Practical Strategies for Suppresssing Hypoxia-Inducible Factor Activity in Cancer Therapy

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Abstract

The utility of anti-angiogenic strategies for cancer control is strongly compromised by hypoxia-driven phenotypic changes in cancer cells, which make cancer cells more invasive and more prone to give rise to metastases. A key mediator of this phenotypic shift is the transcription factor hypoxia-inducible factor-1 (HIF-1), which acts directly and indirectly to promote the epidermal-mesenchymal transition, boost cancer invasiveness, increase production of angiogenic factors, and induce chemoresistance. In some cancers, HIF-1 activity is constitutively elevated even in aerobic environments, making the cancer harder to treat and control. Practical strategies for suppressing HIF-1 activation may include the following: Inhibiting NF-kappaB activation with salicylic acid and/or silibinin, which should decrease transcription of the HIF-1alpha gene; suppressing translation of HIF-1alpha mRNA with drugs that inhibit mTOR or topoisomerase I; supporting the effective activity of prolyl hydroxylases – which promote proteasomal degradation of HIF-1alpha under aerobic conditions – with antioxidant measures, alpha-ketoglutarate, and possibly dichloroacetate; promoting the O2-independent proteasomal degradation of HIF-1alpha with agents that inhibit the chaperone protein Hsp90; and blocking HIF-1 binding to its DNA response elements with anthracyclines. The utility of various combinations of these strategies should be tested in cancer cell cultures and rodent xenograft models; initial efforts in this regard have yielded encouraging results. Comprehensive strategies for suppressing HIF-1 activity can be expected to complement the efficacy of cancer chemotherapy and of effective anti-angiogenic regimens.

Hypoxia Makes Cancers More Invasive and Chemoresistant

Many tumors contain hypoxic regions, reflecting an often chaotic evoked vasculature that does an uneven job of delivering oxygen, as well as large intercapillary distances stemming from exuberant cancer cell proliferation; anemia induced by treatment or malignant progression frequently exacerbates tumor hypoxia.[1-3] It has long been known that hypoxic tumors tend to be resistant to both chemotherapy and radiotherapy, and to have increased capacity for tissue invasion and formation of new metastases. Thus, as has been demonstrated in clinical studies, hypoxic tumors, as contrasted with better aerated tumors, are associated with a poorer prognosis.[4-7] Although considerable effort is being devoted to devising effective anti-angiogenic therapies for cancer, recent rodent studies reveal that successful suppression of angiogenesis

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rapidly induces a phenotypic change in cancer cells which increases their capacity to invade healthy tissue – often co-opting the pre-existing vasculature of that tissue – and to form new metastases.[8-10] Induced hypoxia is likely to be a key driver of this phenotypic change. The relatively modest impact of anti-angiogenic drugs on survival in most clinical trials to date may reflect two phenomena – acquisition by tumors cell of the ability to produce a wider range of angiogenic factors (thereby vitiating the utility of angiostatic therapies targeting VEGF signaling), owing to selection or regulatory responses to hypoxia;[11, 12] and a hypoxia-driven increase in capacity for invasive growth and metastasis formation. Furthermore, it can be anticipated that, when anti-angiogenic measures are used as adjuvants to chemotherapy or radiotherapy, hypoxia-evoked chemo/radio-resistance will compromise the efficacy the efficacy of these therapies. Thus, it is evident that concurrent measures for blunting hypoxia-induced phenotypic changes will be required if anti-angiogenic therapy is ever to achieve its goal of providing long-term cancer control and improved survival.

A Key Role for Hypoxia-Inducible Factor

It is generally acknowledged that one of the key mediators of hypoxia-evoked adverse phenotypic changes in cancers is hypoxia-inducible factor-1 (HIF-1).[13-15] HIF-1 is a heterodimer; the HIF-1beta subunit is expressed constitutively, whereas the expression of HIF-1alpha is regulated in several key ways. HIF-1alpha has a much longer half-life in hypoxic cells, owing to the fact that its chief mechanism for proteasomal degradation is oxygen-dependent – hence its description as "hypoxia-inducible".[16] Prolyl hydroxylase enzymes utilize molecular oxygen to hydroxylate two key prolines in HIF-1alpha; these hydroxylations are prerequisites for interaction of HIF-1 of with the von Hippel-Lindau ubiquitin ligase which enables its proteasomal degradation. Good oxygen availability also compromises the transactivational activity of HIF-1 by supporting the hydroxylase-catalyzed hydroxylation of an asparagine in HIF-1alpha's transactivational region; this hydroxylation blocks the interaction of HIF-1 with it key transcriptional coactivator, p300/CBP. Thus, in well-oxygenated cells, both the level and the transactivational activity of HIF-1 tend to be suppressed. In cells that are relatively hypoxic, an O2-independent pathway for proteasomal degradation of HIF-1alpha, triggered by its association with RACK1, is a major determinant of HIF-1alpha half-life; the molecular chaperone Hsp90 competes with RACK1 for binding to HIF-1alpha, thereby prolonging its half-life.[17] As discussed below, important regulation of HIF-1alpha expression also occurs at the transcriptional and translational level.

Nuclear HIF-1 drives the transcription of at least 40 genes which express hypoxia response elements in their promoters. Most of these genes help cells to cope with chronic hypoxia. A number of proteins involved in glucose uptake and glycolysis are HIF-1-inducible, reflecting the utility of glycolysis as an alternative strategy for ATP generation when oxygen availability is low. Indeed, increased HIF-1 activity is believed to be a key mediator of the "Warburg phenomenon", whereby aggressive cancer cells tend to be characterized by high glycolytic

capacity, resulting in the acidification of the extracellular space in tumors.[18-20] HIF-1 activation also promotes compensatory angiogenesis, in large part by boosting transcription of the VEGF and angiogenin genes.[21, 22] Growth and survival of hypoxic cells is promoted by HIF-1-driven expression of certain autocrine growth and survival factors, and by modulation of the expression of certain apoptosis-regulatory proteins in a direction that tends to inhibit apoptosis.[23-26] The chemoresistance provoked by HIF-1 activity reflects not only a diminished capacity for apoptosis, but also increased expression of the multi-drug resistance protein (MDR) which induces extrusion of many xenobiotic chemicals – including various chemotherapy drugs – from cells.[25, 27] Finally, HIF-1 promotes the invasive capacity and motility of cells, by increasing the production and activity of various proteolytic enzymes that degrade ground substance and basement membranes, and by promoting an epidermal-mesenchymal transition that lessens the homotypic binding of cells to each other.[28-34] The impact of HIF-1 on invasiveness is largely indirect: HIF-1 boosts expression of the tyrosine kinase receptor c-Met, as well as of an enzyme required for extracellular activation of its natural ligand, hepatocyte growth factor/scatter factor (HGF/SF).[35-39] The latter is avidly produced by hypoxic fibroblasts in tumors; thus, a tumor-stroma interaction can induce vigorous activation of c-Met in hypoxic cancer cells. c-Met activation signals by a diverse range of pathways, and markedly amplifies the capacity of cancer cells for invasion and metastasis; indeed, elevated c-Met activity has been observed in many aggressive cancers, and various drugs for blocking c-Met activity are in various stages of development.[40-42]

Not surprisingly, many pharmaceutical companies are also attempting to develop new drugs capable of inhibiting HIF-1 activity.[14, 43] Well-tolerated agents targeting HIF-1 could be expected to slow the spread of many aggressive cancers, and to amplify the efficacy of concurrent chemotherapeutic or anti-angiogenic measures. However, until such agents become clinically available, our growing understanding of how the expression of HIF-1 is regulated allows us to conclude that many currently available agents have the potential to suppress HIF-1 activity in a less direct manner.

The cellular concentration of HIF-1alpha is now known to be regulated on at least three levels: transcription, translation, and proteasomal degradation. Strategies for influencing each of these levels of regulation can be proposed.

NF-kappaB Regulates HIF-1 at the Transcriptional Level

Until recently, little was known about transcriptional regulation of HIF-1alpha. However, it is now clear that the HIF-1alpha promoter contains an NF-kappaB response element, and that NF-kappaB activity drives transcription of the HIF-1alpha gene.[44-46] In some cells, NF-kappaB appears to play an obligate role in this regard, as agents which inhibit NF-kappaB activity tend to suppress the HIF-1 activity evoked by hypoxia. It is interesting to note that NF-kappaB and HIF-1 are somewhat functionally homologous – thus, like HIF-1, NF-kappaB tends to promote

cell invasiveness by increasing the production and activation of certain extracellular proteases; to induce angiogenesis via increased expression of certain angiogenic factors (including VEGF, but also IL-8, which enables cancer cells to execute an "end run" around angiostatic drugs targeting VEGF activity); and to provoke chemoresistance by increasing the expression of various anti-apoptotic proteins and of MDR.[47-55] Indeed, constitutive activation of NF-kappaB is observed in many cancers, and is believed to be a key mediator of aggressive behavior and chemoresistance in these cancers. Moreover, hypoxia induces activation of NF-kappaB in various tissues and cancers;[45, 56-61] while the mechanism behind this effect is still somewhat obscure, it has recently been discovered that oxygen-dependent prolyl hydroxylase activity drives the proteasomal degradation of IkappaB kinase-beta (IKKbeta) , a key mediator of the canonical pathway of NF-kappaB activation; thus, increased expression of IKKbeta would be expected to up-regulate NF-kappaB activation in hypoxic cells.[62, 63] In hypoxic cells, NF-kappaB and HIF-1 can be expected to collaborate as a pernicious "tag team", rendering cancers aggressive and difficult to treat.

Fortunately, several agents currently available have the potential to suppress NF-kappaB activation: most notably, salicylic acid and silibinin. The well documented utility of salicylate in the management of arthritis appears to reflect, not a direct impact of salicylate on cyclooxygenase activity, but rather the ability of therapeutic concentrations of salicylate to bind to the ATP-binding region of IKKbeta, thereby inhibiting its kinase activity.[64-67] Most though not all pathways of NF-kappaB activation require IKKbeta activity, which promotes the phosphorylation and subsequent proteasomal degradation of IkappaB; the resulting decline in levels of IkappaB – which functions to bind NF-kappaB dimers and retain them in the cytoplasm – enables NF-kappaB to translocate to the nucleus and transactivate its many target genes. In dose of 3 g daily (usually 1.5 g b.i.d.), particularly in the well-tolerated delivery form salsalate, salicylate has clinically useful anti-inflammatory activity – presumably reflective of suppressed NF-kappaB activity – and is well tolerated by most patients, the dose-limiting toxicity being ototoxicity (tinnitus, mild hearing loss) that is rapidly reversible.[68-71] (The characteristic toxicities of NSAIDs are not seen with salicylate, presumably because its impact on cyclooxygenase is very weak and reversible.[72-74]) Thus, salsalate may have practical utility in cancer management, both as an adjuvant to chemotherapy, and in the long-term control of cancer aggressiveness.[75]

Another phytochemical with exciting potential for cancer therapy, which likewise has the potential to suppress NF-kappaB activation, is silibinin, the chief component of the traditional herbal extract silymarin (from milk thistle), long used in the treatment of inflammatory hepatic disorders. In cell culture studies, concentrations of silibinin within or close to the range that can be achieved via oral administration have demonstrated a diverse array of beneficial effects on cancer cell signaling pathways, including suppression of NF-kappB activation.[76] Silibinin-mediated suppression of NF-kappaB activity has also been demonstrated in vivo in xenograft models.[77-79] Oral administration of silibinin to nude mice xenografted with a diverse array of

human cancers has achieved a marked reduction in tumor growth rates, without any evident toxicity.[80-85] Indeed, there probably is no other phytochemical which, when administered orally, has been shown to slow the growth of so many types of human cancers in mice. Oral absorption of silibinin is greatly enhanced if it is blended intimately with phosphatidylcholine; a proprietary blend of this sort (Siliphos™ – one-third silibinin by weight) has recently been assessed in a Phase I trial in prostate cancer patients; the dose chosen for Phase II studies was 13 g daily, divided into 3 doses and blended with apple sauce.[86]

The "fly in the ointment" with respect to targeting NF-kappaB in cancer therapy, is that dendritic cell maturation toward a Th1 phenotype required for an effective immune assault on cancers is dependent on NF-kappaB activation.[87, 88] Thus, administration of either salicylate or silibinin may not be consistent with optimal immune scavenging of cancer cells, and may be contraindicated in the context of aggressive cancer immunotherapies.[88-90]

mTOR, Topoisomerase I, and Cardiac Glycosides Regulate Translation of HIF-1alpha

In many cancers, dysregulation of growth factor activity leads to chronic activation of the PI3K-Akt-mTOR pathway; this effect is particularly acute in the significant fraction of cancers in which the natural antagonist of PI3K, the serine phosphatase PTEN, has markedly diminished activity. mTOR activity, in the context of the TORC1 complex, is known to accelerate the translation of HIF-1alpha mRNA – presumably reflecting the fact that the 5' untranslated region of this mRNA contains a 5'-TOP sequence that renders its translation responsive to mTOR activity.[91-93] It follows that rapamycin, and related clinical agents that can inhibit mTOR, have the potential to impede expression of HIF-1alpha at the translational level. This effect should be particularly notable in those cancers in which the PI3K –Akt-mTOR pathway is markedly up-regulated. The mTOR activity of many tumors can be suppressed less directly by agents or strategies which target growth factor activities that trigger PI3K activation. For example, drugs which target EGFR, HER2-Neu, or BCR-Abl receptors may lessen mTOR activation – and hence the translation of HIF-1alpha mRNA – in certain cancers dependent on these growth factor receptors. Down-regulation of systemic levels of insulin and of free IGF-I with a low-fat, whole-food vegan diet and exercise training may also have some utility in this regard, as activation of PI3K is a prominent effect of insulin/IGF receptors.[94-96] It may also be noted that silibinin has been reported to lessen mTOR activation in certain cancer cells in vitro – so silibinin has the potential to down-regulate HIF-1alpha at at least two levels.[93]

A particularly remarkable recent finding is that, in cells that express topoisomerase I, anti-cancer drugs which target this enzyme tend to suppress the translation of HIF-1alpha mRNA.[97-100] This phenomenon is dependent on new transcription of mRNA, but otherwise is poorly understood. Cell cytotoxicity is not requisite for this effect, so it is conceivable that relatively low metronomic doses of topoisomerase I inhibitors that are reasonably well tolerated could be used clinically to suppress HIF-1 activity. One exciting recent study has assessed the impact of

bevacizumab (a monoclonal which suppresses angiogenesis by targeting VEGF) and of metronomic topotecan, singly or jointly, in nude mice implanted with a human cancer cell line derived from a glioblastoma; each agent used singly modestly retarded tumor growth, whereas the combined therapy led to progressive tumor regression.[101] This study may serve as a model for the rational strategy of combining anti-angiogenic measures with agents that suppress HIF-1 activity. This principle is likewise illustrated by a study demonstrating that joint administration of everolimus (a rapamycin analog) and metronomic cyclophosphamide (an anti-angiogenic measure) achieved long-term growth control of a human gastric cancer xenograft, as compared to a modest benefit seen when either agent was administered as monotherapy.[102] Another recent study demonstrates that joint administration of rapamycin and low-dose irinotecan has a much more dramatic impact on the growth of human xenografts than when either agent is used singly.[103] Intriguingly, whereas either drug alone diminished tumor HIF-1 levels by about 50%, the combination essentially abolished HIF-1 expression.

Irinotecan, a structural analogue of topotecan, is metabolized to a potent inhibitor of topoisomerase I, and is approved for parenteral use in various forms of cancer. Phase I studies have demonstrated that irinotecan is well absorbed upon oral administration, and is no more toxic when administered in this way. [104, 105] An evaluation of metronomic oral irinotecan found that it was well tolerated in the range tested – 1.4-4.2 mg/m2/day – and induced significant increases in plasma levels of thrombospondin-1 (suggestive of anti-angiogenic activity) in these doses.[106] Hopefully future trials will assess the impact of such dose schedules on HIF-1 activity. We need to be mindful of the possibility that some cancers could evolve resistance to this effect by suppressing their expression of topoisomerase I (which would be feasible in cancers with high topoisomerase II activity).

Semenza and colleagues have screened over 3,000 drugs for their impact on HIF-1 activity in hypoxic human hepatoblastoma cells; they were able to identify twenty drugs which reduced this activity by >88% at a concentration of 400 nM.[107] Remarkably, 11 of these drugs were cardiac glycosides. Subsequent cell culture studies revealed that this suppressive effect reflected inhibition of HIF-1alpha mRNA translation. However, effective concentrations did not inhibit mTOR activity, and concurrent knock-down of either topoisomerase I or the alpha1 subunit of Na+/K+-ATPase (the target responsible for the cardiac activities of cardiac glycosides) did not influence the impact of cardiac glycosides on HIF-1 activity; hence, this effect is mechanistically distinct from that of mTOR or topoisomerase inhibitors. A concentration of 50 nM or more was required to achieve a substantial decrease of HIF-1 expression within 24 hours in vitro; in mice bearing human tumor xenografts a daily dose of 2 mg/kg digoxin markedly retarded xenograft growth, but had comparatively little impact on a tumor bioengineered to express constitutively high HIF-1 activity. Unfortunately, there is reason to suspect that these exciting findings may not be clinically relevant.[108] The clinically safe and effective serum concentration of digoxin is about 2 nM, far lower than the concentration shown to modulate HIF-1 activity in vitro. And the

efficacy of digoxin in a mouse xenograft model may reflect the fact that rodents are about 100-fold less sensitive to the clinical effects of cardiac glycosides on the sodium pump than are primates, and thus can tolerate doses (such as 2 mg/kg) that would be lethal in humans. Therefore, while other evidence suggests that cardiac glycosides may indeed someday earn a role in human cancer therapy,[109-112] it is doubtful that they could be used to modulate HIF-1 activity.

Regulation of Prolyl Hydroxylase Activity

The prolyl hydroxylases which hydroxylate HIF-1alpha, thereby dooming it to proteasomal destruction, have an obligate dependence on oxygen, since oxygen is used as a substrate for this reaction. Thus, the half-life of HIF-alpha will tend to be high in hypoxic cells (albeit this can be influenced to a degree by modulating heat shock protein activity, as explained below). However, even in cells that are well oxygenated, the activity of the prolyl hydroxylases which target HIF-1alpha can be suppressed, for various reasons. The active site of these hydroxylases contains a ferrous iron atom; oxidation of this atom to a ferrous state renders the enzyme inactive. This may explain why oxidative stress has been found to impair prolyl hydroxylase activity in certain cells lines – an effect that is antagonized by boosting intracellular levels of ascorbate or thiol reductants.[113-117] Indeed, there is evidence that oxidative stress contributes importantly to the loss of prolyl hydroxylase activity associated with hypoxia. Hypoxia – as contrasted with strict anoxia – somewhat paradoxically boosts superoxide generation by mitochondrial complex III; if this superoxide production is blocked, prolyl hydroxylase activity is maintained at an O2 level as low as 1.5%.[118-120]

In many cancers, a constitutive modest elevation of oxidative stress – produced by NADPH oxidase and/or mitochondria – has a favorable effect on cell proliferation and survival, in large part by inhibiting the activity of phosphatases that oppose tyrosine kinase activity.[121-125] This oxidative stress could be expected to boost HIF-1 activity through multiple complementary mechanisms: by inhibiting prolyl hydroxylase activities, by amplifying the PI3K/Akt/mTOR pathway that regulates HIF-1alpha mRNA translation, and by boosting transcription of the HIF-1alpha gene via NF-kappaB activation.[126-128] Phycocyanobilin, the chief phytochemical in spirulina, is a biliverdin derivative, and appears to share the ability of free bilirubin to inhibit certain isoforms of NADPH oxidase.[129] Thus, oral administration of sufficient intakes of spirulina may have the potential to quell oxidative stress in cancer cells with constitutively active NADPH oxidase[125] – an effect which might not only favorably affect cancer cell proliferation, but also lessen HIF-1 activity. Another antioxidant which might have potential for sustaining effective prolyl hydroxylase activity is N-acetylcysteine, which boosts glutathione levels in many tissues.[130] Maintaining good ascorbate status might also be worthwhile in this regard. Indeed, oral administration of high doses of either N-acetylcysteine or sodium ascorbate was found to slow the growth of human xenografts in nude mice – whereas a much lesser impact was noted on growth of cancer cells bioengineered to express constitutively high HIF-1 activity.[131] However, these findings should be viewed in the context of previous controlled clinical studies which

failed to note a clinical impact of either high-dose oral ascorbate or of N-acetylcysteine (600 mg daily, a dose proportionately much smaller than that used in the mouse study) on progression of human cancers.[132, 133] The potential utility of supplemental ascorbate in humans may be compromised by the fact that intracellular ascorbate uptake is nearly saturated by serum concentrations achieved with high-nutritional intakes.[134]

In addition to molecular oxygen, prolyl hydroxylases have an obligate requirement for alpha-ketoglutarate as a substrate. The related alpha-keto acids pyruvate and oxaloacetate can fit into the enzyme pocket reserved for alpha-ketoglutarate, and induce an inhibition of enzyme activity that is reversible with ascorbate or thiol reductants. This suggests that these agents may inhibit prolyl hydroxylase activity by oxidizing its ferrous iron.[117, 135] The reason why this may be functionally significant is that HIF-1 activity, by boosting the expression of glucose transporters, glycolytic enzymes, and pyruvate dehydrogenase kinase-1 (which inhibits pyruvate dehydrogenase activity), increases intracellular levels of both pyruvate and oxaloacetate – which in turn may tend to sustain elevated activity of HIF-1 by suppressing prolyl hydroxylase activities. This mechanism would tend to maintain HIF-1 activity even in well aerated cells. Conversely, the well tolerated drug dichloroacetate, which activates pyruvate dehydrogenase by inhibiting pyruvate dehydrogenase kinase-1,[136]and thereby promotes the oxidative disposal of pyruvate, has shown cancer-inhibitory effects in vitro and in vivo.[137-139] The impact of this drug on HIF-1 activity in cancer cells should be assessed.

Recent Japanese research has revealed that intracellular levels of alph-ketoglutarate can be rate-limiting for prolyl hydroxylase activity, such that exogenous administration of this agent boosts this activity.[140] Remarkably, parenteral administration of 50-100 mg/kg of alpha-ketoglutarate daily has been reported to slow the growth of Lewis lung carcinoma in nude mice dose-dependently, an effect associated with a reduction in tumor vascularity and VEGF expression.[141] Orally administrable alpha-ketoglutarate is commercially available in the U.S. in the form of creatine alpha-ketoglurate, a supplement favored by body builders. If further studies confirm the impact of alpha-ketoglutarate on cancer growth in rodents, the feasibility of using ample doses of this agent in cancer patients should be assessed.

HSP90 Stabilizes HIF-1alpha

Another factor which regulates the half-life of HIF-1alpha, most notably under hypoxic conditions, is heat shock protein 90 (Hsp90), which promotes the stability of this protein by binding to it and acting as a protective chaperone. As noted above, Hsp90 protects HIF-1alpha from a RACK1-dependent, O2-independent pathway of proteasomal degradation.[17] Thus, geldanamycin, a drug which antagonizes the chaperone activity of Hsp90 by binding to it tightly, has been reported to decrease HIF-1alpha levels in cancer cells, under both normoxic and hypoxic conditions.[142-146] If and when a geldamycin analog achieves clinical approval, it might well find a place in multi-modal strategies intended to suppress HIF-1 activity.

HIF-1 Transcriptional Activity is Modulated by Phosphorylation

The constitutive elevation of growth factor activities that is commonly seen in cancers tends to up-regulate, not only the PI3K-Akt-mTOR pathway, but also the Ras-raf-MEK-MAPK signaling module. Serine phosphorylations of HIF-1alpha mediated by p42/p44 MAP kinases promote its nuclear localization and transcriptional activity, apparently by inhibiting its extrusion from the nucleus.[147-149] Thus, measures which antagonize growth factor signaling in cancer cells could be expected, not only to suppress the translation of HIF-1alpha mRNA, but also to diminish the nuclear localization (and hence transcriptional activity) of HIF-1alpha.

Anthracyclines Inhibit HIF-1 DNA Binding

The same drug screening study which identified cardiac glycosides as HIF-1 antagonists, likewise found that several anthracycline cytotoxic agents could likewise inhibit HIF-1 activity; concentrations of about 0.2 µM achieved about a 50% reduction of HIF-1-mediated transcription.[150] Yet these drugs had no impact on the level of HIF-1 protein or mRNA, or on HIF-1's transactivational activity; rather, they were shown to inhibit binding of HIF-1 to its response elements in DNA. Daily doses of doxorubicin or daunorubicin in the range of 0.5-1.5 mg/kg daily were found to markedly suppress the growth of a human prostate cancer xenograft in nude mice, an effect associated with decreased tumor mRNA expression of HIF-1 targets such as VEGF. Adjusting for the 0.75 power of relative body weights, 0,5 mg/kg in a 20 g mouse would correspond to a daily dose of about 5 mg in a 70 kg man – well below the doses used in episodic chemotherapy. Thus, it is conceivable that metronomic low-dose anthracyclines could prove useful for controlling tumor HIF-1 activity. Unlike other anthracyclines, idarubicin has worthwhile oral bioavailability (~30%), and well tolerated chronic daily dosing regimens have been established for this agent.[151-155] Since anthracyclines can produce cumulative cardiac toxicity, concurrent administration of coenzyme Q10 and spirulina might be warranted when theses agents are used metronomically.[156-159] In that regard, it is encouraging that idarubicin appears less prone than other anthracyclines to produce cardiotoxicity.[160]

Overview

In summary, potentially feasible strategies for suppressing HIF-1 activity in aggressive cancers may include: salsalate and silibinin, which may lessen transcription of the HIF-1alpha gene by diminishing NF-kappaB activity (in itself a worthy goal when attempting to diminish cancer aggresssivenesss or chemoresistance); inhibitors of mTOR such as rapamycin and related compounds, which should slow translation of HIF-1alpha mRNA; various measures which target growth factor activities that promote mTOR activation via the PI3K/Akt pathway; topoisomerase I inhibitors, which likewise suppress translation of HIF-1alpha mRNA; agents which promote optimal prolyl hydroxylase activity, such as alpha-ketoglutarate, certain antioxidants (spirulina, N-acetylcysteine), and possibly dichloroacetate; anthracyclines, which inhibit HIF-1 binding to

its DNA response elements; and – when and if they become available – geldanamycin analogs that diminish the half-life of HIF-1alpha by impeding the chaperone function of Hsp90. It would be of interest to test various combinations of such measures in rodent cancer models; the recent study demonstrating the complementarity of rapamycin and low-dose irinotecan for tumor control represents a pioneering effort in this regard.

Effective strategies for suppressing HIF-1 activity may be expected to have particular utility as a complement to effective anti-angiogenic measures, which would be expected to amplify tumor activation of HIF-1 by exacerbating tumor hypoxia. Indeed, without concurrent measures which address hypoxia-evoked mechanisms that render cancer cells more invasive and prone to form metastases, the benefits of anti-angiogenic therapy will likely prove transient. In the many cancers in which NF-kappaB is activated, either constitutively or in response to hypoxia, measures which suppress NF-kappaB activation (salsalate, silibinin, effective antioxidant therapy) can be expected to do double duty in this regard – diminishing transcription of the HIF-1alpha gene, while also working in independent ways to decrease cancer invasiveness and lessen tumor production of a range of pro-angiogenic factors (including IL-8 and others not currently targeted by anti-angiogenic drugs that inhibit VEGF signaling). A global strategy which effectively suppresses angiogenesis while concurrently blocking compensatory signaling pathways that promote cancer invasiveness and metastatic capacity, could be expected to have a dramatic impact on the rate of tumor growth and spread, and perhaps merits its own name – "Stasis Therapy" would be reasonably descriptive. A sketch of a suggested protocol for Stasis Therapy is provided in Table 1.

Measures which suppress HIF-1 activity may also promote the efficacy of concurrent chemotherapy, by lessening cancer chemoresistance. (NF-kappaB inhibition should also have some efficacy in this regard, independent of its impact on HIF-1 activity.) HIF-1 suppression may also aid the efficacy of experimental glucose deprivation therapies, by potentiating the decline in intracellular glucose metabolites and down-regulating the pentose phosphate shunt – effects which could be expected to potentiate the oxidative stress imposed by glucose deprivation.[161-168]

It can be anticipated that immunotherapies will play an increasingly important role in future cancer treatment protocols. Unfortunately, not all of the measures with potential for suppressing angiogenesis or HIF-1 activity are likely to be compatible with an optimally effective immune assault on cancer. Thus, salsalate and silibinin, by blocking NF-kappaB activation, may impair the maturation and activation of dendritic cells.[169-173] Inhibition of mTOR by rapamycin analogs, while it may have favorable effects on the susceptibility of cancer cells to immune killing and on the Th1 differentiation of dendritic cells, could be expected to block T cell activation, and thus likely would not be optimally immunocompatible (as suggested by its utility in preventing graft rejection).[174-177] On the other hand, sunitinib, metronomic low-dose cyclophosphamide, and antioxidant measures could be expected to complement immunotherapy by intervening in

mechanisms which protect cancer cells from immune assault.[178-182] Whether metronomic doses of irinotecan or idarubicin sufficient to suppress HIF-1 activity would be low enough to allow adequate proliferation of immune effector cells remains to be determined. In any case, it should prove feasible to devise Stasis Therapy protocols, perhaps somewhat less effective than optimal regimens, which would be reasonably compatible with effective cancer immunotherapy. It stands to reason that cancer immunotherapy will have a far better chance of achieving progressive tumor regression and perhaps cure, if concurrent immunocompatible measures markedly slow the proliferation, invasion, and metastatic spread of cancer cells.

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