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The HIF pathway as a therapeutic target

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Hypoxia-inducible factor (HIF) is an α,β-heterodimeric transcription factor that mediates cellular responses to low oxygen concentration via the transcriptional activation of specific genes involved in both tumorogenesis and angiogenesis. Manipulation of the HIF pathway has potential use for the treatment of ischemic disease and cancer. Unlike HIF-β, which is constitutively expressed, the levels and activity of the HIF-α subunit are regulated by processes involving posttranslational hydroxylation, catalyzed by Fe(II)- and 2-oxoglutarate-dependent oxygenases. This review focuses on the HIF pathway as a therapeutic target.

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▼ The adaptation of many multicellular organisms to dioxygen availability is mediated by hypoxia-inducible factor (HIF), a heterodimeric α,β transcription factor [1]. Both HIF subunits are members of the basic helix–loop–helix (bHLH)-PAS (period circadian protein, arylhydrocarbon-receptor nuclear translocator, single-minded protein) family [2]; although the β-subunit (or the aryl hydrocarbon nuclear translocator, ARNT) is a constitutive nuclear protein, the α subunit is regulated by oxygen levels. Under hypoxic conditions dimerization of the HIF α- and β-subunits enables a transcriptional response involving the coactivator p300 (Figure 1). More than 40 target genes associated with pentanucleoside hypoxic response elements that enable binding to HIF have been identified, including erythropoietin (EPO) and vascular endothelial growth factor (VEGF). Three HIF-α isoforms have been identified; whereas HIF-1α is expressed ubiquitously, the other two isoforms appear to have restricted expression patterns [3–5].

Under normal oxidation ('normoxic') conditions, posttranslational hydroxylation of either of three residues in HIF-α enables repression of the HIF transcriptional response (Figure 1). HIF-1α and HIF-2α possess an oxygen-dependent degradation domain (ODDD) [6] containing two specific prolyl residues (P402 and P564 in human HIF-1α) as part of a conserved LxxLAP motif [7,8]. Two subdomains exist within the ODDD; the more N-terminal is termed the NODDD (containing P402) and the more C-terminal the CODDD (containing P564). Hydroxylation at either of these sites targets the HIF-α subunit to the von Hippel Lindau tumour suppressor protein (pVHL) E3 ubiquitin ligase complex, which mediates ubiquitination and subsequent proteasomal destruction of HIF-α [7,8]. Three human enzymes, PHD1–3 [9] (for prolyl hydroxylase domain containing, also independently identified as HPH1–3 and EGLN 1–3 [10,11]), have been identified as being responsible for this prolyl hydroxylation. Of these, PHD2 has been shown to have the highest specific activity towards HIF-1α and is ubiquitously expressed [12]. Whereas PHD2 and 3 (the latter corresponding to rat SM20) are predominantly cytoplasmically localized, PHD1 is predominantly localized in the nucleus [13].

In addition to the targeted prolyl residues of the ODDD, human HIF-1α and HIF-2α contain a specific asparaginyl residue in their C-terminal transactivation domains (CAD) [14], the activity of which is also regulated by oxidative modification [15]. Hydroxylation by factor inhibiting HIF (FIH) [16] of Asn803 in HIF-1α ablates the interaction between HIF-α and p300 [17,18].

Because the HIF hydroxylases have a requirement for dioxygen as a cosubstrate, under hypoxic conditions hydroxylation is suppressed, allowing nuclear accumulation of the HIF-α subunits and recruitment of p300. The relative importance of the individual PHD isozymes and FIH in the hypoxic response in different cell types has yet to be fully defined. Individuals with the hereditary von Hippel Lindau disease (inactivation of the *VHL* allele) are predisposed to highly angiogenic tumours [19]. Furthermore,

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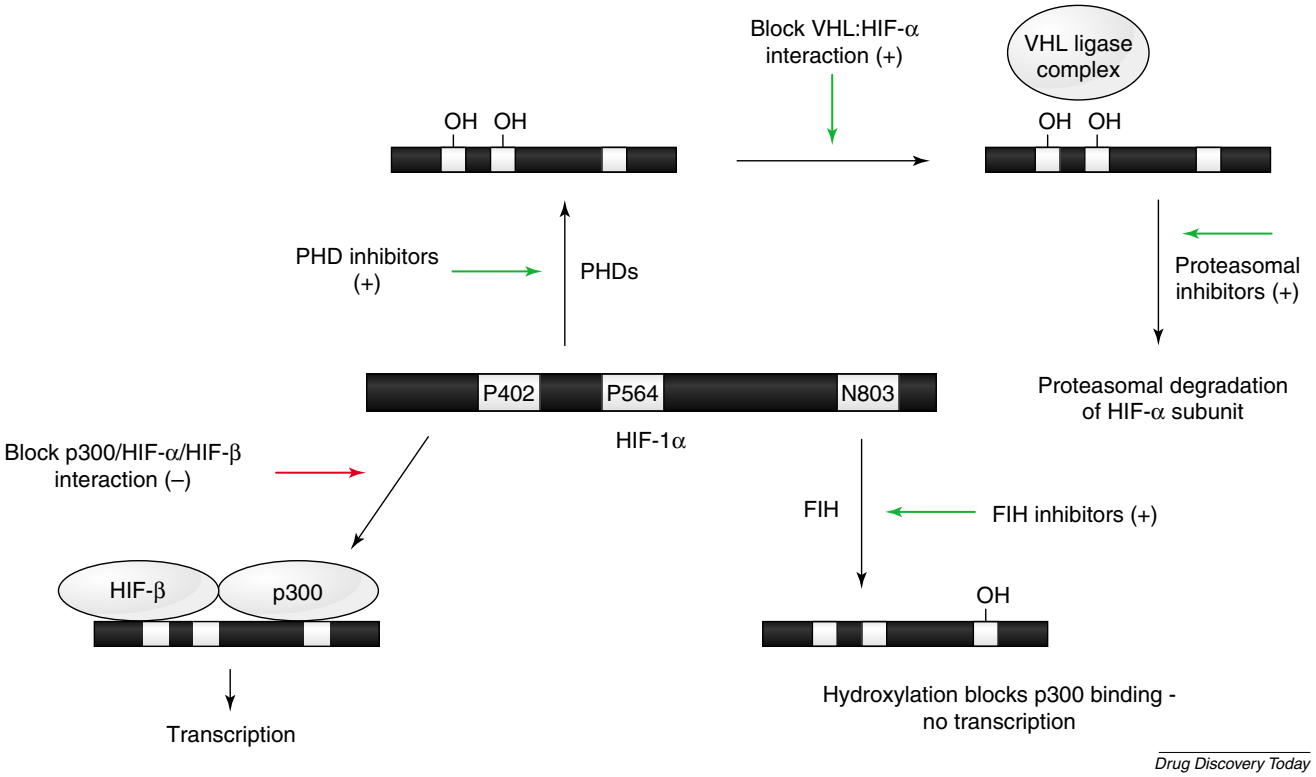

Figure 1. The HIF pathway. The coloured arrows indicate possible points of therapeutic intervention by small molecules, either to increase (green; +) or decrease (red; –) HIF levels or activity.

upregulation of HIF target genes occurs in *VHL* defective cells [20] suggesting that FIH cannot fully compensate for the loss of PHD effectiveness.

Biological significance of the HIF pathway

HIF-1α and HIF-2α have been shown to play roles in development, because their inactivation in mice results in embryonic lethality [4,21]. HIF-1α$^{-/-}$ embryos have numerous defects in cardiovascular development, whereas HIF-2α$^{-/-}$ embryos have normal vasculature, but defects in fetal lung maturation. These phenotypical differences might be indicative of differential target gene expression profiles, which could prove to be important from the perspective of therapeutic exploitation.

Rapidly growing cancer cells induce a hypoxic environment and efficient oxygenation is required to support tumour growth; this is enabled, at least in part, by the HIF system. The HIF pathway is activated in various cancers, suggesting that blocking it is a useful therapy for cancer treatment. Conversely, activation of the HIF system could be useful in the treatment of ischemic disease and numerous other diseases involving inflammatory and hypoxic conditions (see below).

There are several attractive points in the HIF pathway at which therapeutic intervention could be employed to exact either an increase or decrease in HIF activity (Figure 1). This review focuses on the activation of the HIF transcriptional response for the treatment of ischemic disease and in particular via inhibition of the HIF hydroxylases.

Upregulation of HIF activity in the treatment of ischemic and other disease states

Activation of the HIF system has been implicated in the treatment of a wide range of conditions including ischemia, heart attack, stroke, wounding and inflammation. In some cases, surgical intervention is not possible (for example in the treatment of critical limb ischemia for medical or anatomical reasons) and hence development of a pharmaceutical alternative is desirable. Activation of the HIF system for preconditioning before surgery to prevent ischemic injury is also an attractive goal.

HIF-mediated angiogenesis results from the transcriptional activation of several angiogenic factors including the various VEGF isoforms. Current pro-angiogenic treatments have focused on the delivery of a single VEGF isoform, either as naked plasmid DNA or as an adenovirus

[22–25]. Problems incurred with this methodology include the production of leaky blood vessels, which regress following termination of treatment, and associated edema.

In this respect, the modulation of HIF activity might be a more useful target to elicit a 'fuller' or more natural angiogenic response and initial results using this approach seem promising. Transgenic mice expressing constitutively active HIF-1α displayed a 13-fold elevation of total VEGF expression and a 6–9-fold induction of each VEGF isoform [26]. Additionally, injection of naked DNA encoding a HIF-1 (lacking the ODDD)–VP16 hybrid into a rabbit hind-limb ischemia model led to an increase in local blood supply [27]. A similar effect has also been observed in a rat myocardial model [28]. Studies with the rabbit hind-limb model showed no increase in the red blood cell count suggesting that EPO transcription had not been stimulated by treatment with the HIF-1–VP16 hybrid. However, modulation of the HIF system to increase levels of EPO is of interest, as EPO production remains a pharmaceutically useful target for the treatment of renal anaemia. By the end of 2001, the world market for recombinant human EPO was valued at US$5.3 billion.

Stabilization of the HIF-α subunit

Binding of the HIF-α subunit to the VHL ubiquitin ligase complex under normoxic conditions mediates its destruction (Figure 1). Stabilization of the HIF-α subunit through inhibition of this mechanism, or by inhibition of the PHDs, is thus a means whereby HIF activation, and hence therapeutic control, could be achieved. One view is that inhibition of the PHDs could be regarded as indirect proteasomal inhibition and could be more selective than direct inhibition of proteasomes.

When cells were transfected with plasmids encoding for the CODDD and NODDD regions of HIF-1α, endogenous HIF-1α together with transcriptional upregulation of carbonic anhydrase IX (CAIX) and Glut-1 (both HIF-regulated transcripts), was observed [29]. The effect of polypeptide induced HIF-α stabilization was further assessed using an *in vitro* angiogenesis assay. *Tat*-fused peptides were injected into polyurethane sponges and implanted subcutaneously in mice. Vessels of increasing density and complexity were produced following several days of injection, together with increased expression of Glut-1 and VEGF. The vessels produced were considered to be less leaky than those generated through conventional VEGF therapy, again suggesting activation of several angiogenic factors. It was proposed that the NODDD and CODDD peptides led to stabilization of the HIF-α subunits, either through saturation of the PHD isozymes or VHL binding.

PR39, a proline- and arginine-rich macrophage derived polypeptide, has been shown to stabilize HIF-1α by preventing its degradation via the proteasome pathway [30]. Although the mechanism by which PR39 acts is unclear, transgenic mice lines expressing PR39 did exhibit increased myocardial vasculature with the production of functional blood vessels.

The HIF hydroxylases

The HIF hydroxylases belong to the Fe(II)- and 2-oxoglutarate (2OG) -dependent oxygenase superfamily, which is involved in a range of biosynthetic and metabolic pathways. In humans these include collagen biosynthesis (procollagen prolyl-4-hydroxylase, CPH), DNA repair (AlkB, a demethylase) and fatty acid metabolism (γ-butyrobetaine hydroxylase and phytanoyl CoA hydroxylase). Structurally the 2OG oxygenase family is characterized by a double-stranded β-helix (DSBH or jelly roll) core formed from eight β-strands and three Fe-binding residues that form a HxD/E…H motif [31–33]. Most members of the family couple the oxidative decarboxylation of 2OG – to give succinate and carbon dioxide – with that of substrate oxidation.

Crystal structures are available for various 2OG oxygenases including FIH [34–36]. These analyses identified FIH as a member of a new sub-family of 2OG oxygenases. FIH is also one of the jumonji (JmjC) transcription factors that are also characterized by the presence of a DSBH [18,37]. Many of the putative proteins identified as JmjC transcription factors contain the conserved 2OG binding residues found in FIH and also the Fe(II)-binding residues. The JmjC proteins have been implicated in cell growth and heart development. It could be that many of the JmjC proteins are 2OG oxygenases that are involved in transcriptional regulation but, excepting FIH, their substrates are currently unknown.

Mechanism of the 2OG oxygenases

Although variations occur, a combination of kinetic, spectroscopic and structural studies suggest a common mechanism for the 2OG oxygenases (Figure 2). Following formation of an Fe(II)-enzyme complex, binding of 2OG and then substrate primes the enzyme for dioxygen binding. In some cases there might be prior association of the substrate with the enzyme. Substitution of an Fe-bound water molecule by dioxygen and subsequent decarboxylation of 2OG produces carbon dioxide, succinate and a ferryl intermediate [Fe^{IV}=O] that mediates substrate oxidation [31–33].

Small-molecule inhibition of 2OG oxygenases

The ferrous ion bound at the active site of all 2OG oxygenases is essential for activity. Cobalt(II) and nickel(II) ions have been shown to induce erythrocytosis [38], and activation of the HIF system through inhalation of nickel dust

increased the incidence of pulmonary fibrosis [39]. It seems likely that the mechanism involves displacement of the active iron site by cobalt(II) or nickel(II) ions. Treatment of cells with iron chelators such as desferrioxamine (DFX) stimulates HIF activity and EPO transcription, presumably by decreasing the amount of bound active site iron [40]. Although iron chelators and metal ions can be used to generate a HIF response, their use in therapy would not be confined to inhibition of the HIF hydroxylases, but would affect other 2OG oxygenases and other biomolecules that use metals.

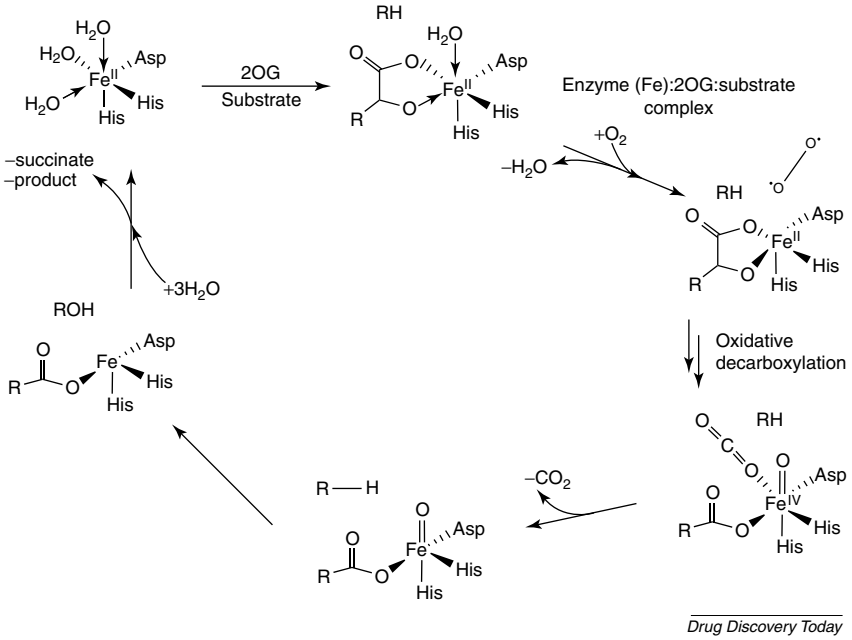

Figure 2. A general reaction scheme for hydroxylation at a saturated carbon as catalyzed by a 2-oxoglutarate (2OG) oxygenase. Oxidative decarboxylation of 2OG enables formation of a reactive ferryl intermediate [Fe^{IV}=O] that in turn reacts with substrate, RH. The point at which CO_2 leaves the active site is uncertain. In some cases the substrate might associate with the enzyme prior to 2OG binding.

2OG analogues and related compounds as HIF hydroxylase inhibitors

Previous small molecules used to target the HIF hydroxylases and other members of the 2OG superfamily have included 2OG analogues. In all cases so far, studies indicate that the 2OG binds in a bidentate manner, via its 1-carboxylate and 2-oxo groups to the active site iron. However, there are variations in the way in which the two methylenes and 5-carboxylate of 2OG are bound. In the case of one sub-family (including the bacterial enzyme deacetoxycephalosporin C synthase and the plant enzyme anthocyanidin synthase) the 5-carboxylate is bound by a conserved Y….RXS motif. However, the crystal structure of FIH [34–36] has revealed that instead of this motif, the 5-carboxylate of 2OG is bound by Lys, Thr and Tyr residues from non-conserved positions with respect to the Y….RXS sub-family.

The most widely used HIF hydroxylase inhibitor in research is the 2OG analogue *N*-oxaloylglycine (NOG) [7], in which an NH has been substituted for a methylene group at the 2OG 3-position (Figure 3a). NOG was originally developed as a CPH inhibitor [41] and presumably works by decreasing the susceptibility to nucleophilic attack by an oxygen-activated species at the 2-carbonyl group functionality or, by virtue of its different electronic properties, hindering oxygen binding. NOG inhibits all 2OG oxygenases for which it has been tested, but is also known to inhibit other enzymes. Because its diester derivative is taken up by cells, it has use as a generic inhibitor. Thus, care must be taken in assuming that observations at the cellular level or above are due to its action as a HIF hydroxylase inhibitor.

N-Oxalyl derivatives of other amino acids were also synthesized as CPH inhibitors, but displayed lowered activity relative to NOG [41]. Different enantiomers of *N*-oxalylalanine appear to show different selectivity for both FIH and the PHDs [7,36]. This presumably reflects differences in the 2OG 5-carboxylate-binding residues of the enzymes and the amino acid architecture in the immediate vicinity. In this respect, it is possible that potent and selective inhibitors of the PHDs versus FIH might be found that are based solely on binding to Fe and the 2OG-binding pocket. However, the use of other interactions, such as those involving binding of the HIF-α substrate, might also be required to obtain inhibitors that are selective for different PHD enzymes.

In addition to the *N*-oxalyl amino acid derivatives, a recent study explored various other 2OG (**1**) analogues as PHD inhibitors (Figure 3) [42]. Substitution of the nitrogen of the amide of NOG (**2**) with a sulfur to give a thiol ester (**3**) gave a similar level of activity as NOG, but the thionoamide analogue (**4**) was less active. Inhibitors in which the 2-keto group of 2OG was replaced with either a thiol or an alcohol gave contrasting results, with the former but not the latter, being significantly active. This work also identified various PHD inhibitors with hydrophobic groups, for example *N*-benzoyl-(*S*)-glutamate, leading to the proposal of

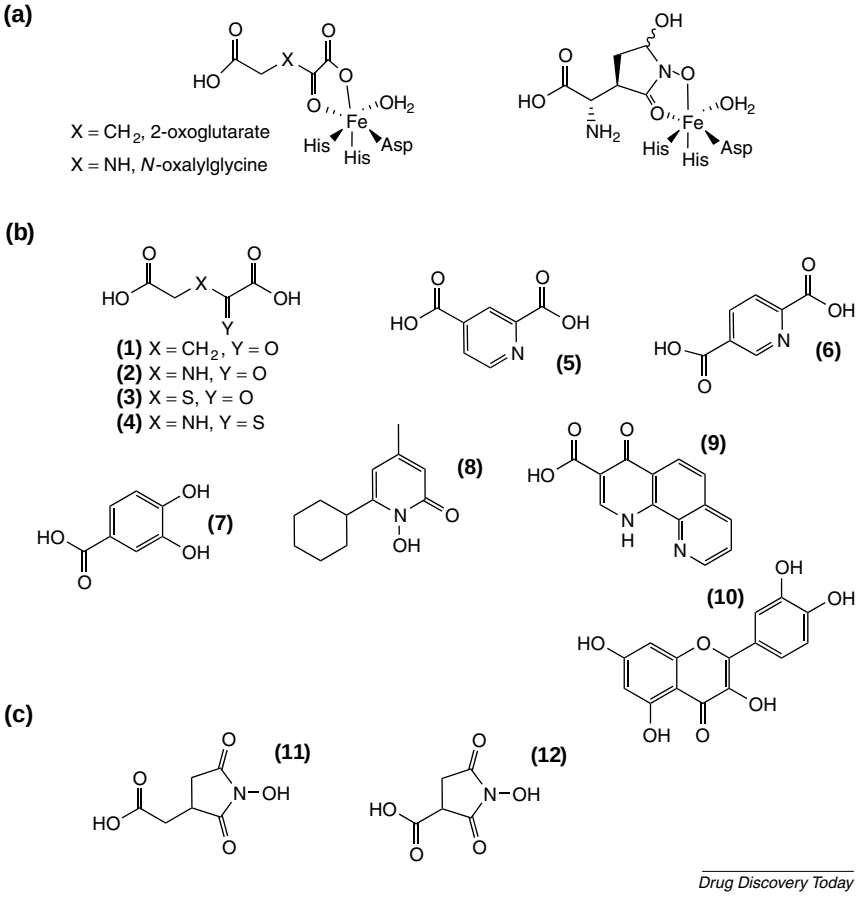

Figure 3. **(a)** Binding mode of 2-oxoglutarate (2OG), *N*-oxalylglycine and dealanylalahopcin at the Fe^{II} active site. **(b)** 2OG analogues and related compounds referred to in the text. **(1)** 2-oxoglutarate, **(2)** *N*-oxalylglycine, **(3)** thiol ester analogue, **(4)** thiono *N*-oxalylglycine, **(5)** pyridine 2,4-dicarboxylate, **(6)** pyridine 2,5-dicarboxylate, **(7)** 3,4-dihydroxybenzoate, **(8)** ciclopirox olamine, **(9)** FG0041, **(10)** quercetin, **(c)** **(11)** 3-carboxymethylene *N*-hydroxy succinimide, **(12)** 3-carboxy-*N*-hydroxy pyrollidone.

a hydrophobic binding pocket at the active site of the PHDs.

Another report describes cyclic hydroxamate inhibitors of the PHDs [43]. These compounds were based on the natural products alahopcin and dealanylalahopcin, that were isolated from *Streptomyces albulus* and previously identified as inhibitors of CPH. As the distance between the side-chain carboxylate of dealanylalahopcin and its hydroxamate was the same as that between the 5-carboxylate of 2OG and its 2-oxo-1-carboxylate iron-binding motif, it was proposed that dealanylalahopcin acted as an analogue of 2OG (Figure 3a). Consistent with this proposal, a dealanylalahopicin analogue with the 'correct' side-chain length (**11**), was shown to be a more potent inhibitor than another analogue that has a side-chain length shorter by a methylene group (**12**, Figure 3c).

Other identified CPH inhibitors that are apparent 2OG analogues have also been tested on the PHDs and FIH with variable effects. Whereas pyridine 2,4-dicarboxylate (**5**) and pyridine 2,5-carboxylate (**6**) both inhibit CPH strongly [44], only the former shows significant inhibition towards the PHDs [45], with both being weak inhibitors of FIH [46]. The differences might reflect different binding pockets and/or conformations of the 2OG in the active sites. Additionally, 3,4-dihydroxybenzoate (**7**) shows comparable inhibition towards FIH and CPH [46], but does not inhibit the PHDs [45,47] (Figure 3b). In each case, as with other supposed 2OG oxygenase inhibitors, care must be taken to exclude all effects due to iron chelation.

Other inhibitors, possibly related to the 2OG-binding mode, include ciclopirox olamine (CPX, **8**) and the bipyridyl FG0041 (**9**, Figure 3b), which was originally proposed as a CPH inhibitor, both of which are proposed to activate the HIF pathway via HIF hydroxylase inhibition. With compound (**8**), symptoms associated with angiogenesis were observed following application of CPX to rabbit skin [48], whereas with (**9**), ventricular function was improved in a rodent model [49]. There is evidence that FG0041 and CPX (identical to FG2229) are *in vitro* PHD inhibitors and that the latter can stimulate VEGF transcription through HIF activation [50,51]. Whereas CPX most probably chelates iron via its hydroxamic acid moiety, the mode of action of FG0041 is unclear, as it contains two possible bidenate iron-binding functionalities – the nitrogens of the bipyridyl and the keto-acid functionality. Intriguingly, quercetin (**10**, Figure 3b), a naturally occurring flavanoid associated with anti-cancer properties, has been shown to inhibit FIH in an iron-concentration-independent manner [52]. This contrasts sharply with AlkB, for which quercetin inhibition could be titrated against iron concentration, again highlighting possible differences between the 2OG oxygenase active sites.

Due to the pivotal position of the 2OG oxygenases in many biochemical pathways, both known (e.g. AlkB, CPH) and unknown (e.g. most JmjC proteins), it is envisaged that simultaneous inactivation of these enzymes would be undesirable. In this respect, the development of inhibitors

based solely upon the 2OG template should be pursued cautiously. Thus, targeting of the substrate binding sites could prove to be a more beneficial focus and might more-readily enable selective inhibition of the HIF hydroxylases and 2OG oxygenases.

Substrate requirements of the HIF hydroxylases

Selective inhibition of the PHDs and FIH might be aided via knowledge of the specific substrate-binding sites in each of the enzymes, enabling differences to be exploited for inhibitor design. Recent work by several groups has investigated the minimal and essential amino-acid substrate requirements of the HIF hydroxylases. FIH shall be considered first because its crystallographic analyses have revealed details of substrate binding.

The crystal structure of FIH with CAD bound at the active site [36] revealed two distinct binding sites (sites 1 and 2). The residues at site 1, corresponding to HIF-1α 795–806, adopted an extended loop conformation forming ten hydrogen bonds to FIH. This contrasts with the α-helical conformation of the same residues in complex with the TAZ1 domain of CBP (p300) [53,54]. It appears that HIF-1α can adopt several conformations that are dependent upon the nature of its binding partner. Whereas free in solution, HIF-α exists predominantly in a disordered state [55], which might explain the difficulty in obtaining a crystal structure for this protein in isolation. At site 2, residues of HIF-1α 813–822 form an α-helix, consistent with the previous structure of CAD bound to CBP, but only make two hydrogen bonds to FIH. The relative contribution of each of these sites to FIH binding has been studied recently. Optimal activity with FIH was observed with a 35-mer peptide incorporating both sites of HIF-1α; removal of seven residues from the N-terminus or four residues from the C-terminus led to significant decreases in activity [46]. The results with FIH contrast with the PHD isozymes (PHD1–3) where optimal activity can be observed with a 20-mer [45]. Alanine scanning mutagenesis of eight conserved residues of HIF-1α CAD [56] revealed that, in addition to the conserved asparagine, the only other mutation to have a significant effect on the hydroxylation activity was that of Val802, the residue immediately preceding the hydroxylated residue, Asn803. The V802A mutant had a similar K_m to wild-type CAD, suggesting that substrate binding had not been affected, but it did have a fourfold lower V_{max}. Additionally, HIF-2α CAD peptides were hydroxylated less efficiently than those of HIF-1α [46]. The major difference between the HIF isoforms in this region is the presence of an alanine residue immediately after Asn803 in HIF-1α; the corresponding residue is a valine in HIF-2α. Mutagenesis studies have yet to be performed on this residue. In the crystal structure of FIH with bound HIF-1α CAD, Asn803 and Ala804 form a tight turn that is stabilized by a hydrogen bond between the backbone carbonyl of Val802 and the NH of Ala804 [36]. Thus, it appears that the VNA motif might be critical for substrate recognition and this binding arrangement should be considered in subsequent inhibitor design.

The substrate selectivity of the PHD enzymes has also been investigated. Although PHD1 and PHD2 are capable of accepting HIF-1α NODDD and CODDD as substrates, PHD3 is reported not to hydroxylate the former, suggesting an element of substrate selectivity that could be exploited [45,57]. All of the PHDs hydroxylate HIF-2α NODDD and CODDD together with HIF-3α CODDD (HIF-3α does not contain a NODDD). Furthermore, mutagenesis of the conserved LxxLAP motif demonstrated that the only obligatory residue for hydroxylation is the hydroxylated proline residue [12]. However, a recent report suggests that Leu574 of HIF-1α (ten residues C-terminal of the hydroxylated Pro564) is essential for binding to PHD2 and is therefore necessary for HIF-α hydroxylation [58].

For the PHDs and FIH it appears that peptides of a significant length (~20 and 35 residues, respectively) are required for optimal substrate hydroxylation. The mutagenesis results suggest that alteration of the amino acids in the immediate vicinity of the prolyl or asparaginyl residue can be tolerated, which could be exploited with respect to substrate inhibitor design. Subtle differences in the architecture of the PHD active sites, as revealed by the lack of activity of PHD3 with HIF-1α NODDD, might allow the selective inhibition of each of the enzymes.

Downregulation of HIF activity in the treatment of cancer

Activation of the HIF system has been observed in numerous cancers and is presumed to be due to the induction of a hypoxic environment as a consequence of the rapidly dividing cells. HIF-α degradation can be stimulated by treatment with iron and ascorbate, which presumably aids in the activation of the HIF hydroxylases [59]. However, it should be noted that different molecular mechanisms have been proposed for the upregulation of the HIF system in tumorogenesis and the individual role of each of these is difficult to assess [21]. However, it is apparent that downregulation of the HIF system is an attractive target for cancer therapy.

One strategy to achieve inhibition of the hypoxic response in tumours is to target the binding interaction between the CH1 domain of CBP and the CAD of the HIF-α subunit. In proof-of-principle studies, overexpression of polypeptides encoding the minimal binding domain of HIF-1α CAD led

to a decrease in hypoxia-inducible reporter activity [60]. Retroviral infection of colon and breast carcinomas with the CAD polypeptide led to a significant decrease in tumour growth in both cases. These experiments demonstrate the possibility of interference with HIF-binding partners as a medicinal target. In this context, targeting of the HIF-α and HIF-β interaction should also be considered.

Small-molecule therapy for the downregulation of HIF activity

A cell-based HTS has been recently developed to identify small-molecule inhibitors of the HIF system [61]. A human glioma cell line was genetically engineered to express a luciferase reporter gene under the control of three canonical copies of a hypoxia response element. The screen used the Diversity Set of 2000 compounds available from the National Cancer Institute and identified four compounds that specifically inhibited HIF-driven luciferase expression. Of these, one compound (NSC-607097, also known as DX-52–1) was a stable analogue of quinocarmycin that had previously been identified as having melanoma specificity [62]. DX-52–1 had previously been developed to Phase I clinical trials, but unexpected problems with toxicity were encountered and the trial was discontinued. The remaining three compounds identified, topotecan and two camptothecin analogues, are drugs that inhibit Topo I activity. Inhibition of Topo I activity by topotecan was associated with repression of HIF-1 activity and associated downregulation of hypoxically transcribed genes, including VEGF. This was, at least in part, mediated through inhibition of HIF-α accumulation, but whether this is via a direct mechanism or as a result of Topo I inhibition is currently unclear. Discernment of the molecular mechanism by which HIF inactivation occurs through the use of Topo I inhibitors is crucial to allow progression to further therapeutic developmental stages.

Conclusions

Targeting of the HIF system to exact a therapeutic response for the treatment of ischemic disease and cancer is an attractive goal. Although advances in our knowledge of the manipulation of the HIF system have been made rapidly over the past few years, the emerging complexity involved in regulation and selectivity should not be ignored. Thus far, no pharmaceutical has been identified that directly regulates the activity of a human transcription factor. Selection of the most appropriate point of therapeutic intervention to modulate HIF activity is also an important factor in pharmaceutical development. In this respect, the HIF hydroxylases appear to be an attractive target for upregulation of hypoxically driven transcripts through small-molecule therapy. Selective inhibition of the HIF hydroxylases, without inhibition of any of the other essential 2OG oxygenases, to stimulate a specific gene-expression response is an immediate target. Although inhibition of 2OG oxygenases has not been (knowingly) achieved for therapeutic purposes, good precedent for the selective inhibition of non-heme and iron-dependent oxygenases comes from the cyclooxygenases, where selective inhibitors for a specific enzyme (cyclooxygenase II) are in widespread use.

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