HDAC6-HSP90途径介导蛋白折叠和降解调控肿瘤细胞增殖与转移

田睿[†], 颜晓羽[†], 于司杭, 许龙, 赵元馨, 孙连坤^{*}, 苏静^{*}

吉林大学基础医学院病理生理学教研室, 病理生理学教育部重点实验室, 长春 130000

[†] 同等贡献

^{*} 联系人, E-mail: sunlk@jlu.edu.cn; sujing@jlu.edu.cn

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摘要 目前认为, 关键激酶以及调控蛋白的折叠和降解失调与肿瘤的发生发展密切相关, 分子伴侣通过整合蛋白质组(proteome)的折叠与降解机制在维持蛋白稳态中发挥了至关重要的作用. HSP90等伴侣蛋白的异常高表达被认为是恶性肿瘤的标志, 通过参与细胞生存以及凋亡途径等关键蛋白的调节, 为肿瘤提供了生存优势. HSP90一方面与其他伴侣蛋白相互作用稳定“客户蛋白”的构象, 另一方面还能够辅助蛋白酶体途径介导蛋白的降解. 最近的研究提示, HSP90的乙酰化等翻译后修饰是维持其功能的关键机制之一. 组蛋白去乙酰化酶6(HDAC6)是重要的自噬受体, 通过去乙酰化皮层肌动蛋白(cortactin)参与自噬体与溶酶体的融合. 目前发现, HDAC6通过降低HSP90的乙酰化水平维持了HSP90分子伴侣的功能, 保证客户蛋白的稳定折叠和成熟释放; 同时, HDAC6还作为HSP90的客户蛋白, 其活性也受到HSP90的调节. 因此, 进一步研究HSP90与HDAC6的交互作用可能为理解肿瘤细胞的分子伴侣如何协调蛋白折叠与自噬降解机制维持自身生存提供新的思路. 本文总结了目前对HSP90以及HDAC6在蛋白折叠以及降解机制中的研究进展, 提出HDAC6与HSP90抑制剂联合应用治疗肿瘤, 为靶向HSP90-HDAC6途径抑制肿瘤增殖转移提供新的方向与思路.

关键词 组蛋白去乙酰化酶6, 热休克蛋白90, 蛋白质折叠与降解, 肿瘤增殖与转移

蛋白质的高效生产、折叠和分泌是肿瘤快速增殖的保证. 为应对恶劣的环境, 肿瘤细胞往往通过上调多种分子伴侣的表达来促进蛋白质的折叠^[1]. 然而, 新合成的蛋白质中有近30%的错误折叠蛋白, 这些蛋白将通过泛素-蛋白酶体系统以及细胞自噬过程被降解, 防止形成毒性聚集体导致细胞死亡^[2]. 蛋白质折叠与降解的相互协调是维持细胞内蛋白质质量控制及稳定的关键机制, 靶向蛋白质折叠和降解途径治疗癌症已成为研究的焦点.

热休克蛋白90(heat shock protein 90, HSP90)作为

伴侣蛋白, 既是参与蛋白质折叠的重要分子伴侣, 又发挥了促进蛋白降解的功能^[3]. 在恶性肿瘤中, HSP90作为增殖转移的关键调节因子促进了多种致癌蛋白的成熟与激活^[4]. HSP90结合辅助分子伴侣传递来的客户蛋白形成复合体后进入折叠循环, 经过一系列三磷酸腺苷(ATP)水解供能及辅助分子伴侣的帮助, 改变HSP90蛋白N端的开闭状态, 最终达到促进客户蛋白的正确折叠与成熟的目的. 此外, HSP90还可以携带客户蛋白与蛋白酶体相结合, 并辅助客户蛋白的降解. HSP90的活性受翻译后修饰的调控, 其中HSP90蛋白的乙酰化已被

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广泛研究. 一些研究表明, 沉默组蛋白去乙酰化酶6 (Histone deacetylase 6, HDAC6) 可以获得HSP90的乙酰化产物, 从而减弱HSP90的伴侣功能, 降低客户蛋白的稳定性.

HDAC6是组蛋白去乙酰化酶(Histone deacetylase, HDAC)家族重要的成员, 主要存在于细胞浆中, 可作为自噬受体(autophagy receptor)发挥作用. 而自噬(autophagy)则是细胞中维持内稳态的重要生命过程, 它通过降解错误折叠的蛋白以及损伤的细胞器发挥细胞保护作用. 此外, HDAC6自身还具有一定的独特性: 首先, HDAC6以自己为核心吸引泛素化蛋白成为蛋白聚集体的支架, 并通过去乙酰化皮层肌动蛋白(cortactin)参与自噬体与溶酶体的融合. 除此之外, HDAC6的去乙酰化作用还调节着HSP90的伴侣功能. 近年研究发现, HDAC6的去乙酰化酶特性及HSP90的伴侣功能为肿瘤增殖转移创造了条件, 促进了相关癌蛋白的稳定, 并通过参与自噬调节过程促进肿瘤细胞存活. 目前, 在膀胱癌、肺癌、胃癌、乳腺癌、黑色素瘤、甲状腺等多种肿瘤中, HSP90以及HDAC6异常高表达^[5~10]. 因此, HSP90-HDAC6可能通过参与蛋白质的折叠及降解机制, 维持细胞内的蛋白质稳态, 为肿瘤的存活、增殖及转移创造条件. 进一步探讨HSP90-HDAC6信号的作用

有望为肿瘤治疗提供新的靶点.

本文讨论了肿瘤细胞中HDAC6-HSP90相互作用如何调控蛋白质的折叠与降解(图1), 以及HDAC6抑制剂(HDAC6 inhibitor, HDAC6i)诱导HSP90乙酰化如何调控肿瘤相关蛋白表达, 增强HSP90抑制剂与HSP90的结合能力, 从而达到抑制肿瘤细胞的增殖与侵袭转移的作用.

1 HDAC6与HSP90的调控

1.1 HSP90调控蛋白折叠

HSP90是一种ATP依赖的高度保守伴侣蛋白, 介导多种肿瘤相关蛋白的折叠和修复^[11]. 根据亚细胞定位的不同, 目前将HSP90家族分为4类: 定位于细胞质和细胞核的HSP90 α 和HSP90 β 、定位于内质网的葡萄糖调节蛋白94(GRP94)以及主要分布在线粒体的肿瘤坏死因子受体蛋白1(tumor necrosis factor receptor associated protein 1, TRAP1). 其中HSP90 α 是可诱导形式, 而HSP90 β 是组成性表达形式, 两者均形成二聚体, 并且在结构和功能方面显著相似^[12~14]. 在结构上, 家庭成员由结合ATP的N-末端结构域、包含亚细胞定位序列的C-末端结构域以及中间结构域组成. 其中, 当HSP90缺

图1 (网络版彩色)HDAC6参与HSP90的调控. HDAC6抑制HSP90的乙酰化水平, 促进HSP90与客户蛋白的结合, 从而调控细胞增殖转移相关途径蛋白的折叠; 当抑制HDAC6时, HSP90过度乙酰化, 增强了HSP90与其抑制剂17-AAG(tanespimycin)的相互作用, 减弱了HSP90与ATP结合的能力, 从而影响HSP90的蛋白折叠. 一部分错误折叠的蛋白经蛋白酶体途径降解清除, 而另一部分蛋白因过度堆积形成毒性聚集体导致细胞凋亡

Figure 1 (Color online) HDAC6 participates in the regulation of HSP90. HDAC6 inhibits the acetylation level of HSP90 and promotes the binding of client proteins to HSP90, thereby regulating the folding of proteins related to cell proliferation and metastasis; when HDAC6 is inhibited, HSP90 is over-acetylated, which enhances the interaction between HSP90 and its inhibitor 17-AAG(tanespimycin). It weakens the ability of HSP90 to bind to ATP, thereby affecting the protein folding of HSP90. Part of the misfolded proteins are degraded and eliminated by the proteasome pathway, while the other part of the proteins are excessively accumulated to form toxic aggregates and cause apoptosis

乏C-末端结构域时,其ATP酶活性会受到损害;中间结构对HSP90与客户蛋白的相互作用、自身功能和结构的灵活性至关重要^[11,15~19]。

研究表明,HSP90能够完成如此复杂的调控过程还依赖一些辅助分子伴侣,如HSP40、HSP70、P23、CHOP(C/EBP-homologous protein)等的帮助。首先,辅助分子伴侣HSP40与客户蛋白质结合产生的相互作用导致HSP70蛋白的募集,HSP70在此阶段仅与HSP40较弱结合。HSP70/HSP40复合物的形成触发ATP水解,引发HSP70的构象变化,增加其与底物的亲和力^[20,21]。其次,HSP70与HSP90的相互作用还需要Hop(应激诱导蛋白1, Stt1)的参与,二磷酸腺苷(adenosine diphosphate, ADP)结合的HSP70可以与Hop相互作用,同时Hop也在携带客户蛋白的HSP70/HSP40复合物与HSP90二聚体之间起到了重要的衔接作用^[22,23]。随后,HSP90伴侣复

合物与ATP结合,迫使HSP90呈现“封闭”构象,p23通过稳定闭合HSP90二聚体构象起到减慢ATP水解的作用,从而减弱HSP90的功能活性。最后,ATP水解为ADP导致HSP90再一次变为开放构象并释放成熟的客户蛋白质(图2)^[24]。这些ATP依赖的构象排列对客户蛋白质的正确折叠和随后的稳定性至关重要。

研究发现,HSP90通过调控客户蛋白折叠参与肿瘤细胞增殖、凋亡以及血管生成等多条信号^[25]。HSP90维持生长因子受体生存信号激酶,如AKT、磷脂酰肌醇-3-激酶(lecithin exception inositol-3-kinase, PI3K),以及癌基因的稳定性^[26,27]。在伯基特(Burkitt)淋巴瘤中,HSP90抑制剂PU-H71处理24 h后,PI3K下游的4E结合蛋白1(4E-binding protein 1, 4EBP1)蛋白表达显著降低,表明HSP90参与了PI3K途径关键蛋白的调控^[28]。应激状态下HSP90-AKT与促凋亡激酶ASK1(apoptosis

图 2 (网络版彩色)HSP90协助客户蛋白质折叠的机制。首先,伴侣蛋白HSP40与客户蛋白结合产生的相互作用募集了HSP70蛋白,HSP70/HSP40复合物由此形成并触发ATP水解,这引发了HSP70的构象变化并且增强了HSP70与底物的亲和力。其次,与ADP结合的HSP70可以与Hop相互作用,通过Hop的衔接作用与HSP90结合,将客户蛋白成功运送至HSP90。此时,HSP90与ATP结合再次引起自身构象变化,HSP90呈现“封闭”构象,辅助分子伴侣p23通过稳定HSP90的闭合构象,达到减缓ATP水解的目的。最后,当ATP又一次水解为ADP导致HSP90变为开放构象,成熟的客户蛋白质也由此释放。

Figure 2 (Color online) The mechanism of HSP90 assists client protein folding. Firstly, the interaction between the chaperone protein HSP40 and the client protein recruits the HSP70 protein, and the HSP70/HSP40 complex forms and triggers ATP hydrolysis, which triggers a conformational change of HSP70 and enhances the affinity of HSP70 to the substrate. Secondly, HSP70 that binds to ADP can interact with Hop, and bind to HSP90 through Hop's cohesion to successfully deliver client protein to HSP90. At this time, the combination of HSP90 and ATP again causes HSP90's own conformational change, HSP90 assumes a closed conformation, and the auxiliary molecular chaperone p23 achieves the purpose of slowing down the hydrolysis of ATP by stably closing the conformation of HSP90. Finally, when ATP is hydrolyzed to ADP again, HSP90 becomes an open conformation, then mature client proteins are released.

signal regulating kinase 1)形成的复合物抑制了ASK1的促凋亡活性;而当抑制HSP90时,该复合物(HSP90-AKT-ASK1)的构象则会发生变化,ASK1的促凋亡活性被重新激活,由此增强了应激物(如H₂O₂)诱导的细胞凋亡. HSP90还可以通过结合血管内皮生长因子(vascular endothelial growth factor, VEGF),激活一氧化氮合酶(nitric oxide synthase, NOS)和基质金属蛋白酶2(matrix metalloproteinase 2, MMP2)促进血管的生成和转移^[11]. VEGF刺激内皮细胞将eNOS和AKT募集到HSP90结构上,促进eNOS磷酸化、酶的激活以及NO的释放^[29]. 线粒体中, HSP90与TRAP1(TNF receptor associated protein 1)、CYLD(cyclophilin D)和survivin相互作用,发挥抗凋亡功能. 研究表明,用HSP90抑制剂GA(geldanamycin)处理HeLa细胞后, HSP90伴侣功能受到抑制,客户蛋白被蛋白酶体降解, survivin的表达随GA浓度的变化呈现依赖性损失,并因此诱导了线粒体凋亡的激活^[30]. 据报道,在氧化过程中由亚基环组成的20S蛋白酶体蛋白复合物通过结合HSP90,选择性识别和降解客户蛋白. 氧化的钙调蛋白(calmodulin, CaM)仅在HSP90存在的情况下才会被20S蛋白酶体显著降解(表1). 以上研究提示, HSP90可能为肿瘤治疗的

潜在靶点.

1.2 HDAC6通过翻译后修饰调控HSP90参与蛋白降解

研究发现, HSP90过度乙酰化会导致其活性受到抑制,直接造成客户蛋白折叠异常. 在胞浆中发挥作用的组蛋白去乙酰化酶HDAC6在维持HSP90的功能和活性中发挥了重要作用. HDAC6是HDAC家族中唯一具有两个串联的含有催化活性的去乙酰化酶结构域(DD1、DD2)的成员,具有独特的结构特征. HDAC6属于Zn²⁺依赖性蛋白酶,可以在细胞质与细胞核之间穿梭. 核输出信号(nuclear export-signal, NES)与含有色氨酸-谷氨酰胺的十四肽确保将HDAC6锚定在细胞质中,保证细胞质中HDAC6的稳定表达. C末端含有的锌指结构域(ZnF-UBP)可与单链或多链泛素相结合形成聚集体,HDAC6可以通过该结构域募集泛素化蛋白促进自噬进程(图3).

HDAC6通过调控HSP90的乙酰化水平影响HSP90客户蛋白的表达. 研究表明,在缺乏HDAC6的小鼠胚胎成纤维细胞中乙酰化的HSP90水平升高,这些细胞中的糖皮质激素受体功能受损,作为HSP90客户蛋白的雄激

表 1 肿瘤相关的HSP90客户蛋白

Table 1 Cancer-related client proteins of HSP90

HSP90客户蛋白	作用位点	分子功能	肿瘤中的功能	文献
VEGF受体	细胞膜	血管生成	新血管形成	
AKT(PDK1、GSK3β、mTOR)	细胞质	通过PI3K通路调节细胞存活信号	抗凋亡细胞存活信号	[31]
DAPK(death associated protein kinase)	细胞质	介导多种细胞死亡相关的过程	肿瘤抑制	
ASK1	细胞质	诱导细胞凋亡、细胞增殖、分化和存活	HSP90-Akt-ASK1形成复合物,抑制促凋亡活性	[32]
CYLD	线粒体	介导氧化应激条件下异常蛋白的降解	肿瘤抑制	[33]
Survivin	线粒体	抑制细胞凋亡	促进肿瘤的增殖、分化和侵袭	
20S蛋白酶体	细胞质	介导氧化应激条件下异常蛋白的降解	HSP90增强了20S蛋白酶体对氧化钙调蛋白的降解	[34]

图 3 (网络版彩色)HDAC6的功能性结构域

Figure 3 (Color online) The functional domains of HDAC6

素受体也在HDAC6抑制后蛋白表达下调^[35,36]。HDAC6表达的减少还影响了另一种HSP90客户蛋白HIF- α (hypoxia inducible factor-1)的稳定性^[37]。癌症相关信号通路EGFR-RAS-RAF-MEK诱导HDAC6在Ser1035位的磷酸化,当抑制HDAC6阻断这一途径促使白血病细胞和肺癌细胞中的HSP90乙酰化,并抑制HSP90与ATP结合,使其与客户蛋白的相互作用丧失或减弱^[38,39]。研究表明,HDAC6介导的HSP90去乙酰化可以结合并保护这些癌蛋白免受蛋白酶体的降解^[40]。同时,HDAC6可以调节HSP90介导的VEGF受体的稳定,血管内皮生长因子受体(vascular endothelial growth factor receptor, VEGFR)在肿瘤生长和血管生成中起关键作用,HDAC6的敲除会导致HSP90的客户蛋白VEGFR降解^[41]。癌症相关的转录因子 β -catenin也与HDAC6和HSP90相关。PKC α (protein kinase C α)通过磷酸化HDAC6增强HDAC6去乙酰化 β -catenin的活性, β -catenin的去乙酰化支持其核转位与抗病毒基因表达^[42]。有证据证明,HSP90活性在维持PKC α 磷酸化中起着重要作用,HSP90保护PKC α 不受HSP70/HSC70依赖性蛋白酶体途径参与的降解^[43]。此外,HSP90有助于LFP、突变体AKT、c-KIT(v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog)、白血病标记蛋白WT1(Wilms tumor protein)和P53突变体的折叠与稳定性^[44-47]。有关白血病细胞的研究表明,HDAC6调节HSP90的乙酰化从而调节BCR-ABL融合蛋白和FLT3-ITD的蛋白酶体降解^[48]。在急性髓细胞白血病(acute myeloid leukemia, AML)中,G蛋白偶联受体激酶2(G protein-coupled receptor kinases 2, GRK2)与HSP90相关,而GRK2可磷酸化HDAC6从而激活 α -tubulin^[49]。由此可见,HDAC6与HSP90之间的翻译后修饰关系影响着癌症的发生发展,肿瘤的增殖、侵袭转移,进而有可能导致癌症药物的耐受。

2 HDAC6-HSP90调控自噬途径

自噬过程是动态变化的,通过双层膜结构包裹待降解的蛋白形成自噬体,将自噬底物运送向溶酶体,在溶酶体内完成降解,分解出的大分子物质最终释放回细胞质,而这一系列动态的过程也被称为自噬流(autophagy flux)。在该过程中HDAC6作为自噬受体,帮助泛素化蛋白转运至自噬体降解。同时,HDAC6还能够通过其去乙酰化酶活性调控自噬体的运动^[47,50]。研究表明,当蛋白酶体功能受到抑制时,HDAC6通过与泛素

结合来感知错误折叠的泛素化蛋白,同时激酶ERK1 (extracellular-regulated kinase 1)通过磷酸化HDAC6,增强 α -微管蛋白的乙酰化来维持微管的稳定性并促进细胞的转移^[39]。随后,HDAC6通过与动力蛋白的相互作用将结合的泛素蛋白沿着微管中心逆行运输,最终促进泛素化蛋白积累形成聚集体^[51,52]。蛋白酶体受抑制时,通过去乙酰化cortactin引起F-actin聚集形成肌动蛋白网络,HDAC6促进自噬小体与溶酶体的融合从而调节自噬成熟(图4)^[53]。有研究表明,自噬促进了细胞侵袭转移。研究结果提示,自噬促进了基质金属蛋白酶MMP2在肝细胞癌(hepatocellular carcinoma, HCC)细胞中的表达,从而促进了细胞外基质(extracellular matrix, ECM)的降解,增强了HCC细胞的转移能力^[54]。当抑制HDAC6时,自噬流量受阻导致自噬相关蛋白p62积累,通过活化caspas8启动细胞凋亡信号^[55]。过度积累的p62可以通过与致癌蛋白Twist1相结合,稳定Twist1蛋白的表达阻止其降解,进而达到促进肿瘤细胞增殖与转移的目的^[56]。以上证据证明,HDAC6可能通过细胞自噬参与了肿瘤细胞存活、增殖以及转移机制的调控。

尽管自噬具有双重作用,但多数研究认为,通畅的自噬流可能作为一种保护机制促进肿瘤细胞的存活^[57-59]。我们实验组发现,索拉非尼能够增加肝癌细胞的自噬水平,同时能够通过上调HDAC6去乙酰化酶活性促进HSP90的折叠功能,导致HSP90的客户蛋白PKM2(M2型丙酮酸激酶)的活性增强,降低了HCC细胞对索拉非尼的敏感性(工作未发表)。值得注意的是,近年来的研究发现,HDAC6与自噬相关蛋白p62的交互作用对自噬流的调节至关重要。自噬相关蛋白p62可能参与了HDAC6去乙酰化酶活性的调节。在前列腺癌中,p62的过表达导致HDAC6蛋白水平的增加,酶活性增强, α -微管蛋白的乙酰化和微管稳定性降低,促进了前列腺癌细胞发生上皮-间质的转化;然而,HDAC6携带泛素化蛋白通过微管运输的途径受到阻碍,这不利于自噬体完成降解过程^[60]。另一方面,研究发现,p62通过与HDAC6酶催化结构域DD2相互作用,抑制了HDAC6的酶活性^[61]。在小鼠胚胎成纤维细胞中抑制p62的表达能够增强HDAC6的去乙酰化酶活性,导致cortactin的募集急剧增加,但p62缺失抑制了F-actin的重塑,进而抑制了自噬小体与溶酶体的融合,阻断了自噬流。

最近的研究发现,HSP90也参与了细胞自噬中多个关键分子的调控。例如,自噬抑制途径AKT/mTOR (mammalian target of rapamycin)中的AKT蛋白、自噬

图4 (网络版彩色)HDAC6与HSP90参与肿瘤细胞蛋白质折叠及降解。(1) 细胞中错误折叠的蛋白经蛋白酶体途径降解。(2) 一些不能被蛋白酶体清除的大分子蛋白, 被HDAC6募集经自噬途径降解。HDAC6还能通过降低cortactin的乙酰化水平促进溶酶体与自噬体融合。(3) 当HDAC6识别错误折叠蛋白并与其结合, 复合物(VCP-HSF1-HSP90-HDAC6)开始解离, HSF1由此释放, 诱导热休克因子与HSP90转录促进细胞存活。(4) HSP90帮助20S蛋白酶体选择性识别HSP90客户蛋白, 并促进客户蛋白降解

Figure 4 (Color online) HDAC6 and HSP90 are involved in protein folding and degradation of tumor cells. (1) Misfolded proteins in cells are degraded by the proteasome pathway. (2) Some macromolecular proteins that cannot be cleared by the proteasome are recruited by HDAC6 and degraded by the autophagy pathway. HDAC6 can also promote the fusion of lysosomes and autophagosomes by reducing the acetylation level of cortactin. (3) When HDAC6 recognizes the misfolded protein and binds to it, the complex (VCP-HSF1-HSP90-HDAC6) begins to dissociate, and HSF1 is released, which induces heat shock factor and HSP90 transcription to promote cell survival. (4) HSP90 helps 20S proteasome to selectively recognize HSP90 client protein and promote client protein degradation

相关蛋白Beclin1、Ulk1(unc-51 like autophagy activating kinase 1)以及LAMP2A(lysosomal associated protein 2A)等均作为HSP90的客户蛋白, 受HSP90的调控^[62-65]。过表达HSP90AA1显著降低了AKT和mTOR的磷酸化水平, 导致PI3K/AKT/mTOR途径受到抑制, 进而上调细胞自噬, 导致骨肉瘤细胞产生化疗耐受^[66]。非小细胞肺癌中, HSP90抑制剂ganetespib通过下调HSP90的非典型客户蛋白ATG7(autophagy-related gene 7)的表达抑制自噬^[67]。另外, HDAC6-HSP90-VCP-HSF1复合体也参与了自噬的调控。当HDAC6识别错误折叠蛋白与其结合时, 该复合物解离, 解离出的VCP(valosin-containing protein)不仅是错误折叠蛋白降解的调节剂, 同时VCP利用其ATP酶活性解离HSP90-HSF1复合物, 释放的HSF1(热休克因子)诱导热休克因子与HSP90转录, 促进客户蛋白折叠并激活HSP基因促进细胞存活(图4)^[47,52,68]。以上研究提示, 探讨HDAC6-HSP90之间的交

互作用可能成为靶向自噬治疗肿瘤的潜在途径, 有望帮助我们更全面地理解蛋白降解途径在肿瘤细胞存活、增殖以及转移中的作用。

3 HDAC6-HSP90介导肿瘤增殖转移

3.1 HDAC6-HSP90对增殖转移相关靶蛋白的调控

目前研究认为, HDAC6与HSP90均在肿瘤增殖和转移信号中发挥了关键作用。首先, HDAC6通过去乙酰化丝裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK)信号转导途径下游激酶ERK1, 促进ERK1的激酶活性^[69]。此外, HDAC6还能调节表皮生长因子受体(epidermal growth factor receptor, EGFR)信号, HDAC6通过对 α -微管蛋白的去乙酰化作用控制EGFR的运输和降解^[70]。HDAC6缺失, 促进乙酰化 α -微管蛋白

积累, 导致依赖微管的蛋白运输能力增强, 加速了EGFR的降解^[71-73]。另一方面, HSP90通过维持AKT的磷酸化水平, 上调PI3K/AKT途径促进肿瘤细胞的存活和转移^[74,75]。同时HSP90抑制剂AUY922可以通过下调EGFR、Met(mesenchymal to epithelial transition factor)和AXL(AXL receptor tyrosine kinase), 破坏AKT信号, 抑制肺癌细胞增殖, 诱导细胞凋亡^[76]。

以上只是HDAC6与HSP90自身对EGFR、ERK和AKT等增殖相关蛋白的调控。最近的研究发现, HDAC6与HSP90的相互作用也参与了肿瘤增殖转移关键蛋白的调控。Tao等人^[77]的实验表明, HDAC6通过抑制HSP90的乙酰化水平, 促进客户蛋白EGFR活性, 激活下游AKT与ERK通路, 从而促进了肿瘤细胞的增殖。此外, HDAC6还可以通过去乙酰化HSP90稳定促肿瘤细胞存活的巨噬细胞迁移抑制因子(macrophage migration inhibitory factor, MIF)的表达。Schulz等人^[78]在研究中表明, 当抑制HDAC6时HSP90的过度乙酰化会严重破坏MIF蛋白, 从而达到抑制肿瘤生长的目的。另一方面, 在乳腺癌中, 抑制HSP90促进了HDAC6的降解, 导致微管蛋白的过度乙酰化, 进而抑制了乳腺癌细胞的转移(表2)。因此, HDAC6-HSP90途径可能涉及多个肿瘤增殖转移相关靶点的调控, 而靶向HDAC6-HSP90的药物也成为近年来的研究热点。

3.2 靶向HDAC6-HSP90抑制肿瘤增殖转移的药物

目前已报道了多种具有不同帽基团(cap group)、接头和锌离子结合基团(zinc binding group, ZBG)的HDAC6抑制剂^[84]。HDAC6抑制剂虽然可以通过阻断

自噬流促进了肿瘤细胞的药物敏感性, 但同时过度乙酰化的微管蛋白也促进了肿瘤细胞的侵袭转移。因此针对HDAC6抑制剂的双重作用, 越来越多的研究倾向于将其他药物同HDAC6抑制剂的联合应用, 提升药物治疗效果。HSP90抑制剂主要靶向分子伴侣N末端的ATP结构域, 也有少部分抑制剂是针对蛋白质的C端结构域, 但由于C端缺乏明确的配体结合口袋, 限制了这类抑制剂的效果^[85]。

随着HDAC6与HSP90之间相互作用的深入研究, HDAC6抑制剂与HSP90抑制剂的联合应用成为了癌症治疗中更为有效的新突破点。GA及其衍生物(如17-AAG、Alvespimycin)相比于核苷酸以更高的亲和力与ATP/ADP结合口袋相结合, 从而抑制了HSP90与客户蛋白的结合能力。同时有研究表明, 抑制HDAC6可增强HSP90与17-AAG的结合, HSP90高度乙酰化使ATP与HSP90的结合减弱, 导致HSP90与AR(androgen receptor)、GR(glucocorticoid receptor)和ER α (estrogen receptor)及其他相关癌蛋白激酶, 如Bar-Abl、FLT-3(FMS-like tyrosine kinase-3)、AKT和HER-2(表皮生长因子受体)等客户蛋白的结合被破坏^[86-89]。在白血病细胞中, HSP90抑制剂17-AAG联合HDAC6抑制剂, 通过促进HSP90高度乙酰化, 更有效地抑制HSP90与客户蛋白的结合, 从而达到抑制肿瘤细胞增殖转移的目的(表2)。抑制HDAC6可以破坏HSP90与客户蛋白的结合能力, 抑制食管鳞状细胞癌(esophageal squamous carcinoma, ESCC)的增殖和转移。在ER α 高表达的乳腺癌细胞系T47D中, HDAC6与HSP90抑制剂的联用导致ER α 的表达显著降低(表2)。尽管人们对HDAC6与HSP90抑制

表 2 HDAC6与HSP90抑制剂联用治疗肿瘤

Table 2 HDAC6 inhibitors combined with HSP90 inhibitors for tumor treatment

癌症	联合应用		相关通路	文献
	HDAC6抑制剂	HSP90抑制剂		
乳腺癌	Tubacin	17-DMAG	抑制微管蛋白去乙酰化, 增强微管稳定性	[79]
尿路上皮癌	Tubacin	17-AAG	减少ERK磷酸化	[80]
白血病	Tubacin	17-AAG	HSP90的高乙酰化可以使17-AAG更有效地抑制HSP90与p23、ATP和客户蛋白的结合	[81]
甲状腺癌	PXD101	NVP-AUY922	Survivin的失活和DNA损伤相关蛋白的活化	[82]
	PXD101	SNX5422		
	SAHA	SNX5422	PI3K/AKT/mTOR信号通路	[83]
	TSA	SNX5422		
食管癌	Tubastatin A	PU-H71	PI3K/AKT信号通路 MAPK/ERK信号通路	[77]

剂联合应用治疗肿瘤有了一定认识(表2), 但还存在许多问题. 目前已经发现了许多癌症相关蛋白受到HSP90的调控, 例如AKT、c-Raf(Ras-associated factor-1)、HER-2等, 这为HSP90抑制剂在肿瘤中的应用提供了理论依据. 但HSP90作用范围的广泛性导致其在癌细胞中发挥的作用远比预期的更为复杂, 可能引起“脱靶效应”(off target)^[90]. 同时, 还应考虑到不同类型的肿瘤对于HSP90抑制剂的敏感性是否存在差异? 如何降低抑制剂对于正常细胞的毒性作用? 解决以上问题为HDAC6-HSP90抑制剂的临床应用以及肿瘤的精准治疗提供理论依据.

4 讨论

靶向HDAC6-HSP90调控蛋白折叠和降解途径为抑制肿瘤的增殖和转移拓展了思路. 蛋白质的折叠与降解维持了癌细胞各种功能蛋白质在细胞内的正常运转, 为肿瘤的生存发展提供适宜条件. 通过抑制HDAC6促进HSP90过度乙酰化, 可有效地抑制HSP90与ATP之间的结合, 影响HSP90对肿瘤增殖、转移相关蛋白的折

叠和降解^[44]. 同时, HDAC抑制剂可以帮助HSP90与其抑制剂更加紧密地结合. 目前, 无论是单独抑制HDAC6还是单独抑制HSP90, 在癌症治疗方面都存在不足. HDAC6的抑制会导致微管蛋白(tubulin)的高度乙酰化, 增加原发性乳腺肿瘤细胞的侵袭转移. 此外, 在2型神经纤维瘤(neurofibromatosis type 2, NF2)细胞中, 微管的去乙酰化会损害Hippo信号通路并影响细胞的接触抑制. 而单独抑制HDAC6还可能增强调节性T细胞功能, 从而导致肿瘤发生免疫逃逸. 与之类似, HSP90抑制剂可以通过调控AKT/mTOR信号通路诱导自噬, 进而触发细胞保护机制. 因此, HDAC6-HSP90双靶点抑制, 一方面能够减少自噬等代偿途径的活化, 另一方面减弱了HSP90抑制剂可能造成的脱靶效应. 毫无疑问, HDAC6-HSP90双靶点的肿瘤治疗还有很长的路要走, 今后随着HDAC6、HSP90抑制剂在不同肿瘤细胞中研究的深入, 弄清楚HDAC6-HSP90与自噬之间复杂的网络关系, 有助于人们进一步了解蛋白折叠与降解途径在肿瘤细胞增殖以及转移中的作用, 为增加肿瘤的治疗效果提供新的靶点.

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Summary for “HDAC6-HSP90途径介导蛋白折叠和降解调控肿瘤细胞增殖与转移”

HDAC6-HSP90 pathway-mediated protein folding and degradation regulates tumor cell proliferation and metastasis

Rui Tian[†], Xiaoyu Yan[†], Sihang Yu, Long Xu, Yuanxin Zhao, Liankun Sun^{*} & Jing Su^{*}

Key Laboratory of Pathophysiology, Ministry of Education, Department of Pathophysiology, Basic Medical College, Jilin University, Changchun 130000, China

[†] Equally contributed to this work

^{*} Corresponding authors, E-mail: sunlk@jlu.edu.cn; sujing@jlu.edu.cn

The folding and degradation of proteins in tumor cells are the key mechanisms maintaining their survival, and functions of the proteins involved are often related to the prevailing tumor resistance. With further scientific research, an increasing number of auxiliary proteins involved in protein folding and degradation have been reported in the literature. The chaperone heat shock protein 90 (HSP90) not only promotes protein folding and plays a role in maintaining protein conformation stability but also assists the proteasomal degradation of client proteins. Current research suggests that the function of HSP90 is regulated by post-translational modifications, such as acetylation. Histone deacetylase 6 (HDAC6) can inhibit the over-acetylation of HSP90 and maintain the chaperone function of HSP90 to ensure that the client protein of the chaperone HSP90 can be stably folded and maturely released, which creates conditions for tumor survival, proliferation, and metastasis. Furthermore, it can act as the client protein and autophagy receptor of HSP90. It recruits ubiquitinated proteins and deacetylates and modifies autophagy-related proteins, thus playing important roles in protein degradation and autophagy. Recently, studies revealed HDAC6, a client protein of HSP90, which plays an important role in protein degradation, cell autophagy, and maintenance of the activity of HSP90 by preventing its excessive acetylation. By inhibiting HDAC6 to promote HSP90 over-acetylation, it can effectively inhibit the binding between HSP90 and ATP, thereby affecting the folding and degradation of HSP90 on tumor proliferation and metastasis-related proteins. Simultaneously, HDAC inhibitors can help HSP90 bind more closely to its inhibitors. Therefore, HDAC6 and HSP90 inhibitors have also been widely used in related research on tumor treatment, and studies have shown that the combined application of HDAC6 and HSP90 inhibitors has a significant effect on inhibiting tumor proliferation and metastasis. Whether it is to inhibit HDAC6 alone or HSP90 alone, there are deficiencies in cancer treatment. The use of inhibitors alone may lead to the activation of other pathways related to tumor cell proliferation and survival. HDAC6-HSP90 dual-target inhibition can reduce the activation of compensatory pathways, such as autophagy, and reduce the possibility of off-target effects of HSP90 inhibitors. In this review, we focus on the crosstalk between HDAC6 and HSP90 to further explain how tumor cells initiate proliferation and metastasis signals by regulating protein folding and degradation mechanisms and discuss the research applications of HDAC6 and HSP90 inhibitors. The advantages and disadvantages are assessed to obtain novel strategies for cancer therapy by targeting the HSP90-HDAC6 pathway.

HDAC6, HSP90, protein folding and degradation, tumor proliferation and metastasis

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