RIPK3翻译后修饰调控细胞程序性坏死的研究进展

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摘要 细胞程序性坏死是一种不依赖于Caspase、可调控的细胞死亡方式, 参与多种疾病的病理过程, 如病毒或病原菌感染、动脉硬化、心脏缺血再灌注和肿瘤等。受体相互作用蛋白激酶3(receptor-interacting protein kinase 3, RIPK3)是细胞程序性坏死的关键调控分子, 可与受体相互作用蛋白激酶1(receptor-interacting protein kinase 1, RIPK1)形成坏死小体, 激活混合谱系激酶结构域样蛋白(mixed lineage kinase domain-like pseudokinase, MLKL), 导致细胞膜破裂和细胞死亡。近年来, 越来越多研究发现RIPK3活性可受多种翻译后修饰如磷酸化、泛素化、糖基化和蛋白水解切割等调控。该文就RIPK3翻译后修饰在调控细胞程序性坏死信号转导中的作用进行综述, 期望为靶向RIPK3的药物设计及细胞程序性坏死相关疾病的治疗提供理论依据。

关键词 细胞程序性坏死; RIPK3; 翻译后修饰; 磷酸化; 泛素化

The Regulation of Necroptosis by Post-Translational Modifications of RIPK3

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Abstract Necroptosis is a caspase-independent programmed cell death which has been widely implicated in many pathologies, such as viral or pathogen infection, atherosclerosis, cardiac ischemia-reperfusion and cancer. RIPK3 (receptor-interacting protein kinase 3) has emerged as a critical regulator of necroptosis, which can interact with RIPK1 (receptor-interacting protein kinase 1) to form a protein complex called necrosome and then active MLKL (mixed lineage kinase domain-like pseudokinase) to cause plasma membrane rupture and cell death. In recent years, increasing studies have found that the activity of RIPK3 is regulated by multiple post-translational modifications, including phosphorylation, ubiquitylation, GlcNAcylation and proteolytic cleavage. This article reviews the role of post-translational modifications of RIPK3 in necroptosis, which may provide theoretical basis for drug design targeting RIPK3 and treatment of necroptosis-related diseases.

Keywords necroptosis; RIPK3; post-translational modifications; phosphorylation; ubiquitylation

传统观念认为, 细胞死亡分为细胞凋亡和细胞坏死。细胞凋亡是为维持体内稳态, 细胞自主有序的死亡, 而细胞坏死最初被认为是被动的、不可调控的细胞死亡方式。然而随着研究的不断深入, 学者们发现细胞坏死也可以被调控。2005年, DEGTEREV等[1]发现细胞坏死可以被受体相互作用蛋白

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激酶1(receptor-interacting protein kinase 1, RIPK1)的小分子抑制剂Nec-1(necrostatin-1)所抑制, 并将其命名为细胞程序性坏死(necroptosis)。细胞程序性死亡是一种依赖于RIPK1和受体相互作用蛋白激酶3(receptor-interacting protein kinase 3, RIPK3)的细胞死亡方式。死亡信号激活RIPK3的激酶活性, 进而磷酸化混合谱系激酶结构域样蛋白(mixed lineage kinase domain-like pseudokinase, MLKL), 引起细胞膜破裂和细胞死亡。因此, RIPK3的表达水平和活性情况可以决定细胞的命运。

1 细胞程序性坏死及其关键调控分子RIPK3

多种细胞受体, 包括肿瘤坏死因子(tumor necrosis factor, TNF)超家族受体、Toll样受体(toll-like receptor, TLR)和干扰素受体等, 参与细胞程序性坏死的起始[2]。目前, 对细胞程序性坏死的了解主要来自TNF-α信号通路的研究(图1)。TNF-α与其受体肿瘤坏死因子受体1(tumor necrosis factor receptor 1, TNFR1)结合后, TNFR1通过其胞内结构域募集一系列蛋白, 如TNF受体相关死亡结构域蛋白(TNFR-associated death domain protein, TRADD)、RIPK1、TNF受体相关因子2(TNF receptor associated factor 2, TRAF2)、凋亡蛋白抑制因子(cellular inhibitor of apoptosis proteins 1 and 2, cIAP1/cIAP2)与线性泛素链组装复合物(linear ubiquitin chain assembly complex, LUBAC)等形成复合物I(complex I)。若cIAP1/2被抑制或者RIPK1被去泛素化酶头帕肿瘤综合征蛋白(cylindromatosis, CYLD)和肿瘤坏死因子α诱导蛋白3(tumor necrosis factor alpha-induced protein 3, TNFAIP3/A20)去泛素化, RIPK1将从复合物I上解离下来, 与TRADD、Fas相关死亡结构域蛋白(Fas-associating protein with death domain, FADD)及半胱天冬酶原8(pro-caspase-8)结合, 形成复合物II(complex II)。当caspase-8活化后, 可以切割RIPK1和RIPK3, 使细胞发生凋亡; 而当caspase-8被抑制时, RIPK1发生自磷酸化。磷酸化的RIPK1通过其RIP同型相互作用基序(RIP homotypic interaction motif, RHIM)结构域与RIPK3结合, 形成坏死小体。随后, 坏死小体中活化的RIPK3招募并磷酸化MLKL, 磷酸化的MLKL形成二硫键连接的寡聚体, 然后转移到细胞膜并破坏其完整性, 最终导致细胞程序性坏死[3-5]。

RIPK1、RIPK3和MLKL是参与程序性坏死通路中的主要分子。然而, 某些非TNF-α诱导的细胞程序性坏死[如poly(I:C)或LPS诱导的细胞程序性坏死)]并不依赖于RIPK1, RIPK3可以通过结合其他含有RHIM结构域的蛋白, 如Z-DNA结合蛋白1(Z-DNA binding protein 1, ZBP1/DAI)或TRIF等激活下游的细胞死亡信号通路[6-8]。最近YUAN等[8]发现, 热应激通过热休克转录因子1(heat shock transcription factor 1, HSF1)增加ZBP1的表达量, 促进ZBP1与RIPK3相互作用, 导致细胞程序性坏死, 进而引起器官损伤和循环衰竭等。此外, 研究表明RIPK1对细胞程序性坏死也具有抑制作用。RIPK1通过阻碍细胞质内受体非依赖的RIPK3的寡聚化或ZBP1与RIPK3的相互作用从而抑制RIPK3的自发活化[9-10]。表皮细胞特异性*RIPK1*敲除可以诱导角质细胞发生RIPK3依赖的细胞程序性坏死和凋亡[11]; *Ripk1*敲除小鼠在临产期死亡, 表现为过度的细胞程序性坏死和凋亡[12-14]。MLKL对于细胞程序性坏死也并非不可或缺, 缺血缺氧条件或金黄色葡萄球菌感染可以活化RIPK3-CaMKII信号通路进而诱导细胞程序性坏死[15-16]。因此, 目前的研究报道, RIPK3是细胞程序性坏死的关键调控分子[17]。

2 RIPK3翻译后修饰对细胞程序性坏死的调控

RIPKs是一类丝氨酸/苏氨酸激酶, 在病原体感染、炎症、DNA损伤和细胞信号转导中发挥重要作用。RIPK3是RIPK家族成员之一, 具有N-端的激酶结构域和C-端的RHIM结构域(图2)。2009年, RIPK3被证实是细胞程序性坏死的关键信号分子, 可以整合多种上游信号通路诱导细胞死亡[7,14]。RIPK3的磷酸化、泛素化和糖基化等多种翻译后修饰可调控其活性、蛋白稳定性以及蛋白复合物形成, 从而决定细胞的反应和命运(表1和图1)。

2.1 RIPK3的磷酸化修饰

RIPK3自磷酸化对细胞程序性坏死的诱导至关重要。目前已报道的RIPK3的磷酸化修饰都发生在其激酶结构域上(图2)。有研究表明, 在RIPK1激酶活性被抑制条件下, RIPK3不能导致TNF-α诱导的细胞死亡[33]。然而, 体外激酶活性检测实验发现RIPK1并不能直接磷酸化RIPK3[34]。这些研究表明, RIPK1可能通过其他分子间接影响RIPK3磷酸化。值得注意的是, RIPK3磷酸化也可以由含有RHIM结

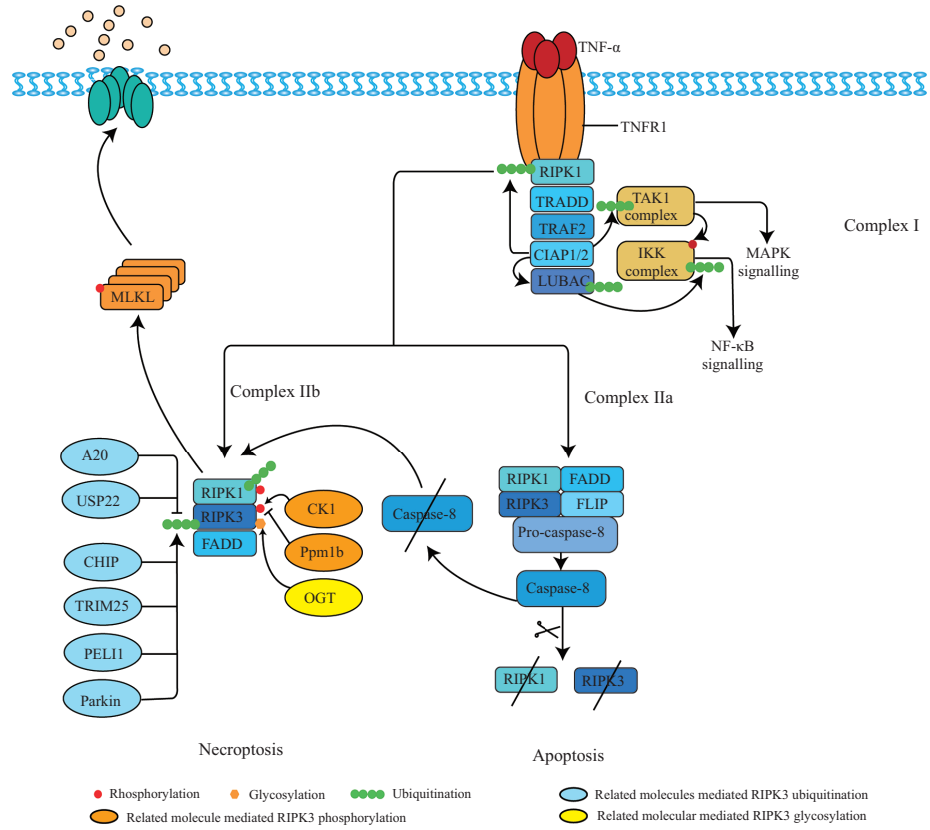
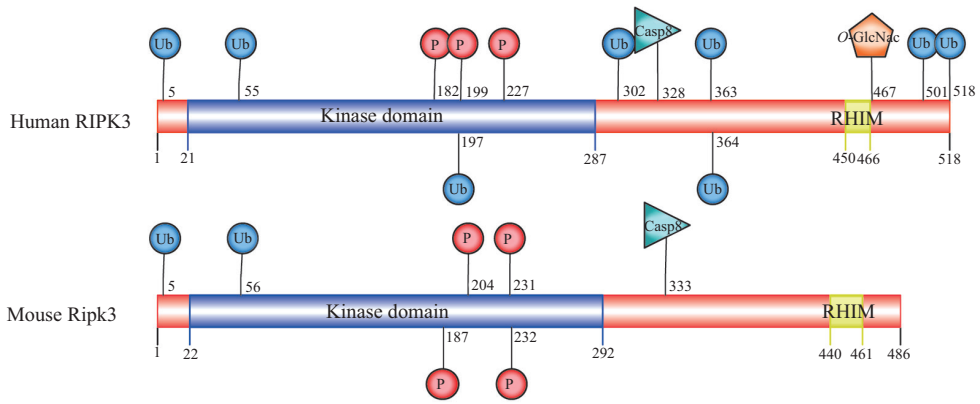


图1 RIPK3翻译后修饰调控TNFα诱导的程序性坏死

Fig.1 TNFα-induced necroptosis is regulated by RIPK3 post-translational modifications



Kinase domain: *N*-terminal kinase domain; RHIM: RIP homotypic interaction motif; P: phosphorylation; Ub: ubiquitination; Casp8: Caspase8 cleavage; *O*-GlcNac: glycosylation

图2 RIPK3分子结构及其修饰位点(根据参考文献[25]修改)

Fig.2 RIPK3 domain architecture and post-translational modification sites (modified from reference [25])

构域的其他蛋白质如TRIF和ZBP1调控, 表明RIPK3的激活也可以不依赖于RIPK1[6,35]。

在细胞程序性坏死过程中, RIPK3 S227(小鼠T231/S232)位点的磷酸化是细胞程序性坏死发生的重要标志物。S227位点磷酸化的RIPK3可以招募并激活MLKL, 从而诱导细胞死亡。小鼠Ripk3的活化需要S232和T231两个位点的磷酸化[18]。与RIPK3激酶失活突变体D142N相似, S227A-RIPK3突变/小鼠S232A-Ripk3突变不能诱导NIH3T3细胞死亡。此外, S227A-RIPK3突变也无法诱导HeLa细胞发生程序性坏死[19]。

RIPK3 S227(小鼠T231/S232)位点的磷酸化分别受酪蛋白激酶1(casein kinase 1, CK1)和蛋白磷酸酶1B(protein phosphatase 1B, Ppm1b)调控。HANNA等[20]的研究表明, CK1可以通过其激酶结构域N末端内的β折叠与寡聚化RIPK3 C末端的β折叠相互作用, 直接磷酸化RIPK3 S227位点。与CK1结合的区域突

表1 RIPK3的翻译后修饰位点

Table 1 Post-translational modifications of RIPK3

修饰位点 Residue		修饰类型 Type	刺激方式 Mode	功能 Function	调控方式 Regulation	机制 Mechanism	模型 Model	方法 Method	参考文献 Reference
人源 Human	鼠源 Mouse								
S227	T231/S232	Phosphorylation	TSZ	Promotes Necroptosis	-	Recruitment of MLKL	Cell: Jurkat, HeLa, HT-29, 293T	BD	[18-19]
S227	T231/S232	Phosphorylation	TSZ	Promotes Necroptosis	CK1	CK1α, CK1δ and CK1ε promote phosphorylation of RIPK3	Cell: HT-29, HeLa	ABCD	[20]
S227	T231/S232	Dephosphorylation	T	Inhibits necroptosis	Ppm1b	Ppm1b dephosphorylates RIPK3	Mouse: *RIPK3*$^{-/-}$, Ppm1b$^{d/d}$, *RIPK3*$^{-/-}$Ppm1b$^{d/d}$; cell: 293T, L929, HeLa	ACDE	[21]
T182	T187	Phosphorylation	TSZ	No effect?	-	Promotes RIPK3 kinase activity; PELI1 is recruited to degrade RIPK3	Cell: HT-29, 293T, HeLa	ABCD	[22]
S199	S204	Phosphorylation	TZ	Promotes necroptosis	-	Promotes RIPK3 kinase activity	Cell: 293T, Jurkat	B	[23]
K5	K5	Deubiquitination	TCZ	Inhibits necroptosis	A20	A20 deubiquitinates RIPK3 to impede necrosome formation	Cell: T cell, MEF	AB	[24]
K55 /K501	K56	Ubiquitination	TSZ/TCZ	Inhibits necroptosis	CHIP	CHIP mediates RIPK3 degradation	Mouse: *CHIP*$^{-/-}$,*CHIP*$^{-/-}$ *RIPK3*$^{-/-}$; cell: MEF, L929, HT-29, HeLa	ABCDE	[25]
K363	-	Ubiquitination	TSZ	Inhibits necroptosis	PEIL1	PEIL1 mediates RIPK3 degradation	Cell: HT-29, 293T, HeLa	ABCD	[22]
K501	-	Ubiquitination	TSZ/TZ	Inhibits necroptosis	TRIM25	TRIM25 mediates RIPK3 degradation	Cell: HT-29, L929, 293T	ABCD	[26]
K518	-	Deubiquitination	TBZ	Promotes necroptosis	USP22	USP22 deubiquitinates RIPK3	Cell: HT-29, HeLa	ACD	[27]
K197 /K302 /K364	-	Ubiquitination	TCZ	Inhibits necroptosis	Parkin	Parkin deubiquitinates RIPK3 to impede necrosome formation	Mouse: AOM/DSS; cell: HT-29, MEF	ABDE	[28]
T467	-	Glycosylation	LZ	Inhibits necroptosis	OGT	OGT glycosylates RIPK3 to impede necrosome formation	Mouse: CLP; cell: BMMs, THP1	BDE	[29]
D328	D333	Cleavage	TZ	Inhibits necroptosis	Casp8	Casp8 hydrolyzes RIPK3	Mouse: *RIPK3*$^{-/-}$*Casp8*$^{-/-}$, *RIPK3*$^{-/-}$Casp8$^{+/-}$; cell: 293T, HeLa, NH3T3	BCDE	[30-31]
-	-	Oligomerization	TZ	Inhibits necroptosis	PP2	PP2 disrupts RIPK3 oligomerization	Cell: L929	AD	[32]

A:构建相关敲除细胞系; B: 构建相关蛋白位点突变细胞系; C: 构建相关敲减细胞系; D: 构建相关过表达细胞系; E: 构建相关敲除小鼠。

A: construct relevant knockout cell lines; B: construct a cell line with associated protein site mutations; C: construct relevant knockdown cell lines; D: construct relevant overexpressed cell lines; E: construct relevant knockout mice. T: TNF-α; TZ: TNF-α+zVAD; TSZ: TNF-α+Smac+zVAD; TCZ: TNF-α+CHX+zVAD; TBZ: TNF-α+BV6+zVAD.

变后, RIPK3 S227位点不能被磷酸化, RIPK3也不能结合以及磷酸化MLKL。CK1缺失阻碍RIPK3 S227磷酸化并抑制细胞程序性坏死的发生。该研究还表明, 在体内外条件下, CK1均可直接磷酸化RIPK3 S227位点。除了可以被CK1直接磷酸化外, RIPK3 T182位点的磷酸化也可以引发其S227位点的自磷酸化。T182A-RIPK3突变可以抑制RIPK3 S227位点的磷酸化, 阻碍RIPK3-MLKL的相互作用, 最终抑制细胞程序性坏死的发生[22]。CHEN等[21]的研究发现Ppm1b是小鼠Ripk3的磷酸酶。该研究表明在Ripk3磷酸化引发的自发细胞程序性坏死和TNF诱导的细胞程序性坏死过程中, Ppm1b可催化Ripk3 T231/S232去磷酸化, 从而抑制Mlkl的募集。此外, 小鼠*Ppm1b*敲除可以增加Ripk3磷酸化水平以及加重TNF处理导致的组织损伤。

RIPK3 S199(小鼠S204)磷酸化也可以促进细胞程序性坏死。S199A-RIPK3(小鼠S204A)突变后丧失了体外激酶活性, 细胞程序性坏死受到抑制。然而, S199D-RIPK3(小鼠S204D)突变后却保留了其激酶活性, 且S204D-Ripk3突变的小鼠纤维细胞可以发生细胞程序性坏死。有意思的是, Ripk1抑制剂Nec-1并不能抑制该细胞发生细胞程序性坏死。此外, 在表达野生型Ripk3和S204D-mRipk3的小鼠纤维细胞中敲低*Ripk1*发现, 表达野生型Ripk3的纤维细胞细胞死亡受到抑制, 而S204D-Ripk3突变细胞仍可诱导较高水平的细胞程序性坏死, 这说明在S204D-Ripk3介导的细胞程序性坏死中, Ripk1不是必需的[23]。

RIPK3 S227(小鼠T231/S232)和RIPK3 S199(小鼠S204)的磷酸化都可以促进细胞程序性坏死的发生, 然而它们的作用机制并不相同。体外激酶活性检测表明, S232A-Ripk3和S232E-Ripk3都存在激酶活性, 然而S227A-RIPK3和S232A-Ripk3不能结合MLKL。因此, RIPK3激酶活性不依赖于S227(小鼠T231/S232)位点的磷酸化, 该位点的磷酸化影响RIPK3与MLKL的招募。而S199A-RIPK3(小鼠S204A)突变的RIPK3丧失了体外激酶活性, 进而影响了细胞程序性坏死的发生[19,23]。

2.2 RIPK3的泛素化修饰

泛素化, 即一个或多个泛素分子对底物进行共价翻译后修饰, 调控蛋白质降解、细胞信号转导和其他细胞过程。泛素连接酶, 包括热休克蛋白70羧基末端相互作用蛋白(carboxyl terminus of Hsp70-interacting protein, CHIP)、三基序蛋白25(tripartite motif 25, TRIM25)、帕金森氏病蛋白2(Parkinson juvenile disease protein 2, PARK2/Parkin)和E3泛素蛋白连接酶pellino同源物1(pellino E3 ubiquitin protein ligase 1, PELI1)等, 通过泛素化RIPK3抑制细胞程序性坏死; 而去泛素化酶, 如泛素特异性蛋白酶22(ubiquitin-specific peptidase 22, USP22)、A20等可以将RIPK3去泛素化, 从而介导细胞死亡[22,25-28]。

CHIP是一种E3泛素连接酶, 可作为共伴侣分子蛋白, 也能泛素化RIPK3, 影响其稳定性, 进而调节细胞程序性坏死。CHIP可泛素化RIPK3多个位点, 包括K55、K89、K363、K501。K55/363R突变虽然不影响RIPK3被CHIP泛素化, 但阻碍了RIPK3的溶酶体定位, 从而保护RIPK3不被降解; 而K89/501R突变不影响CHIP介导的溶酶体途径降解RIPK3。此外该研究还发现, *CHIP*敲除小鼠较野生型小鼠表现出寿命缩短、过早衰老和免疫系统功能障碍等情况, 而*RIPK3*和*CHIP*双敲除的小鼠则改善了这一状况[25]。

E3泛素连接酶PELI1可以与T182位点磷酸化的RIPK3直接相互作用并催化其K363位点K48连接的多聚泛素化, 使得RIPK3以泛素−蛋白酶体途径降解, 从而抑制细胞程序性坏死[22]。有趣的是, 在细胞程序性坏死发生过程中, PELI1也可以介导RIPK1 K115位点K63连接的泛素化, 进而促进RIPK1与RIPK3相互作用, 正向调控细胞程序性坏死[36-37]。因此, PELI1调控细胞程序性坏死依赖于其介导的泛素化类型, 不同类型的泛素化甚至可以导致完全不同的结果。PELI1如何选择泛素化底物及泛素化类型? 在细胞程序性坏死过程中PELI1是否可以同时泛素化RIPK1和RIPK3 ? PELI1在整个细胞程序性坏死网络通路中到底扮演着怎样的角色? 这些问题值得进一步研究。

TRIM25, 又名雌激素反应性指蛋白(estrogen-responsive finger protein, EFP), 是三联基序包含(TRIM)蛋白家族成员。TRIM25通过其SPRY结构域直接与RIPK3相互作用, 介导RIPK3 K501位点发生K48连接的多聚泛素化, 促进RIPK3以蛋白酶体途径降解[26]。*Parkin*是常染色体隐性遗传性少年型帕金森综合征(autosomal recessive juvenile parkinsonism, AR-JP)的致病基因, 其表达产物Parkin蛋白具有E3泛素连接酶活性。研究表明, Parkin可促进RIPK3 K33连接的多泛素化, 从而阻碍RIPK1-RIPK3相互作用[28]。由此可见, 目前发现的RIPK3泛素化酶, 包括CHIP、PELI1、

TRIM25和Parkin, 主要通过介导RIPK3以泛素−溶酶体途径或泛素−蛋白酶体途径降解, 或者通过阻碍RIPK1-RIPK3相互作用, 从而抑制细胞程序性坏死。

RIPK3除了受泛素化酶调控外, 也受去泛素化酶如A20和USP22[24, 27]的调节。A20是一种与多种人类疾病相关的抗炎蛋白, 也是一种去泛素化酶, 可抑制NF-κB活化以及TNF诱导的细胞凋亡[38]。此外, 有研究发现, 在细胞程序性坏死发生过程中, RIPK3 K5位点会被泛素化, 进而促进RIPK1-RIPK3相互作用以及细胞程序性坏死的发生。尽管RIPK3 K5位点是如何泛素化的并不清楚, 但研究发现A20可以抑制RIPK3 K5位点的泛素化, 进而阻碍RIPK1-RIPK3的相互作用。该研究还发现, A20可以抑制RIPK3 K63连接的多聚泛素化, 然而RIPK3 K5位点上是否发生了K63连接的多聚泛素化还有待进一步研究[24]。尽管目前关于K63连接多聚泛素化RIPK3的研究报道较少[39], 但RIPK1不同位点发生的K63连接多聚泛素化调控RIPK1活性的研究很多。K63连接多聚泛素化可以发生在RIPK1的K27、K29、K33和K377位点[40-42]。RIPK1 K377(小鼠K376)位点K63连接的泛素化促进招募肿瘤生长因子-β-活化激酶1(tumor growth factor-β-activated kinase 1, TAK1)和IκB激酶(iKK)复合物, 从而激活NF-κB信号通路[41-42]。小鼠*Ripk1*$^{K376R/K376R}$突变通过减弱TAK1对RIPK1激酶活性的抑制作用, 进而导致大量细胞死亡, 最终使得小鼠在胚胎早期死亡[43]。

USP22可催化组蛋白H2A和H2B去泛素化, 从而调节基因转录, 参与调控细胞生长和分化、肿瘤发展和细胞死亡等[44-46]。近期ROEDIG等[27]的研究发现, 细胞程序性坏死诱导后, USP22通过降低RIPK3 K518位点的泛素化水平促进坏死小体形成及细胞程序性坏死的发生。有趣的是, RIPK3 K518R突变可以增加RIPK3的磷酸化水平并促进细胞程序性坏死。据此我们猜测RIPK3的磷酸化可能需要USP22介导的RIPK3去泛素化修饰。

2.3 其他翻译后修饰

除了磷酸化和泛素化修饰外, RIPK3还可以发生糖基化、蛋白水解切割以及寡聚化修饰。*O*-GlcNAc转移酶(*O*-glcNAc transferase, OGT)是蛋白质*O*-乙酰葡糖胺糖基化(*O*-glcNAcylation)的关键酶。OGT可以催化RIPK3 T467位点*O*-乙酰葡糖胺糖基化, 进而阻碍RIPK1-RIPK3及RIPK3-RIPK3的相互作用, 并抑制细胞程序性坏死信号的转导。值得注意的是, 小鼠Ripk3中的OGT底物残基T467在RIPK3直系同源物中保守性较差。因此, 类似位点是否可以被其他物种中的OGT或其他糖基化酶修饰, 并调节RIPK3介导的细胞程序性坏死仍有待确定[29]。

在TNF诱导条件下, 细胞质内会形成TNFR1复合物II, 通常由TRADD、含有死亡结构域的FAS相关蛋白(FAS-associated protein with a death domain, FADD)、caspase-8、RIP1和RIPK3构成。Caspase 8通过活化经典的caspase级联反应诱导细胞凋亡, 也可以通过切割RIPK3激酶和RHIM结构域之间的中间区域的第328位天冬氨酸位点(D328, 小鼠RIPK3 D333位点)从而抑制细胞程序性坏死的发生[30-31]。在一定条件下, 如LPS诱导凋亡抑制蛋白(inhibitor of apoptosis protein, IAP)缺失的细胞中, RIPK3可以活化caspase 8, 促进caspase 8依赖的凋亡及IL-1β的产生, 但RIPK3活化caspase 8的具体机制目前并不清楚[47-48]。

RIPK3活化依赖于与自身或其他含有RHIM结构域的蛋白结合与寡聚化, 进而调控细胞程序性坏死。例如RIPK3的RHIM结构域与RIPK1的RHIM结构域相互作用形成异质性淀粉样信号复合物, 促进RIPK3磷酸化以及下游信号分子的募集[49]。许多病原体感染也可通过含有RHIM的蛋白质, 如TRIF和ZBP1/DAI等, 与RIPK3的RHIM结构域相互作用进而促进RIPK3寡聚化[7,50-52]。LI等[32]研究发现了一种Src家族抑制剂PP2, 在不影响RIPK3自磷酸化的前提下抑制RIPK3的寡聚化, 从而阻碍MLKL的磷酸化和寡聚化, 最终在不启动RIPK3依赖的细胞凋亡的情况下减轻细胞程序性坏死。这为治疗细胞程序性坏死相关疾病提出了一种潜在的靶向RIPK3寡聚化的策略。

3 RIPK3的生理及病理功能

作为一种内在免疫机制, 细胞程序性坏死在保护人体免于病原菌感染中起到关键作用[53]。细胞程序性坏死异常活化以及和凋亡之间的平衡失调会导致许多疾病的发生。因此, 多种病理过程, 如动脉硬化、脑缺血和肿瘤等涉及细胞程序性坏死调控的异常[54-58]。

凋亡是宿主防御病毒感染的主要机制。为了避免被感染的细胞发生凋亡, 进而成功感染宿主, 许多病毒可以编码caspase抑制剂。当凋亡通路被抑制, RIPK3依赖的细胞程序性坏死可以作为备用防御机制限制病毒感染。有研究表明, *Ripk3*$^{-/-}$小鼠不能消

除牛痘病毒, 继而死于感染[34]。当然, 有些病毒在进化过程中也会产生坏死抑制剂, 如MCMV病毒可以抑制细胞凋亡和坏死的发生[35]。

细胞程序性坏死会致使细胞膜破裂, 释放细胞内容物, 可直接激活和调节炎症反应。严重的细菌感染可以导致机体产生大量炎症因子, 进而导致脓毒症的发生。*Ripk3*$^{-/-}$小鼠可以保护TNF或者盲肠结扎手术(cecum ligation and puncture, CLP)诱导的脓毒症反应[59-60]。此外, 有些病原菌, 如金黄色葡萄球菌、肺炎链球菌和单核细胞增生李斯特菌等, 可诱导巨噬细胞发生RIPK3依赖的细胞程序性坏死, 从而有助于其免疫逃逸[61]。

细胞程序性坏死关键蛋白已被认为是多种心血管疾病包括动脉粥样硬化、主动脉瘤和缺血再灌注损伤等的治疗靶点。有研究发现, 在动脉粥样硬化的小鼠模型中, Ripk3缺失抑制巨噬细胞坏死, 进而减弱该小鼠病程后期的动脉粥样硬化病变[54]。此外, *Ripk3*和载脂蛋白E(apolipoprotein E, *ApoE*)双敲除可以减少淋巴细胞浸润减低单核细胞数量和小鼠致死率[54,62]。在弹性蛋白酶诱导的腹主动脉瘤小鼠模型中, Ripk3缺失减少主动脉瘤的形成, 并在平滑肌细胞中抑制了TNF信号通路[63-64]。此外, 有研究发现, 氧气或者葡萄糖缺乏的海马神经元以及局灶性脑缺血小鼠中Ripk3表达增强, 细胞程序性坏死通路被激活。而在大脑中动脉闭塞小鼠模型中, Ripk3抑制剂GSK'872可以减弱小鼠缺血性大脑损伤[65]。Ripk3抑制剂dabrafenib也可以减弱小鼠局灶性脑缺血损伤[66]。

长久以来细胞凋亡被认为是机体抵抗肿瘤形成的自然屏障, 然而目前人们对细胞程序性坏死在肿瘤发生发展中的作用了解并不多。值得注意的是, 在许多晚期实体瘤中可发现细胞程序性坏死的发生[67]。然而, 目前还没有明确的证据表明坏死对肿瘤是利还是弊。临床肿瘤治疗的一大难题就是癌细胞对凋亡的耐受。目前许多抗癌药物都是通过诱导癌细胞凋亡从而起到抗癌作用的, 因此诱导RIPK3依赖的坏死可能是避开癌细胞凋亡耐受的新型抗癌策略。

4 RIPK3抑制剂的研究进展

RIPK3在程序性坏死中的重要作用促使了该激酶小分子抑制剂的开发与研究(表2)。KAISER等[6]首次报道了RIPK3的几种不同抑制剂: GSK'840、GSK'843、GSK'872[6,68]。其中GSK'840虽然与人RIPK3有着最高亲和力, 但对小鼠Ripk3缺乏活性; 而GSK'843和GSK'872既可以靶向人源RIPK3, 也可以靶向小鼠Ripk3。在体外条件下, 这些分子对人源RIPK3激酶结构域均表现出高亲和力。然而, 高浓度的GSK'840和GSK'843可以诱导细胞凋亡, 存在细胞毒性, 对开发靶向RIPK3的抗炎疗法形成具大挑战[68]。

RODRIGUEZ等[69]筛选出一种与GSK'872结构相似的RIPK3抑制剂: GW440139B。在人源和小鼠细胞中, GW440139B均可通过阻碍RIPK3-MLKL复合体的形成抑制MLKL的磷酸化与寡聚化, 从而抑制细胞程序性坏死[69]。GW440139B在人源细胞中的活性与GSK'840相当, 而在小鼠细胞中也显示出良好的活性。然而, 高浓度的GW440139B是否存在细胞毒性目前并不清楚[69]。

最新研究报道, Zharp-99和HG-9-91-01是RIPK3激酶抑制剂, 可以靶向RIPK3的激酶活性减轻程序性坏死相关的炎症损伤。在TNF诱导的全身炎症反应综合征(systemic inflammatory response syndrome, SIRS)的小鼠模型中, Zharp99处理可以减少小鼠血清中IL-6的含量, 改善TNF-α诱导的致死性休克症状[70]。同样, HG-9-91-01处理可以提高小鼠生存率, 减轻肝脏和盲肠损伤, 降低炎性因子IL-1β量。此外, 在金黄色葡萄球菌引起的肺炎模型中, HG-9-91-01可以减少小鼠肺组织和支气管肺泡灌洗液的载菌量以及抑制促炎因子和趋化因子的表达, 改善小鼠肺部损伤。然而, HG-9-91-01与GSK'872相似, 浓度高时会诱导细胞凋亡和焦亡, 这也为抗炎治疗带来挑战[71]。

LI等[72]发现, 用于临床治疗转移性黑色素瘤的药物B-Raf抑制剂dabrafenib可与ATP竞争性结合RIPK3, 抑制RIPK3的激酶活性, 进而抑制程序性坏死。Dabrafenib可改善对乙酰氨基酚对正常人肝细胞所诱导的坏死, 减轻肝损伤[72]; 对RIPK3依赖的中毒性表皮坏死松解症的也具有保护作用[72-73]。然而, Dabrafenib是临床使用抗癌药物, 是否存在副作用, 能否用于治疗RIPK3依赖的程序性坏死相关疾病还有待研究。

除了直接靶向RIPK3之外, 也有间接靶向RIPK3的抑制剂。LI等[74]研究发现, 在细胞程序性坏死诱导过程中, RIPK3的活化依赖于HSP90和CDC37分子伴侣复合物的活性, 而抑制HSP90可以间接抑制RIPK3活性。如HSP90抑制剂17DMAG可以有效地通过抑制RIPK3的活性从而阻碍细胞程序性坏死的发生, 阻碍大鼠TNF-α诱导的系统性炎症反应[74]。

表2 RIPK3抑制剂

Table 2 RIPK3 inhibitors

名称 Name	作用机制 Mechanism	半最大效应浓度/半抑制浓度 EC_{50}/IC_{50}	范围 Range	应用 Application	其他 Other	参考文献 Reference
GSK'872	Binding to the RIPK3 kinase domain	IC_{50}=1.3 nmol/L	Inhibits human or murine RIPK3	Cell model: concentration-dependent inhibition of TNF-induced necroptosis of HT-29 cells; inhibits necroptosis of primary human neutrophils isolated from whole blood; inhibition of necroptosis in mouse cells (BMDM, PECs, 3T3SA); inhibits TLR3 or DAI-induced necroptosis. Mouse model: reduces the expression of HIF-1α and brain damage in the MCAO model	Only used for scientific research currently; induce apoptosis in a concentration-dependent manner	[6,68]
GSK'843	Binding to the RIPK3 kinase domain	IC_{50}=6.5 nmol/L	Inhibits human or murine RIPK3	Cell model: concentration-dependent inhibition of TNF-induced necroptosis of HT-29 cells; inhibits necroptosis of primary human neutrophils isolated from whole blood; inhibition of necroptosis in mouse cells (BMDM, PECs, 3T3SA); inhibits TLR3 or DAI-induced necroptosis	Only used for scientific research currently; induce apoptosis in a concentration-dependent manner	[68]
GSK'840	Binding to the RIPK3 kinase domain; hinders MLKL phosphorylation and oligomerization	IC_{50}=0.3 nmol/L	Inhibits human RIPK3	Cell model: concentration-dependent inhibition of TNF-induced necroptosis of HT-29 cells	Only used for scientific research currently; induce apoptosis in a concentration-dependent manner	[68]
GW440139B	Destruction of the RIPK3-MLKL complex	EC_{50} =73.6 nmol/L (NIH 3T3 cell)	Inhibits human or murine RIPK3	Cell model: inhibition of TNF-induced necroptosis of SVEC, MEF, L929, HT-29 and HeLa-RIPK3; inhibit Poly I:C-induced necroptosis of MEF and HeLa-RIPK3; inhibits necroptosis of bone marrow macrophages induced by LPS	Only used for scientific research currently	[69]
Dabrafenib	Compete with ATP in combination with RIPK3	EC_{50} =0.75 μmol/L (HT-29 cell)	Inhibits human or murine RIPK3	Cell model: inhibition of TNF-induced necroptosis of HT-29, HEKn and HaCaT; inhibits acetaminophen-induced necrosis of QSG-7701 and HL-7702; inhibits SNP-induced apoptosis and necroptosis of HEKn, HaCaT Mouse model: reduces brain damage in the MCAO model	FDA approved drugs; the inhibitory effect on RIPK3 is its off-target effect of inhibiting B-Raf	[66,72-73]
Zharp-99	Inhibits RIPK3 kinase activity	-	Inhibits human or murine RIPK3	Cell model: inhibit TNF-induced necroptosis of HT-29 and MEF; inhibits TLR-induced necroptosis of BMDM; inhibits necroptosis of L929 cells induced by HSV-1 infection Mouse model: reduces TNF-induced inflammation in SIRS	Only used for scientific research currently; good *in vitro* safety and *in vivo* pharmacokinetic parameters	[70]
HG-9-91-01	Inhibits RIPK3 kinase activity; inhibits RIPK3 binding to MLKL; inhibits MLKL oligomerization	-	Inhibits human or murine RIPK3	Cell model: inhibits TNF and TLR-induced necroptosis Mouse model: reduces TNF-induced inflammation in SIRS; lung damage is reduced in the *Staphylococcus aureus* pneumonia model	Only used for scientific research currently; promotes apoptosis and pyrozoism	[71]

IC_{50}: 被测量的拮抗剂的半抑制浓度; EC_{50}: 半最大效应浓度, 是指能引起50%最大效应的浓度。

IC_{50}: half maximal inhibitory concentration; EC_{50}: concentration for 50% of maximal effect.

但间接靶向RIPK3的抑制剂也势必会影响HSP90功能, 这是否会导致一些副作用还有待研究。

除了在程序性坏死过程中起到关键作用外, RIPK3也参与其他细胞死亡。如有研究发现, 某些RIPK3激酶失活突变体的表达可以诱导细胞凋亡的发生[33,68]; RIPK3可以促进炎症小体的形成以及IL-1β的分泌[47-48,75]。此外, 也有研究发现RIPK3参与细胞自噬的发生。自噬是一种催化细胞内物质降解的过程, 在多种生理和病理过程中均起到至关重要的作用[76]。在能量或者营养不足的条件下, 能量感应蛋白腺苷酸活化蛋白激酶(AMP-activated protein kinase, AMPK)可以被激活, 活化的AMPK随后可以调控其下游蛋白如unc-51样自噬活化激酶1(unc-51 like autophagy activating kinase 1, ULK1)的磷酸化水平, 从而促进细胞自噬的起始[77]。有研究发现, 在TNF诱导的程序性坏死过程中, RIPK3可以与AMPK相互作用, 进而直接磷酸化AMPK, 磷酸化的AMPK通过活化下游蛋白ULK1从而启动自噬的发生。然而有趣的是, TNF诱导的程序性坏死会抑制自噬小体和溶酶体的融合[78]。此外, TORII等[79]的研究表明, 在基因毒性应激下, RIPK3可以与ULK1直接相互作用, 并磷酸化ULK1 Ser^{746}位点, 从而诱导ULK1依赖的替代自噬的发生。值得注意的是, RIPK3与ULK1的结合不依赖于其RHIM结构域, 但其激酶活性影响ULK1磷酸化水平; 基因毒性应激作用不能活化RIPK3以及MLKL[79]。此外, 在TCZ诱导的程序性坏死过程中, 并没有发现ULK1 Ser^{746}位点的磷酸化。以上结果表明, TCZ诱导的RIPK3依赖的程序性坏死和基因毒性应激诱导的RIPK3依赖的替代自噬通路之间没有信号交叉[79]。WU等[80]的研究发现, ULK1可以直接和RIPK1相互作用, 进而将RIPK1 Ser357位点磷酸化。该位点的磷酸化可以抑制RIPK1的活化, 进而抑制RIPK3的磷酸化以及坏死小体的形成, 最终阻碍TNF诱导的细胞程序性坏死的发生。以上结果表明, RIPK3在多种细胞死亡中均有重要作用。RIPK3如何选择它所扮演的角色? 哪些因素决定了RIPK3的功能? 这些问题还有待深入研究。因此, 我们在研究靶向RIPK3的抑制剂或激动剂时, 要考虑到RIPK3的多功能性。

5 总结与展望

本文概述了RIPK3翻译后修饰对细胞程序性坏死的调控, 以及RIPK3及其抑制剂在多种疾病中的作用。目前研究表明, 细胞程序性坏死及其关键蛋白对机体组织损伤存在复杂的作用。RIPK1可以调控多种生物学过程, 其缺失会导致小鼠出生后很快死亡, 而RIPK3缺失的小鼠可正常生长。我们推测通过抑制RIPK1治疗细胞程序性坏死相关疾病可能存在巨大的副作用, 而RIPK3更适合作为细胞程序性坏死相关疾病的靶标。目前已有多个靶向RIPK3的小分子(GSK'872、GSK'843和Dabrafenib等)投入实验研究。这些小分子可能在特定的疾病治疗中有一定的疗效, 然而还需大量研究以及临床试验的验证。此外, 不断探究新的RIPK3调控因子和修饰方式将为发现新型RIPK3抑制剂和靶向细胞程序性坏死治疗人类疾病提供一定理论依据。

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