



低氧诱导因子-1：细胞适应氧供应改变的关键蛋白*

梁楚婷^{1,2)**} 郭炜骅^{1,2)**} 谭理^{1,2)} 贺筠博^{1,2)} 熊芳^{1,2)}张姗姗¹⁾ 曾朝阳^{1,2)} 熊炜^{1,2)} 李桂源^{1,2)} 郭灿^{1,2)***}⁽¹⁾ 中南大学湘雅医院口腔科, 长沙 410008;⁽²⁾ 中南大学肿瘤研究所, 国家卫健委癌变原理重点实验室和教育部癌变与侵袭原理重点实验室, 长沙 410008)

摘要 2019年诺贝尔生理学或医学奖授予威廉·凯林 (William Kaelin Jr)、彼得·拉特克里夫爵士 (Sir Peter Ratcliffe) 和格雷格·赛门扎 (Gregg Semenza), 以表彰他们在细胞感知和适应缺氧机制上做出的重要贡献. 低氧诱导因子-1 (hypoxia-inducible factor-1, HIF-1) 在细胞适应氧供应改变中起关键作用, 可作为转录因子改变基因表达, 通过提高机体携氧能力、增加血液供应、改变代谢方式等途径来适应缺氧环境. 而 HIF-1 的功能也受到各种机制调控: 泛素化-蛋白酶体途径降解和转录因子活性抑制. HIF-1 与抑癌蛋白 (protein von Hippel-Lindau, pVHL)、脯氨酸羟化酶 (proline hydroxylase, PHD)、HIF 抑制因子 (factor inhibiting HIF, FIH) 等构成了严密有序的调节网络. 本文总结了3位诺贝尔奖获得者的研究成果, 并结合最新的研究进展, 系统阐述了 HIF-1 表达量调节机制和 HIF-1 介导的细胞适应缺氧环境机制.

关键词 低氧诱导因子-1, 缺氧, EPO, VEGF, VHL, PHD, FIH

中图分类号 Q2, Q5, R3

DOI: 10.16476/j.pibb.2019.0250

2019年10月7日, 威廉·凯林 (William G. Kaelin Jr)、彼得·拉特克里夫爵士 (Sir Peter J. Ratcliffe) 和格雷格·赛门扎 (Gregg L. Semenza) 被授予2019年诺贝尔生理学或医学奖, 以表彰他们在细胞感知和适应缺氧机制上做出的重要贡献. 氧气是生存必不可少的物质, 可以作为电子受体参与合成ATP, 为生命活动提供能量. 缺氧对机体生理和病理变化有重要影响. 早在20世纪20年代, 就有研究者 (Otto Heinrich Warburg, 1931年诺贝尔生理学与医学奖获得者) 发现缺氧的组织细胞比正常的组织细胞更容易发生癌变^[1]. 在 Warburg 之后, 研究者们相继发现了慢性缺氧还会导致红细胞增多症、肺动脉高压、右心室肥大^[2]. 为了适应缺氧, 机体具有氧气感应的功能, 当机体暴露于低氧环境, 或者是局部组织细胞处于低氧张力的环境时, 可以通过一系列基因表达的改变来影响细胞代谢与机体反应. 这种分子机制在维持机体稳态, 以及贫血、心血管疾病、肿瘤等疾病中都发挥着重要

作用^[3-6].

格雷格·赛门扎最重要的贡献是发现了适应氧变化机制中的核心分子——低氧诱导因子-1 (hypoxia-inducible factor 1, HIF-1). HIF-1 是作为促红细胞生成素 (erythropoietin, EPO) 的转录激活因子被发现的^[7]. 在缺氧和贫血的情况下, 肝脏和肾脏 EPO 基因表达会上升, 从而促进红细胞生成, 提高氧气的运输能力. 赛门扎的研究解释了其中的分子机制. 他通过分析转基因小鼠中人 EPO 基因的表达发现, 3'端侧翼 DNA 中一段序列可以作为缺氧诱导的增强子控制肝细胞 EPO 的基因表达^[8-9]. 1992年, 赛门扎证实了 EPO 基因 3'端存在约 50 个碱基对的增强子, 并且存在缺氧诱导的核

* 国家自然科学基金(81672993, 81772928, 81702907, 81772901, 81803025)和湖南省自然科学基金(2018JJ3704, 2018JJ3815, 2018SK21210, 2018SK21211)资助项目.

** 并列第一作者. *** 通讯联系人.

Tel: 0731-8480412, E-mail: guocde@csu.edu.cn

收稿日期: 2019-10-21, 接受日期: 2019-10-24

因子与之结合来调控转录激活, 这种核因子就是 HIF-1^[10]. 1995 年, 赛门扎等通过纯化分析得出, HIF-1 主要以异二聚体形式存在, 由 HIF-1 α 和 HIF-1 β 两个亚基组成^[11], 这两个亚基都是含有 PAS 域的基本螺旋-环-螺旋蛋白质. HIF-1 β 是一种芳烃受体核转运子 (aryl hydrocarbon receptor nuclear translocator, ARNT), 其表达与氧气联系不强, HIF-1 α 才是其中对氧敏感的成分^[12]. 编码 HIF-1 α 的基因定位于人染色体 14q21-q24^[13]. 另外两位获奖者拉特克里夫和凯林, 他们的主要贡献是发现 HIF-1 α 的羟基化和 VHL (von Hippel-Lindau) 在 HIF-1 α 泛素化降解中发挥的作用. 1998 年有研究者发现 HIF-1 α 可以通过泛素化-蛋白酶体途径被降解, 并且这种方式是氧依赖的^[14]. 随即于 1999 年,

拉特克里夫在肿瘤抑制蛋白基因 VHL 缺陷的细胞中发现, HIF-1 α 表达稳定, 而重新表达 VHL 基因产物 pVHL 后, HIF-1 α 出现了氧依赖的降解导致表达下降^[15]. 凯林发现, 这种 pVHL 介导的 HIF-1 α 破坏必须在脯氨酸羟化酶 (prolyl hydroxylases, PHDs) 的羟基化作用存在下进行^[16], 并于 2002 年对人 HIF-PHD 进行了生化纯化^[17].

三位获奖者的研究共同揭示了缺氧时细胞维持氧稳态的机制 (图 1). 随着研究的不断发展, HIF-1 的生理和病理作用逐渐探明, HIF-1 除了调节 EPO 基因表达外, 还对多种基因表达有影响^[18-19]. 目前, HIF-1 在疾病中的作用被更深入理解, 促进或抑制 HIF-1 功能的药物将会改善多种疾病疗效.

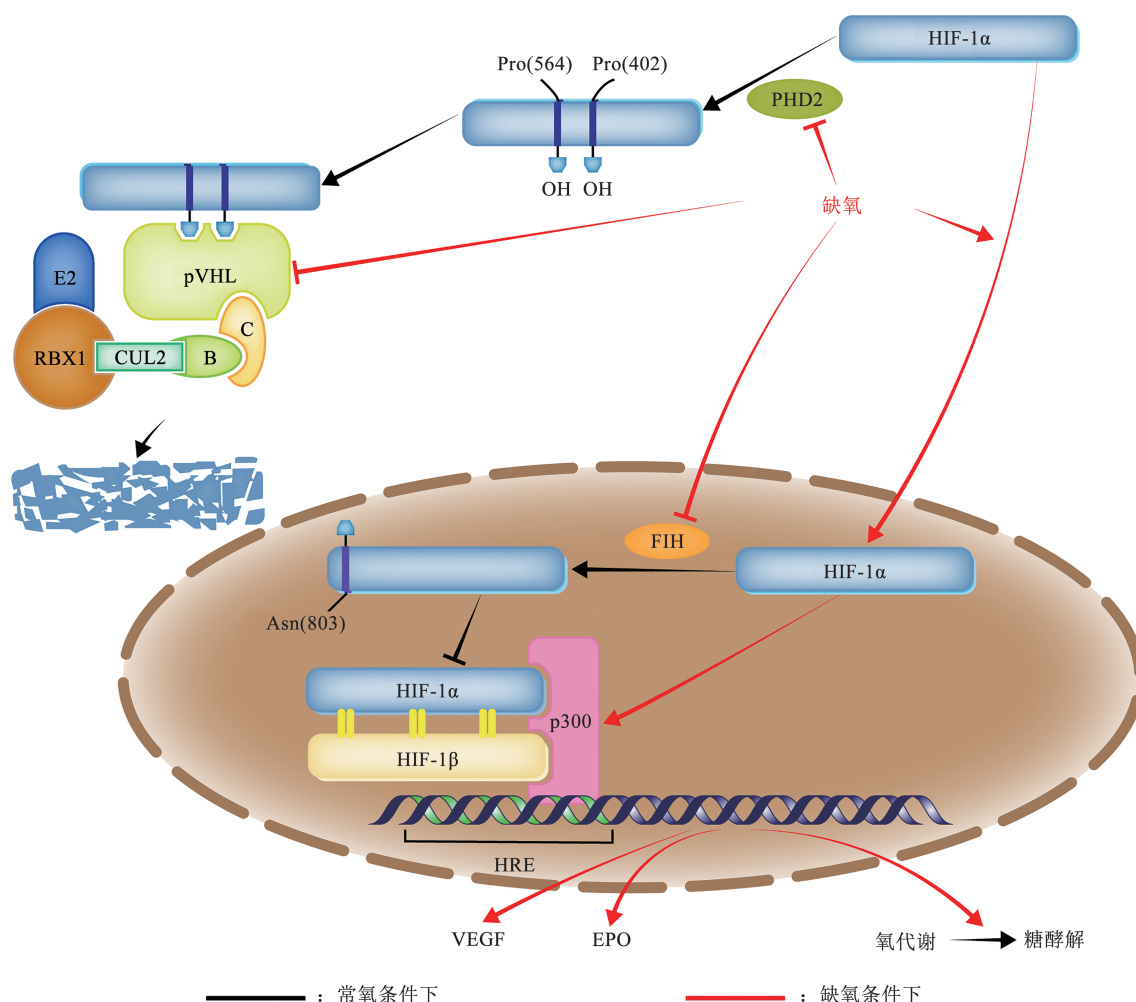


Fig. 1 Mechanism of HIF-1 maintaining cellular oxygen homeostasis

图1 HIF-1维持细胞氧稳态机制

常氧情况下, PHD2羟基化修饰HIF-1 α , 后者经pVHL组成的E3泛素连接酶复合物泛素化修饰后被蛋白酶体降解, HIF抑制因子 (FIH) 羟基化修饰HIF-1 α 使其失活. 缺氧环境中, pVHL、PHD2、FIH被抑制, HIF-1 α 在细胞核中积聚并与HIF-1 β 形成HIF-1二聚体. HIF-1作为转录因子发挥其转录活性, 调控血管生成、红细胞生成、代谢相关基因的转录, 从而适应缺氧环境.

1 缺氧抑制HIF-1的降解

HIF-1是由两个亚基组成的异源二聚体——O₂调节的HIF-1 α 亚基和组成性表达的HIF-1 β 亚基^[3].在正常氧浓度的环境下,HIF-1 α 合成与降解的动态平衡使之保持在一个低浓度的水平,而在缺氧环境下,HIF-1 α 的降解受到抑制,在细胞内积累,结合靶基因内顺式缺氧反应元件(cisacting hypoxia-response elements, HREs),从而调控靶基因的表达.在控制HIF-1 α 浓度与功能的过程中,VHL和PHD等分子发挥了重要的作用,缺氧时这些分子功能受到抑制,使HIF-1 α 的降解受到抑制,表现为细胞内HIF-1的过表达.

1.1 缺氧导致pVHL失活抑制HIF-1 α 降解

VHL是一个抑癌基因,编码pVHL抑癌蛋白,后者参与E3泛素连接酶复合物(VCBC)的构成,VCBC由pVHL、延长蛋白B、C和Cul2蛋白等组成^[20],VCBC能促进HIF-1 α 的蛋白酶体降解^[21].VCBC和HIF-1 α 的结合依赖于pVHL的疏水间隙结构,后者识别HIF-1 α 的羟基化脯氨酰基后可以与HIF-1 α 结合,二者结合呈延长的 β 线样结构,这种结合方式和HIF-1 α 脯氨酸羟基化是增强pVHL-HIF-1 α 复合物稳定性的关键^[22-23].研究表明,除了羟基化作用,pVHL和HIF-1 α 的结合也依赖于Fe²⁺和O₂的调节^[24].pVHL是在Fe²⁺和O₂作用下与羟基化的HIF-1 α 结合使其降解,而在缺氧条件下细胞中pVHL失活,使之不能与HIF-1 α 的羟基化脯氨酰基结合,进而影响HIF-1 α 的蛋白酶体降解,HIF-1 α 合成与降解的动态平衡被打破,细胞中HIF-1 α 过表达.

另外,VHL基因缺陷会抑制HIF-1 α 的降解使HIF-1 α 在细胞内过表达,引起细胞代谢、EPO、血管内皮生长因子(vascular endothelial growth factor, VEGF)一系列改变,导致肿瘤等疾病的发生发展,von Hippel-Lindau病是典型的例子,研究表明,VHL突变参与的HIF-1过表达还可调节细胞程序性死亡、细胞增殖分化、二级转录级联反应等^[25-26].VHL突变的肾细胞癌中发现HIF-1 α 缺乏蛋白酶体降解,在Fe²⁺和O₂作用下补充外源性pVHL可以恢复HIF-1 α 的降解^[27],这揭示了有氧情况下pVHL在HIF-1 α 降解过程中的重要作用.

1.2 缺氧抑制PHD羟基化作用抑制HIF-1 α 降解

前文提到,HIF-1 α 只有经羟基化后才能与pVHL结合,因此HIF-1 α 的降解与HIF-1 α 脯氨酸

残基羟化密切相关,羟基化作用是pVHL结合HIF-1 α 的前提,从而实现HIF-1 α 的蛋白酶体降解,PHD是羟基化作用的关键酶,并且依赖氧发挥作用.PHD是一种2-氧戊二酸双加氧酶,已知的PHD家族主要包括:PHD1、PHD2、PHD3,他们分别结合HIF-1 α 的不同位点发挥作用^[28],PHD2(也称EGLN1)是主要的HIF-PHD,它可促进HIF-1 α 的降解,这种表型在西藏人中常见,是适应高原地区缺氧的表现^[29].PHD1和PHD3主要发挥与HIF-1无关的生理功能,如PHD3调节神经细胞的程序性死亡,其活性可能被胞质伴侣蛋白TRiC调节^[30],PHD1羟化FOXO3a促进Cyclin D1的表达,促进细胞增殖^[31].研究发现PHD羟化HIF-1 α 是依赖Fe²⁺和O₂的^[16],因此有氧条件下羟基化HIF-1 α 可以募集pVHL实现HIF-1 α 的降解.但PHD在细胞缺氧时失活,进而中断HIF-1 α 羟化过程,HIF-1 α 蛋白酶体降解过程受阻,导致HIF-1在细胞中的积累,激活下游产生一系列细胞缺氧改变^[32].

1.3 缺氧抑制FIH羟基化作用上调HIF-1 α 活性

与PHD相似,HIF抑制因子(factor inhibiting HIF, FIH)也是一种2-氧戊二酸和Fe(II)依赖的双加氧酶,并且都具有羟基化作用^[33].不同的是,FIH不是通过调节HIF-1 α 的降解来发挥作用,而是通过调节HIF-1 α 的转录因子活性来发挥作用^[34].缺氧对于FIH活性的调控,可能是由活性氧(reactive oxygen species, ROS)介导的.缺氧条件下,复合物III处辅酶Q的氧化还原循环使线粒体ROS增加,从而诱导FIH失活^[35].但是这一机制目前仍存在争议.在治疗缺氧时,FIH会快速积聚于细胞核中,这涉及了FIH细胞核与细胞质间转移过程的调控.最新的研究发现,FIH的核输入和核输出分别由HIF-1 α 和exportin 1介导^[36].FIH使HIF-1 α 的C端反激活域(C-terminal transactivation domain, CAD)中的一个天门冬氨酸残基发生羟基化,从而破坏了其与p300的相互作用,阻止转录激活^[37].PHD和FIH都是HIF系统中重要的缺氧感知元件,但他们在诱导细胞缺氧应答基因中的作用中有明显差异.拉特格里夫等利用小分子HIF羟化酶抑制剂,发现选择性PHD和FIH抑制剂的联合应用可以诱导一个单独使用PHD抑制剂无法诱导的基因亚群转录^[38].这提示我们,HIF系统是在PHD和FIH的共同作用下运作的,因此同时抑制PHD和FIH的羟基化作用可能在抑制靶向基因治疗中发挥更大作用.

1.4 缺氧时其他因素对HIF-1的调控

缺氧导致pVHL失活、抑制PHD的羟化作用,最终影响HIF-1 α 的蛋白酶体降解;缺氧还可能通过ROS途径介导FIH失活抑制HIF-1 α 的天冬氨酰羟基化.除此之外,缺氧时HIF-1还受到诸多因素影响:间歇性缺氧时细胞增强钙黏蛋白依赖性激酶活性诱导HIF-1转录^[39];缺氧适应可能与HIF受mTOR (mammalian target of rapamycin) 调控有关^[40];NOC18不通过抑制HIF羟基化影响HIF的降解,但在缺氧条件下它可提供NO促进HIF的合成^[41];治疗方面,以HIF-1为靶点设计抑制HIF-1合成和促进HIF-1羟基化、蛋白酶体降解的药物,从而抑制缺氧条件下HIF-1的过表达,达到治疗疾病的效果,如地高辛抑制HIF-1转录活性减慢了慢性缺氧导致的肺动脉高压的进展^[42].

2 HIF-1通过调控多种途径使细胞适应缺氧环境

在缺氧环境下,氧气的缺乏将会通过上述氧信号通路激发缺氧适应反应,氧信号通路几乎存在于所有细胞中,通过控制基因转录广泛地影响下游通路,协调血液供氧与血管生长和能量代谢作用^[43].以HIF-1为核心的氧信号通路可从多个方面导致机体的缺氧适应发生,例如提高血液的携氧能力、增加缺氧局部的血液供应、引发细胞的缺氧耐受等.这些改变促进了组织与细胞对缺氧的适应能力,使其在变化的环境中保持相对稳定的生理功能^[44].

作为氧信号通路的核心,HIF-1的生物学活性可被多种缺氧导致的因素调节,并影响下游基因的转录.HIF-1可以与靶基因内HREs结合,通过招募共激活蛋白p300和CBP^[45],可以在整个人类基因组的数千个基因座上形成活性转录复合物,激活对缺氧适应性反应蛋白质的基因转录,包括EPO、VEGF和糖酵解酶等^[2],促进机体对缺氧环境的适应.

然而,缺血性疾病和肿瘤是缺氧发生的两个重要的临床背景,组织与细胞的某些缺氧适应反应在这些病理情况下反而会促进疾病的进展,或是导致新的疾病发生.机体的缺氧适应反应在缺血性疾病与肿瘤的发生发展过程中扮演了极其重要的角色,理解缺氧在这些疾病中发挥的作用对这些疾病的治疗具有重要的意义.

2.1 HIF-1提高血液的携氧能力

在缺氧环境下,HIF-1可以通过激活EPO基因

的转录促进EPO的生成,EPO可以通过促进红细胞的生成调节血液携氧能力,缓解机体缺氧状况,在O₂稳态中起关键作用.胎儿的EPO基因在肝脏中表达,而出生后肾脏成为其主要的合成与分泌部位.前文已提到,EPO的转录受HIF-1的调控,而该因子被PHD抑制^[46].因此,脯氨酰羟化酶抑制剂可以稳定HIF-1并刺激包括EPO基因在内的HIF-1靶基因的表达.这些分子可以激活肾源性贫血患者患病肾脏中的EPO基因表达,并且正在开发或已经在临床上用于治疗肾性贫血^[47].

2.2 HIF-1促进血管生成

在缺氧或缺血环境的细胞中HIF-1可诱导VEGF的表达^[48],VEGF在机体血管生成和血管重塑中起重要作用^[49],VEGF可激活缺氧部的血管细胞,动员血管生成细胞进入循环^[50].新生的血管增加缺氧部分的血液供应,缓解了组织细胞的缺氧情况,但这也为肿瘤的生长提供了基础^[51].

缺氧对原发肿瘤的形成及其向致死表型的发展至关重要,而HIF-1活性的增加为VEGF诱导的肿瘤血管生成和癌细胞对缺氧的其他适应提供了分子基础^[52].由于其在肿瘤血管形成中的重要作用,HIF-1现已成为肿瘤治疗的关键靶点之一^[53].抑制HIF-1 α 活性会损害肿瘤的生长、血管生成和血管成熟^[54].除肿瘤的发生发展外,VEGF刺激的新血管形成发生在几个重要的临床环境中,包括视网膜疾病、心肌缺血和伤口愈合.近年来的研究发现HIF-1介导的VEGF生成在翼状胬肉^[55]、视网膜病变^[19, 56]、烧伤创面愈合^[57]等的治疗上具有重要价值.此外,在器官移植方面,增强HIF-1 α 的表达可通过VEGF促进微血管修复,减轻移植植物缺氧^[58].

2.3 HIF-1通过调控细胞代谢适应缺氧环境

HIF-1除通过促进血管生成和红细胞生成以提高氧气的输送外,还可通过调节代谢以降低细胞对氧气的需求来适应缺氧环境.在糖代谢中,HIF-1一方面通过促进葡萄糖转运蛋白1 (glucose transporter 1, GLUT1) 的表达,以促进细胞对葡萄糖的摄取,提高能量供应,另一方面通过促进糖酵解酶的表达使氧化代谢向糖酵解转变,即促进丙酮酸向乳酸的转变并限制向乙酰辅酶A的转变,减少对氧气的需求^[59-60].醛缩酶A (aldolase A, ALDA)、磷酸甘油酸激酶1 (phosphoglycerate kinase 1, PGK1) 和丙酮酸激酶M (pyruvate kinase M, PKM) 是葡萄糖向丙酮酸转化途径中的

几个关键酶, 乳酸脱氢酶A可催化丙酮酸向乳酸转化. 研究发现, 这些基因含有与促红细胞生成素增强子中HIF-1结合位点相似的序列. 在缺氧条件下, HIF-1促进了这些基因的表达, 以促进乳酸的生成^[61]. 同时, HIF-1还激活丙酮酸脱氢酶激酶1 (pyruvate dehydrogenase kinase 1, PDK1) 的表达, 使丙酮酸脱氢酶失活, 从而抑制丙酮酸向乙酰辅酶A的转化^[62]. 在脂肪代谢中, HIF-1通过PGC-1 β 途径抑制中链和长链酰基辅酶A脱氢酶 (medium- and long-chain acyl-CoA dehydrogenases, MCAD and LCAD), 从而抑制脂肪酸 β -氧化, 进而减少乙酰辅酶A的生成^[63]. HIF-1还可通过调控线粒体从而调控细胞代谢. 在缺氧环境下, 线粒体会产生大量活性氧, 进而导致细胞死亡^[64]. HIF-1一方面通过诱导BNIP3和Beclin-1/Atg5复合物的表达, 诱导线粒体自噬^[65], 另一方面通过将细胞色素C氧化酶亚基从COX4-1替换到COX4-2, 从而提高线粒体呼吸的效率^[66]. 在缺氧条件下, HIF-1通过对以上这些基因表达的调控, 使氧化代谢向糖酵解转化, 并增强了糖酵解, 以减少氧气的需求并维持正常的能量供应, 同时抑制了缺氧诱导的活性氧生成对细胞的损伤.

2.4 HIF-1在缺氧环境中是把双刃剑

HIF-1一方面通过血管生成、促红细胞生成素、代谢重编程介导了对缺氧的适应性反应^[67], 这体现在缺血预处理 (ischemic preconditioning, IPC) 上, 组织器官长时间的缺血及长时间的缺血后再灌注易导致器官损伤, 而短暂的缺血和再灌注则保护了器官免受损伤, 这一过程称为缺血预处理. 而HIF-1在这一过程中是必需的^[68-69]. 另一方面, HIF-1则介导了对慢性连续和间歇性缺氧的不良反应^[67]. 在阻塞性睡眠呼吸暂停综合征 (obstructive sleep apnoea, OSA) 患者中, OSA会引起颈动脉体产生慢性间断性缺氧 (chronic intermittent hypoxia, CIH), CIH诱导活性氧的形成并增加细胞内Ca²⁺水平, 从而引起HIF-1 α 表达增加和HIF-2 α 表达降低, 导致HIF-1 α 依赖的促氧化剂和HIF-2 α 依赖的抗氧化剂之间的平衡被破坏, 导致活性氧进一步增加, 进而激活交感神经系统并促进肾上腺髓质分泌儿茶酚胺, 导致高血压和呼吸异常^[70-72]. 慢性缺氧还会引起红细胞增多症和肺动脉高压^[2]. 在肺动脉高压小鼠模型中, HIF-1参与

了右心室质量、右心室压力和肺动脉内侧壁厚度的增加^[73]. 肺动脉平滑肌细胞 (pulmonary arterial smooth muscle cell, PASMC) 增殖引起的血管重塑是肺动脉高压的一个重要发病机制, HIF-1一方面抑制缺氧诱导的K(v)通道活性, 另一方面诱导BMP4, 上调TRPC1、TRPC6, 从而增加细胞内钙离子浓度, 引起PASMC的收缩和增殖^[74-76], HIF-1还可以通过增强Na⁺/H⁺离子交换酶活性, 使细胞内pH呈碱性, 有利于PASMC的增殖^[77].

前文提到, 缺氧可通过HIF-1对肿瘤的发生发展发挥巨大的作用, 除肿瘤血管生成外, 还包括侵袭转移、免疫逃避、化疗抵抗等^[54, 78-80]. 值得注意的是, 肿瘤即使在氧气充足的情况下, 依然进行糖酵解即消耗大量葡萄糖产生乳酸. 这种现象称为Warburg效应^[1]. 其中一种机制就是HIF-1与丙酮酸激酶M2 (pyruvate kinase M2, PKM2) 之间的正反馈调节^[81]. HIF-1诱导PKM2表达, PKM2可直接与HIF-1 α 亚基相互作用从而促进HIF-1与靶基因的结合, 共激活分子的募集, 组蛋白乙酰化和基因转录. PHD3对PKM2的羟基化增强了PKM2与HIF-1 α 的相互作用^[82-83]. 这种正反馈促进了Warburg效应, 促进HIF-1下游包括糖酵解、血管生成基因的表达, 从而促进肿瘤的发展.

3 治 疗

HIF-1在缺氧适应反应中的重要功能, 以及其在多种疾病中的作用, 使得靶向HIF-1来治疗各种疾病成为可能. 目前靶向HIF-1的药物制剂可分为两类: 治疗肿瘤性疾病和缺血性疾病^[84]. 对于肿瘤性疾病, 研究人员想通过抑制HIF-1的活性或表达, 来减弱缺氧对肿瘤发生发展的促进作用^[85-86]. 对于缺血性疾病, 研究人员一方面想通过促进HIF-1的表达来提高缺氧适应性反应, 如IPC^[87], 应用PHD抑制剂来降低HIF-1 α 的降解, 从而治疗贫血和其他低氧性疾病^[88], 基因治疗或化学诱导剂增强HIF-1 α 活性来防止肢体缺血和减少同种异体移植排斥^[89]. 另一方面想通过抑制HIF-1来减弱HIF-1带来的不良反应, 如在缺血性视网膜病中, 地高辛和叶黄素分别抑制HIF-1的合成和转录活性来抑制眼部的新生血管^[90-91], 地高辛通过抑制HIF-1转录活性减缓了慢性低氧引起的肺动脉高压的进展^[42].

参 考 文 献

- [1] Vander Heiden M G, Cantley L C, Thompson C B. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science*, 2009, **324**(5930): 1029-1033
- [2] Yu A Y, Shimoda L A, Iyer N V, *et al.* Impaired physiological responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1 alpha. *J Clin Invest*, 1999, **103**(5): 691-696
- [3] Schodel J, Grampp S, Maher E R, *et al.* Hypoxia, hypoxia-inducible transcription factors, and renal cancer. *Eur Urol*, 2016, **69**(4): 646-657
- [4] Semenza G L. Hypoxia-inducible factor 1 and cardiovascular disease. *Annu Rev Physiol*, 2014, **76**: 39-56
- [5] Semenza G L. Hypoxia-inducible factors: mediators of cancer progression and targets for cancer therapy. *Trends Pharmacol Sci*, 2012, **33**(4): 207-214
- [6] Schito L, Semenza G L. Hypoxia-inducible factors: master regulators of cancer progression. *Trends Cancer*, 2016, **2**(12): 758-770
- [7] Hirota K, Semenza G L. Regulation of angiogenesis by hypoxia-inducible factor 1. *Crit Rev Oncol Hematol*, 2006, **59**(1): 15-26
- [8] Semenza G L, Koury S T, Nejfelt M K, *et al.* Cell-type-specific and hypoxia-inducible expression of the human erythropoietin gene in transgenic mice. *Proc Natl Acad Sci USA*, 1991, **88**(19): 8725-8729
- [9] Semenza G L, Nejfelt M K, Chi S M, *et al.* Hypoxia-inducible nuclear factors bind to an enhancer element located 3' to the human erythropoietin gene. *Proc Natl Acad Sci USA*, 1991, **88**(13): 5680-5684
- [10] Semenza G L, Wang G L. A nuclear factor induced by hypoxia *via* de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. *Mol Cell Biol*, 1992, **12**(12): 5447-5454
- [11] Wang G L, Semenza G L. Purification and characterization of hypoxia-inducible factor 1. *J Biol Chem*, 1995, **270**(3): 1230-1237
- [12] Wang G L, Jiang B H, Rue E A, *et al.* Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O₂ tension. *Proc Natl Acad Sci USA*, 1995, **92**(12): 5510-5514
- [13] Kambe T, Tada J, Chikuma M, *et al.* Embryonal carcinoma P19 cells produce erythropoietin constitutively but express lactate dehydrogenase in an oxygen-dependent manner. *Blood*, 1998, **91**(4): 1185-1195
- [14] Lee J W, Bae S H, Jeong J W, *et al.* Hypoxia-inducible factor (HIF-1)alpha: its protein stability and biological functions. *Exp Mol Med*, 2004, **36**(1): 1-12
- [15] Maxwell P H, Wiesener M S, Chang G W, *et al.* The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature*, 1999, **399**(6733): 271-275
- [16] Ivan M, Kondo K, Yang H, *et al.* HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O₂ sensing. *Science*, 2001, **292**(5516): 464-468
- [17] Ivan M, Haberberger T, Gervasi D C, *et al.* Biochemical purification and pharmacological inhibition of a mammalian prolyl hydroxylase acting on hypoxia-inducible factor. *Proc Natl Acad Sci USA*, 2002, **99**(21): 13459-13464
- [18] Schodel J, Mole D R, Ratcliffe P J. Pan-genomic binding of hypoxia-inducible transcription factors. *Biol Chem*, 2013, **394**(4): 507-517
- [19] Ozaki H, Yu A Y, Della N, *et al.* Hypoxia inducible factor-1alpha is increased in ischemic retina: temporal and spatial correlation with VEGF expression. *Investigative Ophthalmology & Visual Science*, 1999, **40**(1): 182-189
- [20] Clifford S C, Cockman M E, Smallwood A C, *et al.* Contrasting effects on HIF-1alpha regulation by disease-causing pVHL mutations correlate with patterns of tumourigenesis in von Hippel-Lindau disease von Hippel-Lindau. *Hum Mol Genet*, 2001, **10**(10): 1029-1038
- [21] Shen C, Kaelin W G, Jr. The VHL/HIF axis in clear cell renal carcinoma. *Semin Cancer Biol*, 2013, **23**(1): 18-25
- [22] Hon W C, Wilson M I, Harlos K, *et al.* Structural basis for the recognition of hydroxyproline in HIF-1 alpha by pVHL. *Nature*, 2002, **417**(6892): 975-978
- [23] Min J H, Yang H, Ivan M, *et al.* Structure of an HIF-1alpha-pVHL complex: hydroxyproline recognition in signaling. *Science*, 2002, **296**(5574): 1886-1889
- [24] Mole D R, Maxwell P H, Pugh C W, *et al.* Regulation of HIF by the von Hippel-Lindau tumour suppressor: implications for cellular oxygen sensing. *IUBMB Life*, 2001, **52**(1-2): 43-47
- [25] Peng M, Mo Y, Wang Y, *et al.* Neoantigen vaccine: an emerging tumor immunotherapy. *Mol Cancer*, 2019, **18**(1): 128
- [26] Kondo K, Kaelin W G Jr. The von Hippel-Lindau tumor suppressor gene von Hippel-Lindau. *Exp Cell Res*, 2001, **264**(1): 117-125
- [27] Cockman M E, Masson N, Mole D R, *et al.* Hypoxia inducible factor-alpha binding and ubiquitylation by the von Hippel-Lindau tumor suppressor protein. *J Biol Chem*, 2000, **275**(33): 25733-25741
- [28] Appelhoff R J, Tian Y M, Raval R R, *et al.* Differential function of the prolyl hydroxylases PHD1, PHD2, and PHD3 in the regulation of hypoxia-inducible factor. *J Biol Chem*, 2004, **279**(37): 38458-38465
- [29] Lorenzo F R, Huff C, Myllymaki M, *et al.* A genetic mechanism for Tibetan high-altitude adaptation. *Nat Genet*, 2014, **46**(9): 951-956
- [30] Masson N, Appelhoff R J, Tuckerman J R, *et al.* The HIF prolyl hydroxylase PHD3 is a potential substrate of the TRiC chaperonin. *FEBS Lett*, 2004, **570**(1-3): 166-170
- [31] Xiao L, Wei F, Liang F, *et al.* TSC22D2 identified as a candidate susceptibility gene of multi-cancer pedigree using genome-wide linkage analysis and whole-exome sequencing. *Carcinogenesis*, 2019, **40**(7): 819-827
- [32] Tian Y M, Yeoh K K, Lee M K, *et al.* Differential sensitivity of hypoxia inducible factor hydroxylation sites to hypoxia and hydroxylase inhibitors. *J Biol Chem*, 2011, **286**(15): 13041-13051
- [33] Elkins J M, Hewitson K S, Mcneill L A, *et al.* Structure of factor-inhibiting hypoxia-inducible factor (HIF) reveals mechanism of oxidative modification of HIF-1 alpha. *J Biol Chem*, 2003, **278**(3): 1802-1806
- [34] Mahon P C, Hirota K, Semenza G L. FIH-1: a novel protein that

- interacts with HIF-1alpha and VHL to mediate repression of HIF-1 transcriptional activity. *Genes Dev*, 2001, **15**(20): 2675-2686
- [35] Tormos K V, Chandel N S. Inter-connection between mitochondria and HIFs. *J Cell Mol Med*, 2010, **14**(4): 795-804
- [36] Wang Y, Zhong S, Schofield C J, *et al*. Nuclear entry and export of FIH are mediated by HIF1alpha and exportin1, respectively. *J Cell Sci*, 2018, **131**(22): pii: jcs219782
- [37] Hewitson K S, McNeill L A, Riordan M V, *et al*. Hypoxia-inducible factor (HIF) asparagine hydroxylase is identical to factor inhibiting HIF (FIH) and is related to the cupin structural family. *J Biol Chem*, 2002, **277**(29): 26351-26355
- [38] Talks K L, Turley H, Gatter K C, *et al*. The expression and distribution of the hypoxia-inducible factors HIF-1alpha and HIF-2alpha in normal human tissues, cancers, and tumor-associated macrophages. *Am J Pathol*, 2000, **157**(2): 411-421
- [39] Yuan G, Nanduri J, Bhasker C R, *et al*. Ca²⁺/calmodulin kinase-dependent activation of hypoxia inducible factor 1 transcriptional activity in cells subjected to intermittent hypoxia. *J Biol Chem*, 2005, **280**(6): 4321-4328
- [40] Brugarolas J, Kaelin W G, Jr. Dysregulation of HIF and VEGF is a unifying feature of the familial hamartoma syndromes. *Cancer Cell*, 2004, **6**(1): 7-10
- [41] Kasuno K, Takabuchi S, Fukuda K, *et al*. Nitric oxide induces hypoxia-inducible factor 1 activation that is dependent on MAPK and phosphatidylinositol 3-kinase signaling. *J Biol Chem*, 2004, **279**(4): 2550-2558
- [42] Abud E M, Maylor J, Udem C, *et al*. Digoxin inhibits development of hypoxic pulmonary hypertension in mice. *Proc Natl Acad Sci USA*, 2012, **109**(4): 1239-1244
- [43] Xia M, Zhang Y, Jin K, *et al*. Communication between mitochondria and other organelles: a brand-new perspective on mitochondria in cancer. *Cell Biosci*, 2019, **9**: 27
- [44] Formenti F, Constantin-Teodosiu D, Emmanuel Y, *et al*. Regulation of human metabolism by hypoxia-inducible factor. *Proc Natl Acad Sci USA*, 2010, **107**(28): 12722-12727
- [45] Ruas J L, Poellinger L, Pereira T. Role of CBP in regulating HIF-1-mediated activation of transcription. *J Cell Sci*, 2005, **118**(Pt 2): 301-311
- [46] Semenza G L. HIF-1, O(2), and the 3 PHDs: how animal cells signal hypoxia to the nucleus. *Cell*, 2001, **107**(1): 1-3
- [47] Schodel J, Ratcliffe P J. Mechanisms of hypoxia signalling: new implications for nephrology. *Nat Rev Nephrol*, 2019, **15**(10): 641-659
- [48] Forsythe J A, Jiang B H, Iyer N V, *et al*. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol Cell Biol*, 1996, **16**(9): 4604-4613
- [49] Liu Y, Christou H, Morita T, *et al*. Carbon monoxide and nitric oxide suppress the hypoxic induction of vascular endothelial growth factor gene *via* the 5' enhancer. *J Biol Chem*, 1998, **273**(24): 15257-15262
- [50] Bosch-Marce M, Okuyama H, Wesley J B, *et al*. Effects of aging and hypoxia-inducible factor-1 activity on angiogenic cell mobilization and recovery of perfusion after limb ischemia. *Circ Res*, 2007, **101**(12): 1310-1318
- [51] Goel H L, Mercurio A M. VEGF targets the tumour cell. *Nat Rev Cancer*, 2013, **13**(12): 871-882
- [52] Semenza G L. HIF-1: using two hands to flip the angiogenic switch. *Cancer Metastasis Rev*, 2000, **19**(1-2): 59-65
- [53] Semenza G L. Targeting HIF-1 for cancer therapy. *Nat Rev Cancer*, 2003, **3**(10): 721-732
- [54] Fan C, Tang Y, Wang J, *et al*. Long non-coding RNA LOC284454 promotes migration and invasion of nasopharyngeal carcinoma via modulating the Rho/Rac signaling pathway. *Carcinogenesis*, 2019, **40**(2): 380-391
- [55] Deng X, Xiong F, Li X, *et al*. Application of atomic force microscopy in cancer research. *J Nanobiotechnology*, 2018, **16**(1): 102
- [56] Rodrigues M, Kashiwabuchi F, Deshpande M, *et al*. Expression pattern of HIF-1alpha and VEGF supports circumferential application of scatter laser for proliferative sickle retinopathy. *Invest Ophthalmol Vis Sci*, 2016, **57**(15): 6739-6746
- [57] Du J, Liu L, Lay F, *et al*. Combination of HIF-1alpha gene transfection and HIF-1-activated bone marrow-derived angiogenic cell infusion improves burn wound healing in aged mice. *Gene Ther*, 2013, **20**(11): 1070-1076
- [58] Jiang X, Malkovskiy A V, Tian W, *et al*. Promotion of airway anastomotic microvascular regeneration and alleviation of airway ischemia by deferoxamine nanoparticles. *Biomaterials*, 2014, **35**(2): 803-813
- [59] Mo Y, Wang Y, Zhang L, *et al*. The role of Wnt signaling pathway in tumor metabolic reprogramming. *J Cancer*, 2019, **10**(16): 3789-3797
- [60] Fan C, Tu C, Qi P, *et al*. GPC6 promotes cell proliferation, migration, and Invasion in nasopharyngeal carcinoma. *J Cancer*, 2019, **10**(17): 3926-3932
- [61] Semenza G L, Roth P H, Fang H M, *et al*. Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. *J Biol Chem*, 1994, **269**(38): 23757-23763
- [62] Kim J W, Tchernyshyov I, Semenza G L, *et al*. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab*, 2006, **3**(3): 177-185
- [63] Huang, Li T, Li X, *et al*. HIF-1-mediated suppression of acyl-CoA dehydrogenases and fatty acid oxidation is critical for cancer progression. *Cell Rep*, 2014, **8**(6): 1930-1942
- [64] Semenza G L. Hypoxia-inducible factor 1: regulator of mitochondrial metabolism and mediator of ischemic preconditioning. *Biochim Biophys Acta*, 2011, **1813**(7): 1263-1268
- [65] Zhang H, Bosch-Marce M, Shimoda L A, *et al*. Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. *J Biol Chem*, 2008, **283**(16): 10892-10903
- [66] Fukuda R, Zhang H, Kim J W, *et al*. HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells. *Cell*, 2007, **129**(1): 111-122
- [67] Xiong F, Deng S, Huang H B, *et al*. Effects and mechanisms of innate immune molecules on inhibiting nasopharyngeal carcinoma. *Chin Med J (Engl)*, 2019, **132**(6): 749-752

- [68] Sarkar K, Cai Z, Gupta R, *et al.* Hypoxia-inducible factor 1 transcriptional activity in endothelial cells is required for acute phase cardioprotection induced by ischemic preconditioning. *Proc Natl Acad Sci USA*, 2012, **109**(26): 10504-10509
- [69] Cai Z, Zhong H, Bosch-Marce M, *et al.* Complete loss of ischaemic preconditioning-induced cardioprotection in mice with partial deficiency of HIF-1 alpha. *Cardiovasc Res*, 2008, **77**(3): 463-470
- [70] Semenza G L, Prabhakar N R. The role of hypoxia-inducible factors in carotid body (patho) physiology. *J Physiol*, 2018, **596**(15): 2977-2983
- [71] Wu C, Li M, Meng H, *et al.* Analysis of status and countermeasures of cancer incidence and mortality in China. *Sci China Life Sci*, 2019, **62**(5): 640-647
- [72] Peng Y J, Yuan G, Ramakrishnan D, *et al.* Heterozygous HIF-1alpha deficiency impairs carotid body-mediated systemic responses and reactive oxygen species generation in mice exposed to intermittent hypoxia. *J Physiol*, 2006, **577**(Pt2): 705-716
- [73] Bo H, Fan L, Li J, *et al.* High expression of lncRNA AFAP1-AS1 promotes the progression of colon cancer and predicts poor prognosis. *J Cancer*, 2018, **9**(24): 4677-4683
- [74] Whitman E M, Pisarcik S, Luke T, *et al.* Endothelin-1 mediates hypoxia-induced inhibition of voltage-gated K⁺ channel expression in pulmonary arterial myocytes. *Am J Physiol Lung Cell Mol Physiol*, 2008, **294**(2): L309-318
- [75] Wang J, Weigand L, Lu W, *et al.* Hypoxia inducible factor 1 mediates hypoxia-induced TRPC expression and elevated intracellular Ca²⁺ in pulmonary arterial smooth muscle cells. *Circ Res*, 2006, **98**(12): 1528-1537
- [76] Wang J, Fu X, Yang K, *et al.* Hypoxia inducible factor-1-dependent up-regulation of BMP4 mediates hypoxia-induced increase of TRPC expression in PASMCs. *Cardiovasc Res*, 2015, **107**(1): 108-118
- [77] Shimoda L A, Fallon M, Pisarcik S, *et al.* HIF-1 regulates hypoxic induction of NHE1 expression and alkalization of intracellular pH in pulmonary arterial myocytes. *Am J Physiol Lung Cell Mol Physiol*, 2006, **291**(5): L941-949
- [78] Wang W, Zhou R, *et al.* PVT1 promotes cancer progression via microRNAs. *Front Oncol*, 2019, **9**: 609
- [79] Jin K, Wang S, Zhang Y, *et al.* Long non-coding RNA PVT1 interacts with MYC and its downstream molecules to synergistically promote tumorigenesis. *Cell Mol Life Sci*, 2019, **76**(21): 4275-4289
- [80] Mo Y, Wang Y, Xiong F, *et al.* Proteomic analysis of the molecular mechanism of lovastatin inhibiting the growth of nasopharyngeal carcinoma cells. *J Cancer*, 2019, **10**(10): 2342-2349
- [81] Wei F, Jing Y Z, He Y, *et al.* Cloning and characterization of the putative AFAP1-AS1 promoter region. *J Cancer*, 2019, **10**(5): 1145-1153
- [82] Wang Y A, Li X L, Mo Y Z, *et al.* Effects of tumor metabolic microenvironment on regulatory T cells. *Mol Cancer*, 2018, **17**(1): 168
- [83] Tang L, Wei F, Wu Y, *et al.* Role of metabolism in cancer cell radioresistance and radiosensitization methods. *J Exp Clin Cancer Res*, 2018, **37**(1): 87
- [84] Wei F, Tang L, He Y, *et al.* BPIFB1 (LPLUNC1) inhibits radioresistance in nasopharyngeal carcinoma by inhibiting VTN expression. *Cell Death Dis*, 2018, **9**(4): 432
- [85] Wu Y, Wei F, Tang L, *et al.* Herpesvirus acts with the cytoskeleton and promotes cancer progression. *J Cancer*, 2019, **10**(10): 2185-2193
- [86] Fan C M, Wang J P, Tang Y Y, *et al.* circMAN1A2 could serve as a novel serum biomarker for malignant tumors. *Cancer Sci*, 2019, **110**(7): 2180-2188
- [87] Bergeron M, Gidday J M, Yu A Y, *et al.* Role of hypoxia-inducible factor-1 in hypoxia-induced ischemic tolerance in neonatal rat brain. *Ann Neurol*, 2000, **48**(3): 285-296
- [88] Duan S, Guo W, Xu Z, *et al.* Natural killer group 2D receptor and its ligands in cancer immune escape. *Mol Cancer*, 2019, **18**(1): 29
- [89] Jiang X, Wang J, Deng X, *et al.* Role of the tumor microenvironment in PD-L1/PD-1-mediated tumor immune escape. *Mol Cancer*, 2019, **18**(1): 10
- [90] Fan C, Tang Y, Wang J, *et al.* The emerging role of Epstein-Barr virus encoded microRNAs in nasopharyngeal carcinoma. *J Cancer*, 2018, **9**(16): 2852-2864
- [91] Yoshida T, Zhang H, Iwase T, *et al.* Digoxin inhibits retinal ischemia-induced HIF-1alpha expression and ocular neovascularization. *FASEB J*, 2010, **24**(6): 1759-1767

Hypoxia-inducible Factor-1: a Key Protein for Cells Adapting to Changes in Oxygen Supply*

LIANG Chu-Ting^{1,2)**}, GUO Wei-Hua^{1,2)**}, TAN Li^{1,2)}, HE Yun-Bo^{1,2)}, XIONG Fang^{1,2)},
ZHANG Shan-Shan¹⁾, ZENG Zhao-Yang^{1,2)}, XIONG Wei^{1,2)}, LI Gui-Yuan^{1,2)}, GUO Can^{1,2)***}

⁽¹⁾Department of Ophthalmology, Xiangya Hospital, Central South University, Changsha 410008, China;

⁽²⁾NHC Key Laboratory of Carcinogenesis and Key Laboratory of Carcinogenesis and Cancer Invasion of the Chinese Ministry of Education, Cancer Research Institute, Central South University, Changsha 410008, China)

Abstract In 2019, the Nobel Prize in Physiology or Medicine was awarded to William G.Kaelin Jr, Sir Peter J. Ratcliffe and Gregg L.Semenza for their discoveries in mechanism of how cells sense and adapt to oxygen level. Hypoxia-inducible factor-1 (HIF-1) plays a key role in this mechanism. It can act as a transcription factor to alter gene expression and adapt to hypoxia by increasing oxygen carrying capacity, increasing blood supply, and changing metabolic patterns. The function of HIF-1 is also regulated by various mechanisms: ubiquitination-proteasome pathway degradation and inhibition of transcription factor activity under the action of tumor suppressor proteins pVHL, proline hydroxylase (PHD), factor inhibiting HIF (FIH), *etc.* This paper summarizes the research results of three Nobel Prize winners, and introduces the latest progress, systematically expounds the regulation mechanism of HIF-1 and HIF-1 mediated cell adaptation to hypoxia.

Key words hypoxia-inducible infactor-1, hypoxia, EPO, VEGF, VHL, PHD, FIH

DOI: 10.16476/j.pibb.2019.0250

* This work was supported by grants from The National Natural Science Foundation of China (81672993, 81772928, 81702907, 81772901 and 81803025) and the Hunan Province Natural Sciences Foundation of China (2018JJ3704, 2018JJ3815, 2018SK21210 and 2018SK21211).

** These authors contributed equally to this work.

*** Corresponding author.

Tel: 86-731-8480412, E-mail: guocde@csu.edu.cn

Received: October 21, 2019 Accepted: October 24, 2019