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Modeling the regulation of p53 activation by HIF-1 upon hypoxia

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As a famous tumor suppressor, p53 is also activated under hypoxic conditions. Hypoxia-inducuble factor 1, HIF-1, is involved in the activation of p53 upon hypoxia. However, how p53 is modulated by the HIF-1 pathway to decide cell fate is less understood. In this work, we developed a network model including p53 and HIF-1 pathways to clarify the mechanism of cell fate decision in response to hypoxia. We found that HIF-1α and p53 are activated under different conditions. Under moderate hypoxia, HIF-1α is activated to induce glycolysis or angiogenesis, and promotes partial accumulation of p53 by inducing PNUTS. Under severe hypoxia, p53 rises to high levels due to ATR-dependent stabilization and promotes Mdm2-dependent HIF-1α degradation. As a result, fully activated p53 triggers apoptosis. Of note, competition for p300 between HIF-1α and p53 plays a key role in regulating their transcriptional activities. This work may advance the understanding of the mechanism for p53 regulation by HIF-1 in the hypoxic response.

Keywords: cell fate decision; HIF-1α; hypoxia; network model; p53 activation

Cells respond to hypoxia mainly through hypoxia-inducible factor 1 (HIF-1), which is a heterodimer composed of hypoxic response factor HIF-1α and constitutively expressed HIF-1β [1]. Under normoxia, HIF-1α is inactive and kept at very low levels due to oxygen-dependent hydroxylation [2]. HIF-1α is hydroxylated by prolyl hydroxylases (PHDs) on Pro402 and Pro564, and is recognized by the E3 ligase von Hippel-Lindau (VHL) for the proteasomal degradation. Meanwhile, the transcriptional activity of HIF-1α is inhibited by another hydroxylase, factor inhibiting HIF-1 (FIH), which hydroxylates Asn803 and blocks the binding of p300 to C-terminal transactivation domain (C-TAD) [3]. Upon hypoxia, HIF-1α is stabilized and activated, inducing a large number of genes involved in multiple processes including glucose metabolism, cell survival, angiogenesis and invasion [1]. Thus, HIF-1α plays a key role in cellular adaptation and survival under hypoxic conditions.

As a tumor suppressor, p53 becomes stabilized and activated in cellular response to stresses including DNA damage and hypoxia [4]. The mode of p53 regulation is distinct in these two cases. p53 transactivates a number of target genes involved in cell cycle arrest and apoptosis in response to DNA damage [5–7]. By contrast, the activity of p53 in response to hypoxia has been reported controversially. It has been reported that p53 only accumulates under severe hypoxia [8]. It

Abbreviations
C-TAD, C-terminal transactivation domain; FIH, factor inhibiting HIF-1; HIF-1, hypoxia-inducible factor 1; ODEs, ordinary differential equations; PFKL, phosphofructokinase L; PHDs, prolyl hydroxylases; PIP2, phosphatidylinositol-4,5-bisphosphate; PIP3, phosphatidylinositol-3,4,5-trisphosphate; SHIP-1, SH2-containing inositol 5-phosphatase 1; SN, saddle-node; VEGF, vascular endothelial growth factor; VHL, von Hippel-Lindau.

was shown that most common target genes are not induced by p53 upon hypoxia, and p53 mainly acts as a transrepressor to induce apoptosis. But, it was also reported that several proapoptotic target genes, like *bax*, *puma* and *bnip3l*, are induced by p53 in response to hypoxia [9,10]. Moreover, p53 can promote apoptosis through upregulating SH2-containing inositol 5-phosphatase 1 (SHIP-1) [11], which inhibits AKT phosphorylation to further stabilize p53, enclosing a positive feedback loop [11,12]. It is still a challenge to clarify the detailed mechanism of apoptosis induction by p53 under severe hypoxia.

The mechanism of p53 activation upon hypoxia is rather complicated. First, HIF-1α promotes p53 stabilization through different mechanisms. It was reported that HIF-1α promotes the stabilization of wild-type p53 in cells treated by hypoxia-mimicking agents [13]. The direct interaction between HIF-1α and Mdm2 promotes p53 stabilization by repressing Mdm2-mediated degradation [14]. HIF-1α induces PNUTS and promotes p53 phosphorylation by inhibiting the protein phosphatase PP1, thereby preventing p53 from Mdm2-dependent degradation [15]. Second, under severe hypoxia, ATR promotes p53 stabilization by phosphorylation that blocks Mdm2-dependent degradation of p53 [8]. Moreover, HIF-1α represses p53 by competing for limited p300 that is transcriptional coactivator for both p53 and HIF-1α [16]. Finally, p53 promotes its own activation by triggering HIF-1α degradation upon anoxia [17]. It is intriguing to unravel how HIF-1 and ATR coordinate to regulate p53 in cellular response to hypoxia.

A series of models has been constructed to explore the mechanism of cellular response to hypoxia. Qutub *et al.* [18] focused on the detailed mechanism of intracellular oxygen sensing by HIF-1α and reported that the HIF-1-mediated cellular response may exhibit switch-like or gradual behavior depending on the context of hydroxylation. Nguyen *et al.* [19] confirmed that HIF-1α is progressively activated by sequential deactivation of PHDs and FIH activity with enhancing hypoxia. Recently, Bagnall *et al.* [20] developed a minimal model comprising the HIF-1α-PHD negative feedback loop to interpret the pulsing behavior of HIF-1α. In the above, most of those models focused on how the abundance and activity of HIF-1α are regulated by hypoxia, seldom modeling the regulation of p53 upon hypoxia. Recently, we developed a preliminary model to characterize the competition for p300 between p53 and HIF-1α, focusing on the regulation of HIF-1α by p53 [21]. A more elaborate model is required for clarifying how p53 activity is modulated by multiple mechanisms under various hypoxic conditions.

Here, we developed a network model to reveal how p53 and HIF-1α pathways coordinate to regulate cellular response to hypoxia. We proposed that HIF-1α is partially activated to induce genes involved in glycolysis under mild hypoxia. Under moderate hypoxia, HIF-1α is fully activated to induce angiogenesis and promotes partial stabilization of p53 by inducing PNUTS. Under severe hypoxia, p53 accumulates sufficiently due to ATR-dependent phosphorylation and competes with HIF-1α for limiting p300. Under anoxia, p53 is fully activated since HIF-1α is degraded by p53-dependent mechanism, thereby inducing apoptosis. Together, different regulation of p53 by HIF-1α modulates the cellular outcome depending on the severity of hypoxia.

Materials and methods

The p53 and HIF-1α pathways are interlinked in response to hypoxia. Our model characterizes the sensing of hypoxia, activation of p53 and HIF-1α, and decision-making in cell fates (Fig. 1). We focused on the regulation of p53 stability and activity by HIF-1 under various hypoxic conditions. More details of the model are described below.

Regulation of HIF-1α by hypoxia

Under normoxia, HIF-1α is hydroxylated in its N-TAD by oxygen-dependent hydroxylases PHDs, leading to VHL-dependent degradation [2,22]. Moreover, the transcriptional activity of HIF-1α is inhibited due to FIH-mediated hydroxylation in the C-TAD [3]. Under hypoxia, PHDs lose their activity and HIF-1α becomes stable due to repressed degradation. Moreover, the transcriptional activity of HIF-1α is activated due to the deactivation of FIH under severe hypoxia. It was reported that FIH remains active and hydroxylates HIF-1α in its C-TAD under mild hypoxia [23]. The different thresholds of oxygen level for the activation of FIH and PHD-2 necessitate differentiating active forms of HIF-1α. Five forms of HIF-1α are considered here: HIF-$1\alpha_c$ (cytoplasmic dehydroxylated HIF-1α), HIF-1α-aOH_c (cytoplasmic HIF-1α hydroxylated on Asn803 in C-TAD), HIF-$1\alpha_n$ (nuclear dehydroxylated HIF-1α), HIF-$1\alpha_{nAC}$ (nuclear acetylated HIF-1α) and HIF-1α-aOH_n (nuclear HIF-1α hydroxylated on Asn803 in C-TAD) (see Eqs. 1–5 in Method S1).

It is assumed that PHD-2 is the main form of PHDs to promote HIF-1α degradation since its activity is much higher than PHD-1/3 [24,25]. The degradation of HIF-1α by PHD-2 is constrained in cytoplasm due to the cytoplasmic localization of PHD-2 [26]. HIF-1α induces the expression of PHD-2, which in turn targets HIF-1α for degradation, enclosing a negative feedback loop [27]. This feedback loop should contribute to adaptation of HIF-1α

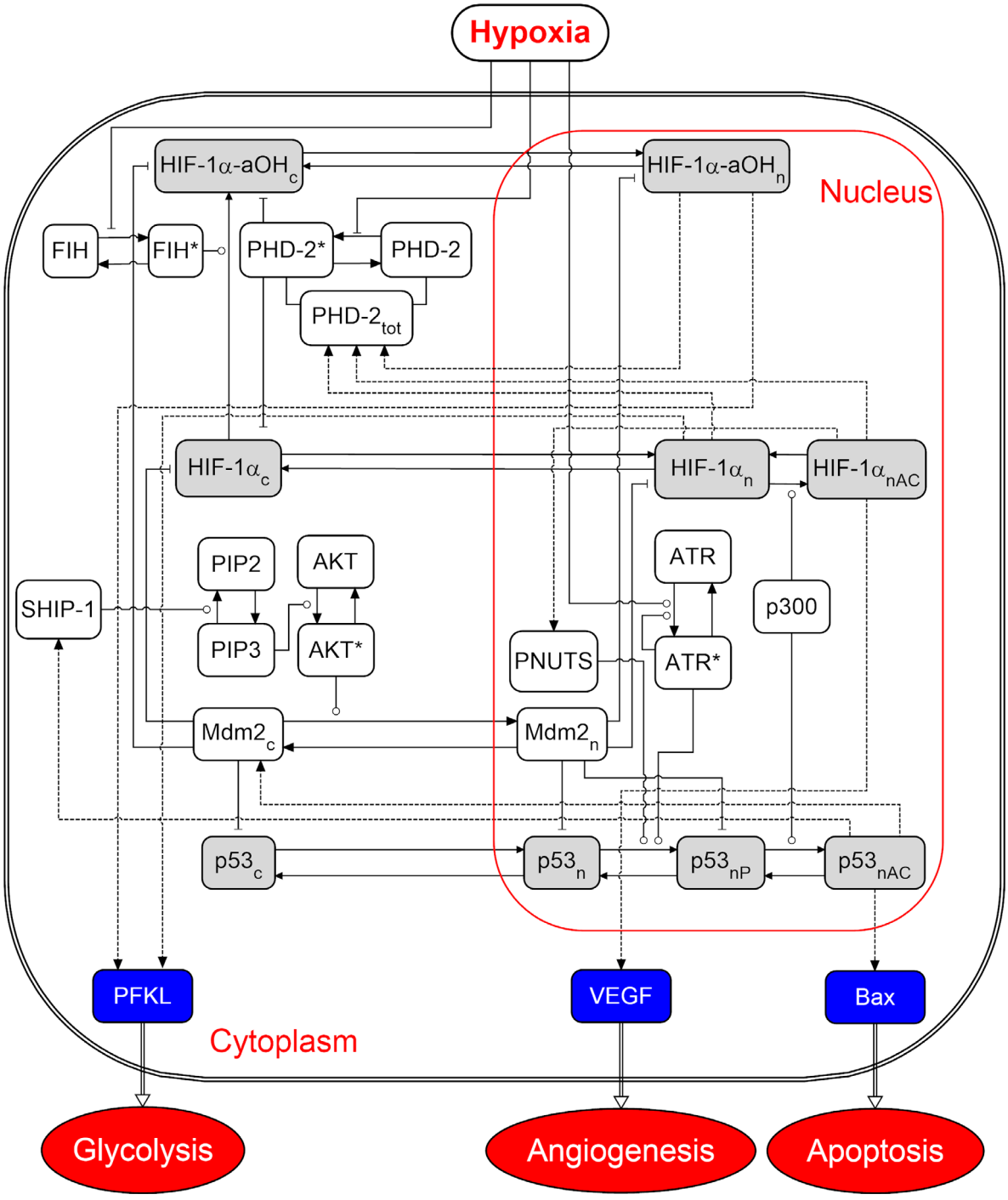

Fig. 1. Schematic diagram of the network model involving HIF-1α and p53. The model focuses on the regulation of p53 by HIF-1α pathway upon hypoxia. The hydroxylases PHD-2 and FIH are progressively deactivated, leading to the stabilization and activation of HIF-1α. Partially active HIF-1α can induce PFKL to trigger glycolysis. The fully activated HIF-1α induces PNUTS and VEGF to promote p53 phosphorylation and angiogenesis, respectively. On the other hand, both ATR and PNUTS promote p53 stabilization by facilitating its phosphorylation. p53 and HIF-1α compete for binding both p300 and Mdm2 in their transactivation and degradation, respectively. Activated p53 induces Bax to initiate apoptosis. Moreover, p53 promotes its own accumulation through the p53-SHIP-1-Mdm2 positive feedback loop. Arrow-headed dotted lines denote the transactivation of target genes by HIF-1α and p53, while the arrow-headed solid lines mark the state transition or the nucleus-cytoplasm trafficking. The circle-headed and bar-headed lines display the promotion and inhibition, respectively.

to hypoxia [20]. Similar to PHD-2, FIH-mediated HIF-1α hydroxylation is also restricted in cytoplasm since FIH is mainly a cytoplasmic protein [26]. Two forms of PHD are considered: PHD (inactive form) and PHD* (active form); FIH is divided into FIH (inactive form) and FIH* (active form) [19]. The oxygen-dependent activation and deactivation of FIH and PHD-2 are described by Michaelis–Menten kinetics (Eqs. 6, 7, 9 and 10 in Method S1). It is assumed that PHD-2 is induced by all the three forms of HIF-1α in the nucleus [20], and the production rate of PHD-2_{tot} is characterized by the Hill functions of HIF-$1\alpha_n$, HIF-1α-aOH_n and HIF-$1\alpha_{nAC}$ levels (Eq. 8 in Method S1). By contrast, FIH expression is HIF-1α-independent, and its total amount is assumed to be constant.

HIF-1α induces a number of genes to modulate cellular adaptation to hypoxia. Among them, phosphofructokinase L (PFKL) contributes to metabolic switch from oxidative phosphorylation to glycolysis, while vascular endothelial growth factor (VEGF) promotes angiogenesis to enhance oxygen supply [1]. PFKL and VEGF are induced by HIF-1α under distinct hypoxic conditions depending on the status of FIH [28]. We assume that PFKL is induced by HIF-1α-aOH_n and HIF-$1\alpha_n$, while VEGF expression is activated by HIF-$1\alpha_{nAC}$. PNUTS is reported to be induced by HIF-1α at 0% O_2 [15], and can be considered a target of HIF-$1\alpha_{nAC}$ [28]. Similar to PHD-2_{tot}, the induction rates of PNUTS, PFKL and VEGF are all characterized by the Hill function (Eqs. 11, 25 and 26 in Method S1).

Activation of p53 upon hypoxia

As a transcription factor, the phosphorylation and acetylation of p53 modulate the selective induction of its target genes [29]. It is assumed that p53 is progressively activated: phosphorylation contributes to its stabilization and partial activation; further acetylation leads to its full activation [21]. Based on its status and subcellular localization, four forms of p53 are included in the model, namely $p53_c$ (cytoplasmic p53), $p53_n$ (nuclear inactive p53), $p53_{nP}$ (nuclear phosphorylated p53) and $p53_{nAC}$ (nuclear acetylated p53) (Eqs. 14–17 in Method S1).

ATR promotes p53 accumulation by phosphorylation under severe hypoxia. ATR can be activated by hypoxia-induced replication arrest and phosphorylate p53 at Ser15 [8]. Moreover, activated ATR promotes its own activation through autophosphorylation [30]. Two forms of ATR are considered: ATR (inactive form) and ATR* (active form); their conversion is oxygen-dependent, and is described by Michaelis–Menten kinetics (Eqs. 12 and 13 in Method S1).

Reciprocal regulation of HIF-1 and p53 pathways

HIF-1α promotes p53 accumulation in two ways. On one hand, HIF-1α induces PNUTS to promote p53 phosphorylation indirectly, protecting p53 from Mdm2-dependent degradation [15]. On the other hand, HIF-1α releases p53 from Mdm2-dependent degradation by direct interaction with Mdm2 [14].

The transcriptional coactivator p300 is shared by multiple transcription factors including HIF-1α, p53, NF-κB and so on, in cells exposed to hypoxia [31–34]. Moreover, p300 is involved in histone acetylation during transcription initiation [35]. Although p300 is abundant in the whole cell, the available p300 for HIF-1α and p53 is limited in the hypoxic response [34]. Therefore, the two transcription factors have to compete for limited p300 to gain transcription activity [34]. We use $p300_{tot}$ to denote the total of p300 available for HIF-1α and p53. For simplicity, $p300_{tot}$ is assumed to be fixed as a parameter. The activation rate of HIF-1α and p53 is proportional to $p300_{tot}$, and is described by the competitive Michaelis–Menten kinetics (Eqs. 5 and 17 in Method S1). It is assumed that $p53_{nAC}$ can induce the transcription of *mdm2*, *SHIP-1* and *bax*, and the transcription rates obey Hill function with Hill coefficient set to 4 (Eqs. 18, 20 and 27 in Method S1).

As an E3 ubiquitin ligase, Mdm2 promotes the degradation of both p53 and HIF-1α [14]. The subcellular localization of Mdm2 significantly influences the degradation of p53 [36,37], whereas Mdm2-regulated degradation of HIF-1α occurs mostly in cytoplasm [38]. Thus, Mdm2 is divided into $Mdm2_c$ (cytoplasmic Mdm2) and $Mdm2_n$ (nuclear Mdm2). Mdm2 promotes the degradation of both p53 and HIF-1α, and they compete for degradation described by competitive Michaelis–Menten kinetics (Eqs. 1–4 and 14–16 in Method S1). Specially, the Mdm2-mediated degradation of $p53_{nAC}$ and HIF-$1\alpha_{nAC}$ is ignored because ubiquitination is excluded by the acetylation [39].

Notably, the subcellular distribution of Mdm2 is also regulated by p53-induced SHIP-1 [11,12]. Given *SHIP-1* is induced by p53 under severe hypoxia [11], we assume that $p53_{nAC}$ controls SHIP-1 production (Eq. 20 in Method S1). Further, AKT activity is repressed due to the deactivation of phosphatidylinositol-3,4,5-trisphosphate (PIP3) to phosphatidylinositol-4,5-bisphosphate (PIP2) by SHIP-1 (Eqs. 21 and 23 in Method S1) [12,40]. Consequently, most of Mdm2 is sequestered in cytoplasm, which stabilizes nuclear p53 [41]. Thus, the p53-SHIP-1-Mdm2 positive feedback loop contributes to p53 stabilization by modulating the subcellular localization of Mdm2. Based on their phosphorylation status, AKT and PIP3 are divided into active (AKT* and PIP3) and inactive (AKT and PIP2) forms. As posttranslational modifications are the main regulation modes of AKT upon hypoxia, the total amount of AKT and AKT* is assumed to be a constant. Similarly, the total amount of PIP2 and PIP3 is also set as a constant. Michaelis–Menten kinetics is exploited to characterize the enzymatic reactions in the p53-SHIP-1 pathway (Eqs. 18, 19, and 21–24 in Method S1).

Methods

The dynamics of all the proteins are described by ordinary differential equations (ODEs), which are listed in Method S1. All the initial values of the variables are set as their steady-state values at 21% O_2, and are listed in Table S1. All the parameters are shown in Table S2. The free software OSCILL8 (http://oscill8.sourceforge.net) is exploited to solve the ODEs and plot bifurcation diagrams. The original codes for the simulation by OSCILL8 are presented in Method S2. Time is in units of minutes, and the units of parameters are set to ensure the concentration of each protein is dimensionless. The level of oxygen is characterized by its volume percentage denoted by L_{O_2}.

Results

Overview of the dynamics of HIF-1α and p53

Heat maps are exploited to elucidate the global properties of [HIF-1α_{tot}] and [p53$_{tot}$] dynamics at various L_{O_2} (Fig. 2A,B). Under mild hypoxia, [HIF-1α_{tot}] shows adaptive behavior with a bell-shaped pulse. This adaptive response has been observed experimentally due to the HIF-1α-PHD negative feedback [19,20]. [HIF-1α_{tot}] rises and settles at high levels under moderate hypoxia (Fig. 2A). Under severe hypoxia, it stays at high levels for a period of time and drops to basal levels finally; the delay in the descending phase of [HIF-1α_{tot}] is due to hypoxia-independent degradation [8]. By contrast, [p53$_{tot}$] still stays at low levels in mild hypoxia, reaches moderate levels under severe hypoxia, and attains high levels upon anoxia (Fig. 2B). The difference in the dynamics of [HIF-1α_{tot}] and [p53$_{tot}$] under different hypoxic conditions shows good agreement with experimental observations [8]. Together, HIF-1α is preferentially activated under mild or moderate hypoxia, whereas p53 accumulates remarkably under severe hypoxia or anoxia.

The bifurcation diagrams reveal the dependency of the steady-state [HIF-1α_{tot}] and [p53$_{tot}$] on L_{O_2}, characterizing the long-time dynamical behaviors of these two proteins (Fig. 2C,D). Both [HIF-1α_{tot}] and [p53$_{tot}$] exhibit two discrete states with varying L_{O_2}. The steady-state level of HIF-1α_{tot} is still rather low at 2% O_2, consistent with the presence of adaptive behavior. [HIF-1α_{tot}] switches to high levels in the range of 0.085–2% O_2 due to repressed degradation, but drops to rather low levels for $L_{O_2} < 0.085\%$ (Fig. 2C). By contrast, [p53$_{tot}$] varies markedly in the range of 0.085–0.4% O_2, and switches to high levels for $L_{O_2} < 0.085\%$ (Fig. 2D). How HIF-1α and p53 dynamics vary and what the underlying mechanism of their reciprocal regulation will be clarified in the following.

Regulation of HIF-1α dynamics by PHD-2

As shown in Fig. 2A, HIF-1α shows adaptive behavior and sustained high levels under mild and moderate hypoxia, respectively. It was reported that HIF-1α induces PHD-2/3 to promote its own degradation [27]. The correlation between HIF-1α-induced PHD-2 expression and HIF-1α dynamics is revealed in Fig. 3. Under mild hypoxia, [PHD-2*] drops quickly due to the deactivation of PHD-2, leading to the stabilization of HIF-1α and a rise in [HIF-1α_{tot}] (Fig. 3A). [PHD-2$_{tot}$] rises gradually due to HIF-1α-dependent induction. Correspondingly, [PHD-2*] increases after the initial decline so that [HIF-1α_{tot}] drops later due to enhanced degradation. Therefore, HIF-1α-PHD feedback contributes to the adaptation of HIF-1α to mild hypoxia. These results are consistent with the adaptive behaviors of HIF-1α reported in Ref. [20].

By contrast, PHD-2* is fully deactivated, and [PHD-2*] drops to rather low levels under moderate hypoxia (Fig. 3B). As a result, HIF-1α_{tot} accumulates and settles at high levels, leading to sustained transactivation of PHD-2. That is, full deactivation of PHD-2* inhibits the HIF-1α-PHD negative feedback, resulting in sustained stabilization of HIF-1α in this case. Similar dynamics of HIF-1α was also observed experimentally [20]. Collectively, both PHD-2 induction by HIF-1α and its deactivation affect the dynamics of HIF-1α.

Requirement of HIF-1α expression for the stabilization of p53

The effects of HIF-1α level on p53 stabilization are shown in Fig. 4. Under anoxia, [p53$_{tot}$] drops remarkably when the production rate of HIF-1α, $k_{sHIF1\alpha_c}$, decreases from 0.4 to 0 (Fig. 4A). Thus, HIF-1α expression contributes to p53 stabilization remarkably. This result is consistent with the data from immunoblotting experiment in which HIF-1α was expressed normally or knocked out in cells treated with hypoxia mimetic desferrioxamine for 6 h [13].

Indeed, under anoxia the steady-state [p53$_{tot}$] drops with decreasing $k_{sHIF1ac}$ (production rate of cytoplasmic HIF-1α) when it is less than 0.5 (Fig. 4B). Nevertheless, [p53$_{tot}$] is still greater than 3.0 with $k_{sHIF1ac} = 0$, suggesting that additional mechanisms also play a role in stabilizing p53. Of note, HIF-1α-dependent p53 stabilization only occurs for smaller $k_{sHIF1ac}$. [p53$_{tot}$] rises

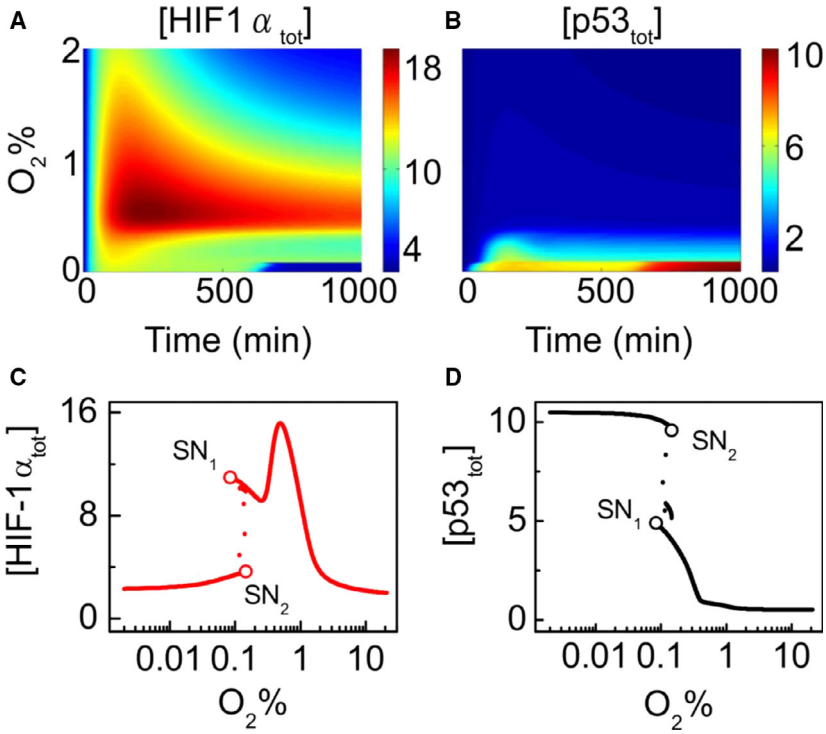

Fig. 2. Different responses of HIF-1α and p53 upon hypoxia. (A, B) Heat maps of [HIF-1α_{tot}] (A) and [p53$_{tot}$] (B) as a function of O_2% and time. (C, D) Bifurcation diagrams of the steady state of [HIF-1$_{tot}$] (C) and [p53$_{tot}$] (D) versus O_2%.

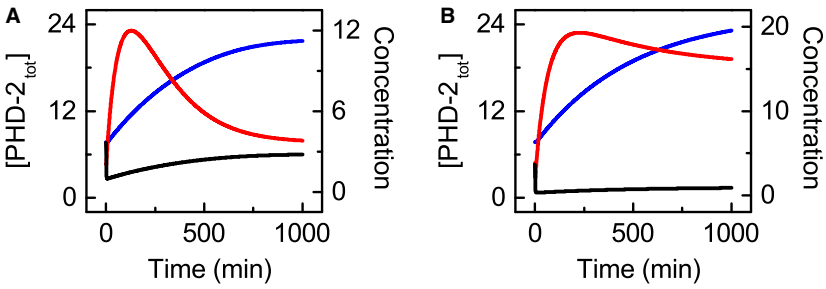

Fig. 3. Regulation of HIF-1α dynamics by PHD-2. Time courses of [HIF-1α_{tot}] (red), [PHD-2*] (black) and [PHD-2$_{tot}$] (blue) for 2% (A) and 0.5% O_2 (B).

quickly to high levels, while it declines slowly for $k_{sHIF1ac} > 0.5$ and switches to lower levels when $k_{sHIF1ac}$ exceeds the threshold corresponding to the saddle-node (SN) bifurcation point. Overexpressed HIF-1α downregulates p53 since most p300 is snatched from p53, deactivating p53 and facilitating its degradation. Thus, HIF-1α may play a dual role in the regulation of p53 level depending on its own induction rate.

Notably, the dynamics of normalized [p53$_{tot}$] and [HIF-1α_{tot}] is comparable to experimental data [8] (Fig. 4C,D). Under severe hypoxia (0.02% O_2), [HIF-1α_{tot}] rises to and stays at high levels in the early period of 12 h, and drops to basal levels in the late phase (Fig. 4C). The upregulation of [HIF-1α_{tot}] results from hypoxia-induced stabilization, while its subsequent downregulation is owing to Mdm2-dependent degradation [17]. The dynamics of [HIF-1α_{tot}] show agreements with experimental data [8]. [p53$_{tot}$] first reaches a moderate level and further rises to a higher level later (Fig. 4D). Together, p53 level rises progressively within 24 h as observed experimentally [8]. Therefore, HIF-1α and p53 exhibit distinct dynamic patterns under hypoxia.

HIF-1α promotes p53 accumulation by inducing PNUTS

We next address the role of HIF-1α-induced PNUTS in the stabilization of p53 (Fig. 5). To assess the significance of HIF-1α transcriptional activity in p53 stabilization, we investigate how p53 expression is influenced by FIH that represses the transcriptional activity of HIF-1α [3] (Fig. 5A). Consistent with experimental data, [p53$_{tot}$] with shRNA treatment for FIH

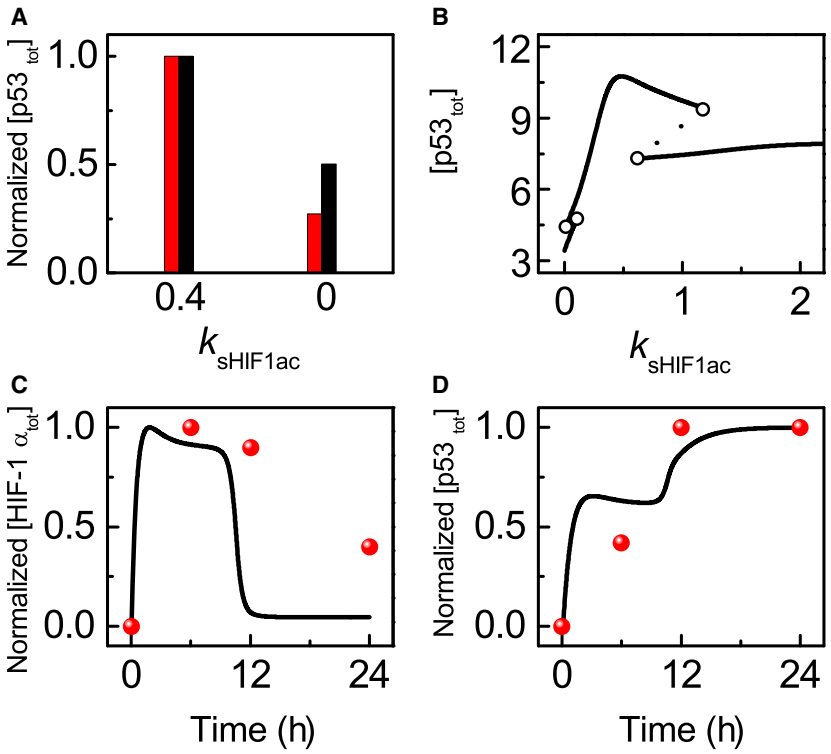

Fig. 4. HIF-1α-dependent accumulation of p53. (A) Normalized [$p53_{tot}$] for 0% O_2 at 6 h. The black bars denote the simulation results and the red bars correspond to experimental data with abundant or deficient HIF-1α in desferrioxamine-treated cells [13]. (B) Steady-state [$p53_{tot}$] as a function of $k_{sHIF1ac}$ (production rate of cytoplasmic HIF-1α) for 0% O_2. The stable and unstable steady states are denoted by solid and dashed lines, respectively. The open circles denote the SN bifurcation points. (C, D) Time courses of normalized [$HIF\text{-}1\alpha_{tot}$] (C) and [$p53_{tot}$] (D) at 0.02% O_2. The black lines denote the simulation results, and the red dots represent the experimental data from RKO cells [8].

is doubled at least compared with the controlled case at 1% O_2 [42]. Similarly, [$p53_{tot}$] also rises markedly under normoxia in the shFIH case. As shown in the figure, our results coincide with experimental data rather well [42]. The dependency of p53 stabilization on FIH expression suggests that the transcriptional activity of HIF-1α is required for p53 stabilization.

The effects of FIH abundance on p53 accumulation under moderate hypoxia are shown in Fig. 5B. The steady-state levels of both $HIF\text{-}1\alpha_{nAC}$ and PNUTS drop toward saturation with increasing FIH_{tot}, while [HIF-1α-aOH] rises toward saturation. These behaviors result from the conversion of $HIF\text{-}1\alpha_{nAC}$ to HIF-1α-aOH depending on FIH. Moreover, $p53_{tot}$ drops significantly with increasing FIH_{tot} due to downregulation of PNUTS under moderate hypoxia. Therefore, FIH abundance plays a significant role in p53 stabilization by modulating HIF-1α activity and PNUTS induction.

To further reveal the significance of PNUTS induction in p53 accumulation, the bifurcation diagram of [$p53_{tot}$] versus k_{sPNUTS} at 0.3% O_2 is plotted (Fig. 5C). The steady-state [$p53_{tot}$] is very low in the absence of PNUTS expression, and rises toward saturation with increasing k_{sPNUTS}. Our results suggest that PNUTS promotes p53 stabilization under moderate hypoxia.

The dynamics of [$p53_{tot}$] and [PNUTS] are compared with experimental data from EC219 cells [15] (Fig. 5D). Upon anoxia (0% O_2), [PNUTS] rises persistently within 12 h due to induction by HIF-1α, and p53 accumulates owing to repression of its dephosphorylation by PNUTS. That is, HIF-1α-induced PNUTS contributes to p53 stabilization in the early phase of cellular response to anoxia as shown in the experiment [15]. In the late phase, PNUTS recovers to basal expression tightly following the behavior of [$HIF\text{-}1\alpha_{tot}$] as shown in Fig. 3E. Therefore, transient PNUTS induction by HIF-1α contributes to the initial accumulation of p53, and other mechanisms are at work to maintain high levels of $p53_{tot}$ in the late phase.

PNUTS and ATR cooperate to facilitate p53 stabilization

Under severe hypoxia, activated ATR phosphorylates p53 and promotes its accumulation in addition to PNUTS [8]. We next characterize the respective role of ATR and PNUTS in p53 stabilization (Fig. 6). Both ATR and PNUTS exhibit bistable behaviors with varying oxygen levels (Fig. 6A). PNUTS is induced gradually under moderate hypoxia, while it drops to basal levels under severe hypoxia. ATR is activated only under severe hypoxia. Thus, PNUTS and ATR

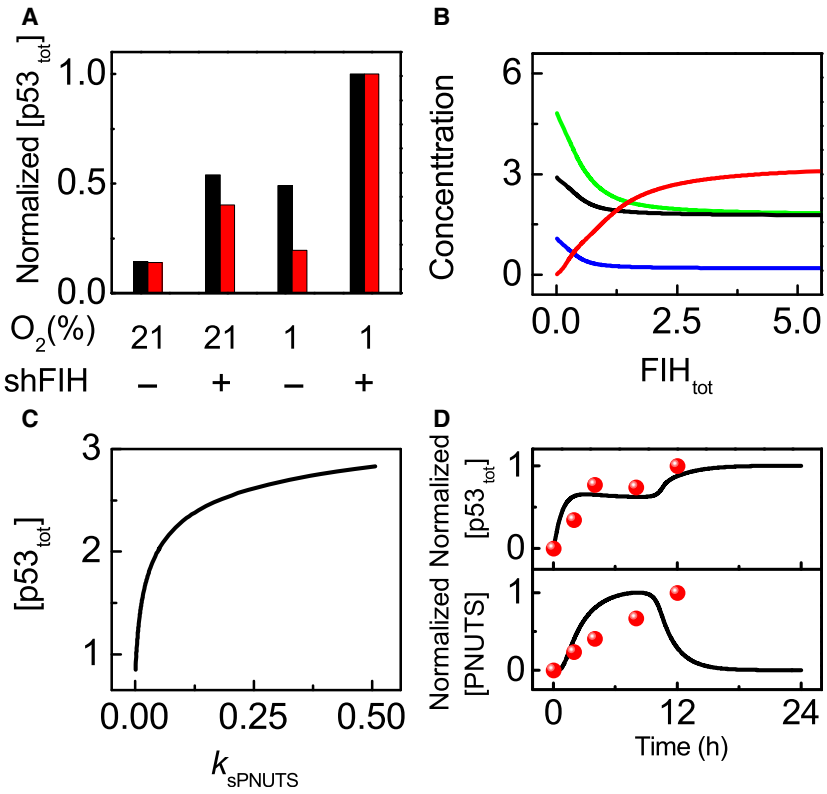

Fig. 5. HIF-1α-induced PNUTS promotes the stabilization of p53. (A) Normalized [$p53_{tot}$] under normoxia (24% O_2) or hypoxia (1% O_2) in the absence or presence of FIH at 24 h. The red bars denote the simulation results and the black bars correspond to experimental data from LS174 [42]. (B) Bifurcation diagrams of [PNUTS] (blue), [$p53_{tot}$] (green), [HIF-1α-aOH_n] (red) and [HIF-$1\alpha_{nAC}$] (black) versus FIH_{tot} at 0.3% O_2. (C) Bifurcation diagram of [$p53_{tot}$] versus the rate constant of PNUTS induction by HIF-$1\alpha_{nAC}$, k_{sPNUTS}, at 0.3% O_2. (D) Time courses of normalized [$p53_{tot}$] and [PNUTS] at 0% O_2. The black lines describe the simulation results, and the red circles represent the experimental data from EC219 cells [15].

contribute to p53 accumulation under different hypoxic conditions.

We further compare the bifurcation diagram of [$p53_{tot}$] versus O_2% without PNUTS or ATR (Fig. 6B). Without PNUTS, p53 still exhibits a bistable switch while it cannot reach sufficiently high levels stimulated by ATR alone. Of note, this result suggests that transient expression of PNUTS contributes to the complete stabilization of p53. Without ATR, the steady-state level of $p53_{tot}$ gradually rises to moderate levels at 0% O_2. Thus, we propose that both PNUTS and ATR are required for the full accumulation of p53 by promoting its phosphorylation upon anoxia.

p53 competes for p300 with HIF-1α

We have verified that HIF-1α is indispensable for high expression of p53 by inducing PNUTS. We will further explore the interplay between the two transcription factors in their activation and investigate the key factors that influence the acetylation of HIF-1α and p53 (Fig. 7).

We first show the bifurcation diagrams of [HIF-$1\alpha_{nAC}$] and [$p53_{nAC}$] versus O_2%, revealing how oxygen levels affect the acetylation of HIF-1α and p53 (Fig. 7A). [$p53_{nAC}$] stays above 4.5 for $L_{O_2} < 0.2\%$ and reduces remarkably for $L_{O_2} > 0.4\%$. By contrast, [HIF-$1\alpha_{nAC}$] rises gradually from 0.4% to 0.085% O_2, while it drops to rather low levels with L_{O_2} below 0.085%. These results can be well explained by higher binding affinity of p53 for p300 that facilitates p53 acetylation [43]. Under moderate hypoxia, HIF-1α is acetylated by p300 and induces PNUTS that promotes p53 stabilization, leading to partial acetylation of p53. Under severe hypoxia, accumulated p53 attenuates HIF-1α activity by competing for p300 and promoting its degradation. That is, p53 promotes its own activation indirectly by repressing HIF-1α, enclosing an implicit positive feedback in the competition between p53 and HIF-$1\alpha_{nAC}$. Together, HIF-1α or p53 predominates in the competition for p300 depending on the severity of hypoxia.

p53 abundance affects the competition for p300 as seen in the bifurcation diagram of [$p53_{nAC}$] and [HIF-$1\alpha_{nAC}$] versus the production rate of p53, k_{sp53_c} (Fig. 7B). Under anoxia, HIF-1α acetylation predominates with very small k_{sp53_c}, and [HIF-$1\alpha_{nAC}$] drops quickly with increasing k_{sp53_c}. When k_{sp53_c} exceeds a certain threshold, p53 prevails over HIF-1α in acetylation. Therefore, the outcome of the competition between p53 and HIF-1α is largely dependent on the

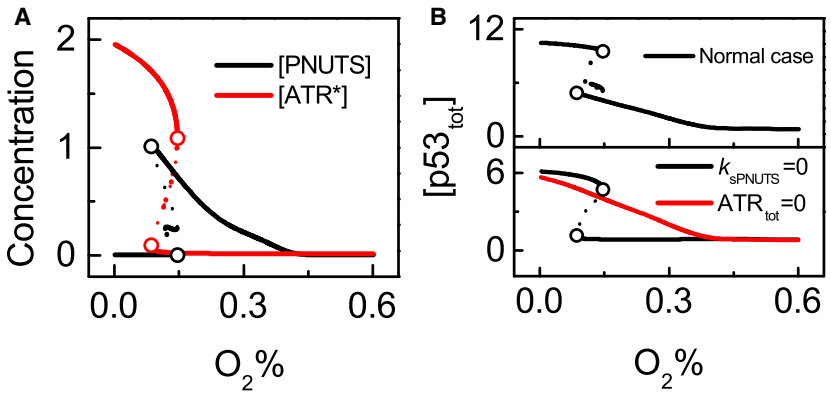

Fig. 6. Roles of PNUTS and ATR in p53 stabilization upon hypoxia. (A) Bifurcation diagrams of [PNUTS] (black) and [ATR*] (red) versus % O_2. (B) Bifurcation diagrams of [$p53_{tot}$] versus % O_2 in the normal case (top), the case of k_{sPNUTS} = 0 or ATR_{tot} = 0 (bottom).

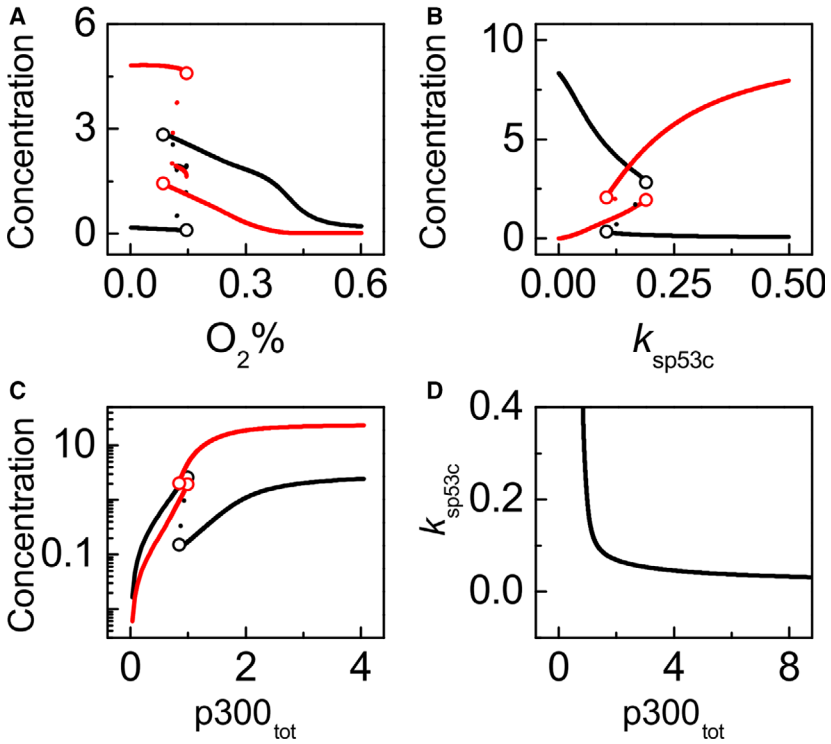

Fig. 7. p53 competes for p300 with HIF-1α upon hypoxia. (A–C) Bifurcation diagrams of [$p53_{nAC}$] (red) and [HIF-$1\alpha_{nAC}$] (black) versus oxygen level (A), k_{sp53_c} (B) and $p300_{tot}$ (C). (D) Two-parameter bifurcation of $p300_{tot}$ and k_{sp53_c}. L_{O_2} is 0% in B–D.

relative proportion of their abundance. Similarly, p300 abundance affects the competition between p53 and HIF-1α (Fig. 7C). Both [$p53_{nAC}$] and [HIF-$1\alpha_{nAC}$] rise with increasing $p300_{tot}$, and both of them become saturated and reach rather high levels when p300 level is very high. Together, intense competition between p53 and HIF-1α appears only for limited p300.

In the two-parameter bifurcation diagram of $p300_{tot}$ versus k_{sp53_c}, the curve shows the threshold of $p300_{tot}$ with varying k_{sp53_c} based on the right SN point in Fig. 7C (Fig. 7D). The phase plane is divided into two regions, p53 is highly (or weakly) acetylated in the upper right (or lower left) region. Both the levels of p53 and p300 significantly affect the outcome of the competition between p53 and HIF-1α. For limited p300, p53 prevails over HIF-1α due to its stabilization under severe hypoxia. When p300 is abundant, both p53 and HIF-1α can be sufficiently acetylated, and HIF-1α level reduces due to p53-dependent degradation. Together, the competition for limited p300 between p53 and HIF-1α significantly influences the cellular outcome upon hypoxia.

p53 promotes its own activation by degrading HIF-1α *via* Mdm2

HIF-1α level is rather low under severe hypoxia [44], which may result from oxygen-independent degradation

modulated by p53 [17,19]. The underlying mechanism is investigated in the following (Fig. 8).

We first show the dynamics of [HIF-1α_{tot}] with different k_{sp53_c} (the rate constants of p53 production) (Fig. 8A). [HIF-1α_{tot}] rises to a high platform followed by returning to basal levels. The transient high platform results from hypoxia-induced stabilization. Increasing the abundance of p53 by increasing k_{sp53_c} largely shortens the platform. Decreasing k_{sp53_c} lengthens the platform; [HIF-1α_{tot}] remains at the high platform when k_{sp53_c} becomes too small. These results can be verified by plotting the bifurcation diagram of [HIF-1α_{tot}] versus k_{sp53_c} (Fig. 8B). Only when k_{sp53_c} exceeds the threshold dose [HIF-1α_{tot}] drop to basal levels.

To explore how the subcellular distribution of Mdm2 affects HIF-1α degradation, we consider three cases: blocking nuclear export of Mdm2 by setting $k_{outM} = 0$, or nuclear entry by setting $k_{inM} = 0$, or normal translocation in the standard parameter setting (Fig. 8C). Restricting Mdm2 to the cytoplasm can effectively decrease [HIF-1α_{tot}], consistent with the experimental data for HIF-1α_{tot} at 4 h after hypoxia treatment in which S166A mutation on Mdm2 inhibits its nuclear entry and promotes HIF-1α_{tot} degradation [38] (see the right histograms). On the contrary, HIF-1α hardly changes if all Mdm2 is confined in the nucleus (experimentally, this was realized by inducing S166E mutation on Mdm2) [38]. Thus, HIF-1α stability is largely affected by the subcellular localization of Mdm2.

p53-induced SHIP-1 can alter the subcellular localization of Mdm2 through the PIP3-AKT cascade and thus participates in regulating HIF-1α stability [12]. p53-dependent induction rate of SHIP-1, k_{sSHIP1}, significantly influences the steady states of [Mdm2$_c$] and [Mdm2$_n$] (Fig. 8D). For small k_{sSHIP1}, most Mdm2 enters the nucleus. Only when k_{sSHIP1} exceeds the threshold, cytoplasmic Mdm2 becomes dominant as its nuclear entry is inhibited by high expression of SHIP-

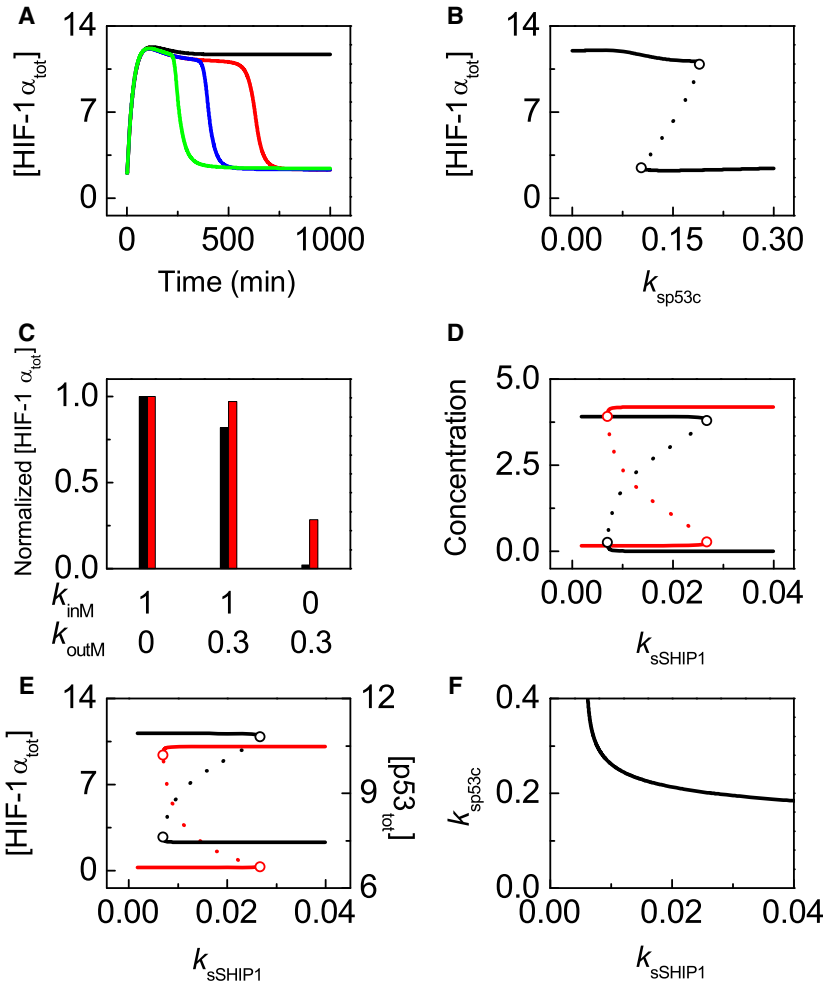

Fig. 8. p53-mediated HIF-1α degradation under anoxia. (A) Time courses of [HIF-1α_{tot}] for k_{sp53_c} = 0.1 (black), 0.2 (red), 0.22 (blue) and 0.3 (green). (B) Steady-state levels of [HIF-1α_{tot}] versus k_{sp53_c}. (C) Normalized [HIF-1α_{tot}] with different settings of k_{inM} and k_{outM} by simulation and experiment at 4 h with k_{sSHIP1} = 0 [38]. (D, E) Steady-state levels of [Mdm2$_n$] (black) and [Mdm2$_c$] (red) (D) and [HIF-1α_{tot}] (black) and [p53$_{tot}$] (red) (E) as a function of k_{sSHIP1}. (F) Two-parameter bifurcation diagram of k_{sp53_c} and k_{sSHIP1}. L_{O_2} is 0% in A–F.

1. As a result, [HIF-1α_{tot}] abruptly drops to low levels when k_{sSHIP1} exceeds the threshold; $p53_{tot}$ further rises to high levels with larger k_{sSHIP1} since SHIP-1 prevents the nuclear entry of Mdm2 (Fig. 8E). Together, SHIP-1 indirectly promotes the degradation of HIF-1α by modulating the subcellular distribution of Mdm2.

k_{sSHIP1} directly determines the level of SHIP-1, while k_{sp53_c} controls the level of p53 and indirectly influences the rate of SHIP-1 production. The two-parameter bifurcation diagram of k_{sp53_c} versus k_{sSHIP1} is plotted to show how they modulate HIF-1α and p53 levels (Fig. 8F). With small k_{sp53_c}, sufficient SHIP-1 can be produced with rather large k_{sSHIP1} so that most Mdm2 can be maintained in the cytoplasm to degrade HIF-1α. With rising k_{sp53_c}, sufficient SHIP-1 can be produced even with smaller k_{sSHIP1} to facilitate HIF-1α degradation by Mdm2. When k_{sSHIP1} becomes rather small, increasing k_{sp53_c} has no marked effects on the threshold since it is rather difficult to produce sufficient SHIP-1 in this case.

Coordination of HIF-1α and p53 guarantees an optimal fate decision

Finally, we reveal how HIF-1α and p53 coordinate to decide cell fate depending on the severity of hypoxia. HIF-1α and p53 are progressively activated to selectively induce their target genes controlling cellular outcome (Fig. 9).

Under mild hypoxia, [HIF-1α_{nT}] (the total level of HIF-1α and HIF-1α-aOH_n) accumulates transiently to induce the phosphofructokinase PFKL to transform metabolism from oxidative phosphorylation to glycolysis, promoting cellular adaptation to hypoxia. Upon moderate hypoxia, [HIF-1α_{nT}] stays at high levels persistently and induces sustained expression of PFKL to trigger glycolysis (Fig. 9A,B). At 0.1% O_2, most HIF-1α converts to acetylated HIF-1α_{nAC} (Fig. 9C). As a result, accumulated HIF-1α_{nAC} induces VEGF (Fig. 9D). Therefore, HIF-1α is partially activated under mild or moderate hypoxia due to deactivation of PHDs,

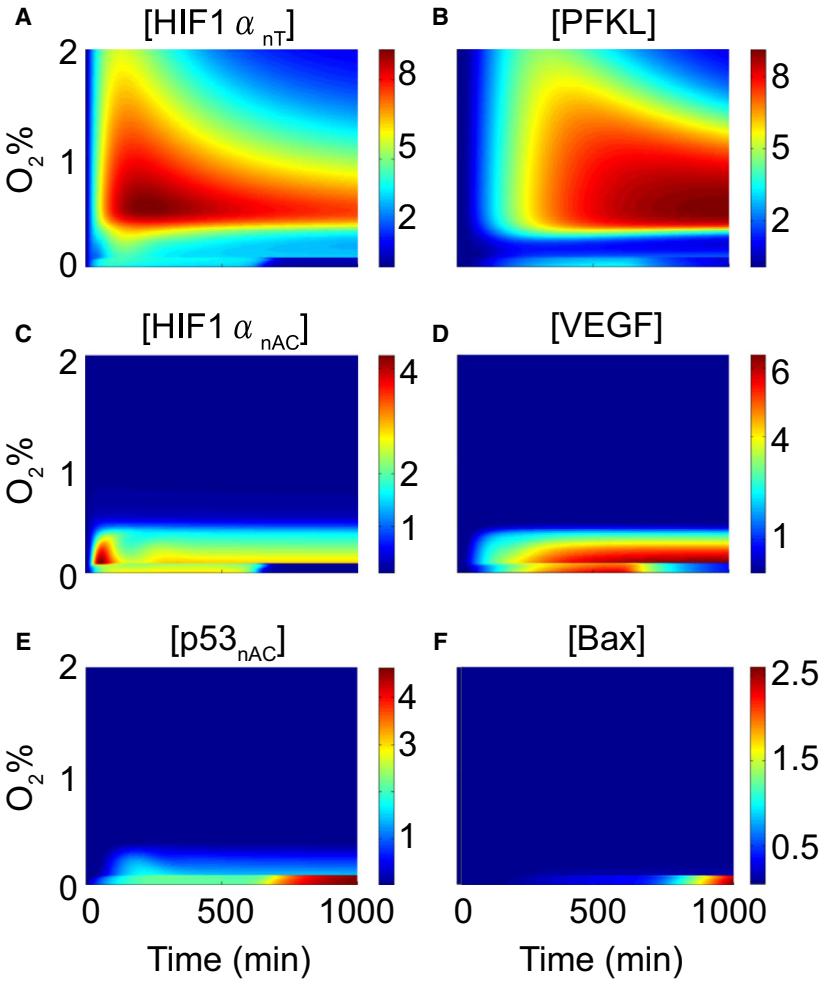

Fig. 9. HIF-1α and p53 cooperate to determine cell fate. (A–F) Color-coded concentration of HIF-1α_{nT} (Sum of HIF-1α-aOH_n and HIF-1α_n) (A), PFKL (B), HIF-1α_{nAC} (C), VEGF (D), $p53_{nAC}$ (E) and Bax (F) as a function of L_{O_2} and time.

inducing a number of genes to facilitate cellular adaptation to hypoxia; further aggravating hypoxia leads to deactivation of FIH and full activation of HIF-1α, promoting angiogenesis that is involved in metastasis [1].

Under moderate hypoxia, p53 is partially activated due to PNUTS-dependent stabilization. Under severe hypoxia, p53 becomes fully activated and accumulates in the form of $p53_{nAC}$ after a delay (Fig. 9E). Correspondingly, Bax is induced by p53 and triggers apoptosis (Fig. 9F). HIF-1α is degraded by Mdm2 after transient activation. Upon anoxia, PNUTS and ATR cooperate to promote p53 phosphorylation and acetylation, activating its proapoptotic activity. Of note, the p53-SHIP-1-Mdm2 positive feedback contributes to the sharp reversion between p53 and HIF-1α. Together, HIF-1α and p53 cooperate to make an optimal fate decision under hypoxia.

Conclusion and Discussion

In this work, we investigated the mechanism of cellular response to hypoxia involving HIF-1α and p53 pathways. The reciprocal regulation of HIF-1α and p53 can be summarized briefly in a schematic diagram (Fig. 10). Under mild hypoxia, HIF-1α is partially activated to trigger glycolysis and does not contribute to p53 stabilization. Under moderate hypoxia, it becomes fully activated and contributes to angiogenesis. At the same time, HIF-1α promotes the partial stabilization of p53 by inducing PNUTS. Accumulated p53 begins to compete for p300 with HIF-1α. Only under severe hypoxia, p53 prevails over HIF-1α in the competition for p300 due to ATR-mediated stabilization. Moreover, activated p53 facilitates its further activation by inducing Mdm2-dependent HIF-1α degradation. Fully activated p53 can initiate apoptosis by inducing Bax. Together, the transition in the predomination of HIF-1α and p53 plays a key role in cell fate decision depending on the severity of hypoxia.

We focused on the modulation of p53 activation by HIF-1α and other factors upon hypoxia. On one hand, HIF-1α contributes to the primary accumulation of p53 by inducing PNUTS under moderate hypoxia. This step was verified indirectly by a recent report that p53 expression could be regulated by FIH, which has been deemed a critical regulator of HIF-1α [42]. On the other hand, HIF-1α represses p53 by competing for p300. Thus, HIF-1α plays a dual role in the regulation of p53 under moderate hypoxia. Under severe hypoxia, ATR promotes the full accumulation of p53 that prevails over HIF-1α in the competition of p300 [8]. Moreover, p53 promotes HIF-1α degradation to maintain its dominant state. Of note, HIF-1α and p53 are predominated under different conditions. Under mild or moderate hypoxia, HIF-1α overwhelms p53 in the competition for p300; under severe hypoxia, a reversion occurs in their competition, and p53 becomes activated. We proposed that HIF-1α-induced PNUTS and ATR coordinate to promote p53 accumulation by regulating its phosphorylation progressively upon hypoxia.

It has been identified experimentally that FIH acts as a repressor of HIF-1α transcriptional activity in cellular response to hypoxia [3,45]. It is broadly expressed in various organs and tissues [46]. Its deletion or downregulation is closely related to tumor progression in multiple human cancers including glioblastoma and colorectal cancer [47,48]. The oncogenic role of FIH depends on its inhibition on HIF-1α transcriptional activity [47,49]. It has been reported that about 40% genes induced by C-TAD of HIF-1α are inhibited by FIH [50]. Although FIH plays a key role in repressing HIF-1α activity in the hypoxic response, FIH-knockout mice exhibit reduced body weight, elevated metabolic rate and improved glucose homeostasis, suggesting that FIH should contribute to metabolism regulation. Of note, the regulation of metabolism by FIH is not dependent on its canonical role in inhibiting HIF-1α-

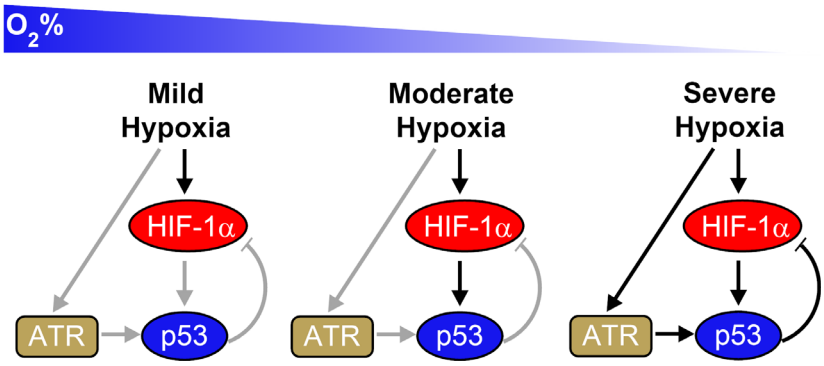

Fig. 10. Schematic of hypoxia-driven cell signaling mediated by p53 and HIF-1α. The black and gray lines denote the activated and inactivated regulation, respectively.

dependent transcription in developmental hypoxia in mice [51]. Thus, FIH performs distinct functions in different context. It is intriguing to characterize the distinct roles of FIH in other contexts in addition to hypoxic response by modeling.

p300 functions as coactivators for the activation of numerous transcription factors in hypoxic response [31–33]. Moreover, p300 also acts as histone acetyltransferases in transcription initiation [35]. It can be considered limiting, sharing by the multiple transcription factors and histones. Especially, the competition of p300 between p53 and HIF-1α significantly influences their transcription activities and is intensively investigated in recent years [32,52]. The induction of the target genes by HIF-1α is modulated by the abundance of p53 and p300 [53]. In our work, it is assumed that HIF-1α and p53 compete for limited p300 upon hypoxia. The amount of available p300 is denoted as $p300_{tot}$ in our model. $p300_{tot}$ is significantly affected by histones and other transcriptional factors. Thus, we have assessed the effects of varying $p300_{tot}$ on the outcome of the competition between HIF-1α and p53. Indeed, it has been revealed experimentally that overexpressed p300 relieves p53-induced repression of HIF-1α activity [34]. Of note, p300 competition should be helpful to maintain the ordered physiological functions induced by various transcription factors [54]. It is still a challenge to construct a more detailed model including more transcription factors to investigate the regulation of HIF-1α and p53 activity in hypoxic response.

The inositol phosphatase SHIP-1 was found in the middle 1990s and shown to be largely expressed in hematopoietic cells [55,56]. Further investigations showed that SHIP-1 is also expressed in other tissues like spermatids, testis, osteoblasts and mesenchymal stem cells [57,58]. Very recently, it has been identified that SHIP-1 is induced by p53 under severe hypoxia in multiple cell lines [11]. We consider p53-induced SHIP-1 as the endogenous repressor of AKT in the present work. In our previous work, PTEN was assumed to be upregulated by p53 by repressing its repressor miR-17-92 [21,59]. SHIP-1 is not considered in the previous model since its induction by p53 in hypoxia has not been reported. We cannot exclude that SHIP-1 and PTEN may cooperate in the inhibition of AKT. Moreover, the additive AKT inhibition pathway may be important in several cancers in which the PTEN is mutated or depleted [60].

There are still some limitations to our model. We did not consider HIF-1-induced autophagy that promotes cell survival and adaptation to hypoxia [61]. It has been reported that Mdm2 competes with FIH for Asn803 at the C-terminal of HIF-1α, facilitating the recruitment of p300 to HIF-1α. The above function of Mdm2 was not considered as the oxygen level required for considerable accumulation of Mdm2 is much less than that of FIH inactivation in our model [62]. Given HIF-1α production is upregulated *via* PI3K pathway [63], p53-induced SHIP-1 should repress HIF-1α production indirectly by inhibiting AKT activation. For simplicity, this indirect repression of HIF-1α by p53 was not included here. Moreover, the transcriptional repression of the target genes by p53 is not considered since we focused on the regulatory mechanism of the transcriptional activity of p53 and HIF-1α.

Hypoxia plays a central role in tumor progression and resistance to therapy [64]. Our work may provide clues for cancer therapy based on the reciprocal regulation of HIF-1α and p53 upon hypoxia. Hypoxic cell killing is significant for cancer therapy [64]. Reactivation of p53 should be an efficient way to remove cancer cells in hypoxic microenvironment and improve treatment effects. Moreover, overexpression of either PNUTS or SHIP-1 may contribute to p53 activation under hypoxic conditions.

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Author contributions

X-PZ designed the research; PW and DG performed numerical simulations; PW, DG, X-PZ and FL analyzed the data; PW, X-PZ, FL and WW wrote the paper; X-PZ coordinated the research. All authors reviewed the manuscript.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Method S1. Equations of the model.

Method S2. Computational code in Oscill8.

Table S1. Description and initial values of variables.

Table S2. Parameters of the model.