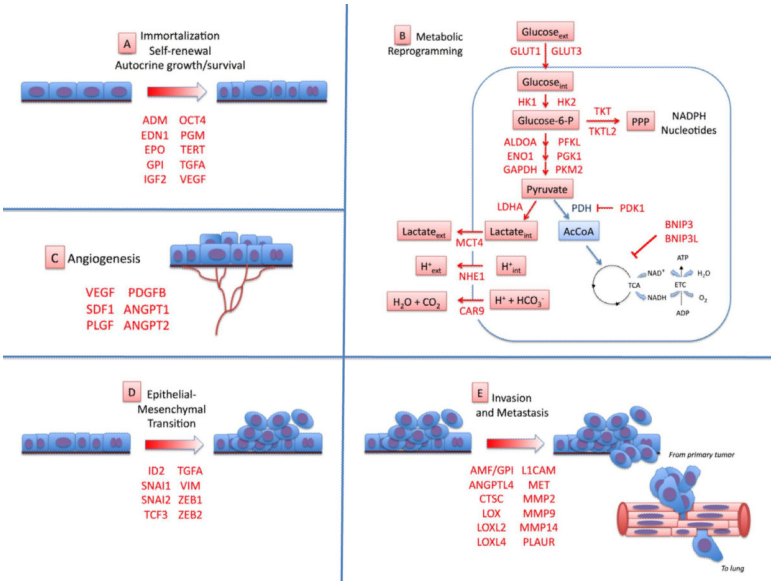

Figure 2.

HIF target genes encode proteins involved in critical aspects of cancer progression. The list of HIF-regulated genes (shown in red) is intended to be illustrative rather than comprehensive. (**A**) HIF target genes (locus abbreviations in parentheses) that promote cell immortalization, stem cell self-renewal, and autocrine growth and survival include those encoding adrenomedullin (*ADM*), endothelin 1 (*EDN1*), erythropoietin (*EPO*), glucose-6-phosphate isomerase (*GPI*), insulin-like growth factor 2 (*IGF2*), octamer binding protein 4 (*OCT4*), phosphoglycerate mutase (*PGM*), telomerase (*TERT*), transforming growth factor α (*TGFA*), and vascular endothelial growth factor (*VEGF*). (**B**) HIF-1 target genes involved in metabolic reprogramming include glucose transporter 1 and 3 (*GLUT1, GLUT3*), hexokinase 1 and 2 (*HK1, HK2*), glycolytic enzymes aldolase A (*ALDOA*), enolase 1 (*ENO1*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), phosphofructokinase L (*PFKL*), phosphoglycerate kinase 1 (*PGK1*), pyruvate kinase M2 (*PKM2*), and lactate dehydrogenase A (*LDHA*). The enzymatic activity of pyruvate dehydrogenase (PDH) is inhibited by PDH kinase 1 (PDK1), thereby blocking the conversion of pyruvate to acetyl coenzyme A (AcCoA) for entry into the tricarboxylic acid (TCA) cycle. Two members of the BCL2 family of mitochondrial proteins (*BNIP3, BNIP3L*) trigger mitochondrial selective autophagy. Lactate and hydrogen ion (H^+) generated by glycolysis are effluxed from the cell through the activity of the monocarboxylate transporter 4 (*MCT4*), sodium-hydrogen exchanger 1 (*NHE1*), and carbonic anhydrase 9 (*CAR9*). HIF-1 may also regulate the expression of the transketolase (*TKT*) and TKT-like 2 (*TKTL2*) enzymes of the non oxidative arm of the pentose phosphate pathway (PPP). (**C**) HIFs stimulate tumor vascularization by activating transcription of the genes encoding VEGF, stromal-derived factor 1 (*SDF1*), placental growth factor (*PGF*), platelet-derived growth factor B (*PDGFB*), and angiopoietin 1 and 2 (*ANGPT1, ANGPT2*). (**D**) HIF target genes that promote epithelial mesenchymal transition include inhibitor of differentiation 2 (*ID2*), snail 1 and 2 (*SNAI1, SNAI2*), transcription factor 3 (*TCF3*), TGFA, vimentin (*VIM*), and zinc finger E-box-binding homeobox 1 and 2 (*ZEB1, ZEB2*). (**E**) HIF target genes promoting invasion and metastasis include those encoding autocrine motility factor (*AMF*; also known as *GPI*), angiopoietin- like 4 (*ANGPTL4*), cathepsin C (*CTSC*), lysyl oxidase (*LOX*), LOX-like 2 and 4 (*LOXL2, LOXL4*), L1 cell adhesion molecule (*L1CAM*), Met proto-oncogene/ hepatocyte growth factor receptor (*MET*), matrix metalloproteinase 2, 9, and 14 (*MMP2, MMP9, MMP14*), and the urokinase plasminogen activator receptor (*PLAUR*).

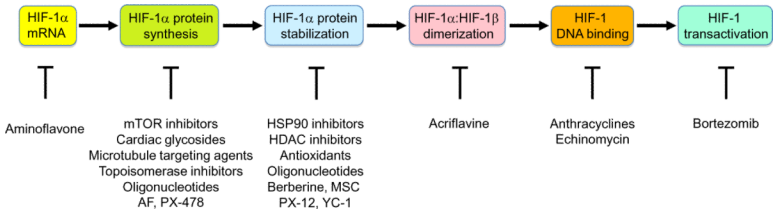

Figure 3.

Molecular mechanism of action of drugs that inhibit HIF-1. The steps required for the transactivation of target genes by HIF-1 are shown in the colored ovals and the drugs that inhibit each step are shown below. Abbreviations: AF, aminoflavone, MSC, Se-methylselenocysteine.