

NLRP3 炎症小体泛素化调控的研究进展

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摘要:由于能被多种类型的病原体或危险信号所激活,NLRP3(NOD-like receptor family pyrin domain-containing protein 3)炎症小体在多种疾病过程中都发挥了关键作用.作为炎症反应的核心,NLRP3 炎症小体可能为各种炎症性疾病的治疗提供新的靶点.泛素化作为一种多功能翻译后修饰,在 NLRP3 介导的炎症免疫反应中起着关键的调节作用.通过对 NLRP3 炎症小体的组成、结构及功能,泛素化过程,泛素化与去泛素化对 NLRP3 炎症小体的正、负性调控的详细阐述,对 NLRP3 泛素化靶向治疗的前景与现状深入分析,为炎症性疾病的治疗提供新思路.

关键词:泛素化;炎症性疾病;NLRP3 炎症小体

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炎症小体是机体抵抗病原体侵害和识别自身危险信号的重要分子结构.当细胞受到病原相关分子模式(pathogen-associated molecular patterns, PAMPs)或者危险相关分子模式(damage-associated molecular patterns, DAMPs)刺激时,炎症小体活化,促使白细胞介素 1 β (Interleukin-1 β , IL-1 β)和 IL-18 等促炎细胞因子成熟释放,诱导细胞焦亡,参与固有免疫防御^[1].NLRP3 炎症小体是迄今为止研究最多最广泛的炎症小体^[2],其激活的分子机制及其与疾病发病机制的关系是新兴的研究热点.泛素化和去泛素化在 NLRP3 降解或激活中起着至关重要的作用^[3-5].泛素 E3 连接酶和去泛素化酶靶向治疗成功地用于癌症的治疗^[6].因此,全面了解泛素系统对 NLRP3 炎症小体级联反应的调节有助于 NLRP3 炎症小体介导疾病的靶向治疗.

1 NLRP3 炎症小体的组成、结构及功能

NLRP3 炎症小体是由识别炎症的 NOD 样受体 3(NLRP3)、接头蛋白即凋亡相关斑点样蛋白(apoptosis-associated speck-like protein containing a CARD, ASC)和效应分子半胱氨酸蛋白酶-1(Caspase-1)组成的炎性复合体,其主要在外周巨噬细胞、单核细胞、常规树突状细胞和中枢小胶质细胞中表达^[7].NLRP3 受体包含 3 个结构域:氨基末端 pyrin 结构域(PYD)、NACHT 结构域(Nucleotide-binding and Oligomerization Domain, NOD)和一个羧基末端富含亮氨酸的重复结构域(Leucine-rich Repeat, LRR)^[8-10].由于 NOD 具有三磷酸腺苷(ATP)酶活性,它对 NLRP3 的自缔合和功能至关重要. LRR 能够通过折叠回到 NACHT 结构域上进行自动抑制. ASC 由 2 个结构域组成:氨基末端 PYD 结构域和羧基末端半胱天冬酶募集结构域(caspase recruitment domain, CARD)^[11]. Caspase-1 由一个氨基末端 CARD 结构域和 2 个催化结构域组成,后者由一个中央大催化(p20)结构域和一个羧基末端催化小亚基(p10)结构域组成.

NLRP3 炎症小体的激活一般认为需要 2 种信号——启动信号或称第 1 信号,以及激活信号或称第 2 信号^[10]. Tumor necrosis factor α (TNF- α)^[12], IL-1^[13], 毒素(MPTP)^[14], DAMPs, PAMPs, 模式识别受体

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(Pattern recognition receptor, PRR). 例如 Toll 样受体(Toll-like receptors, TLR)和 NOD 样受体(NOD-like receptor, NLR)配体^[15]都可作为启动信号. 最常见的 NLRP3 炎症小体激活的第 2 信号包括线粒体活性氧(mtROS)、K⁺ 流出、Ca²⁺ 流通、错误折叠蛋白、溶酶体破坏、ATP、毒素、颗粒物质和病毒 RNA 等^[16-19]. 静息状态下的巨噬细胞中 NLRP3 的基础水平不足以激活 NLRP3 炎症小体, Pro IL-1 β 也不会稳定表达, 而巨噬细胞经 PAMPs 诱导后, 激活转录因子 NF- κ B, 从而诱导 NLRP3 和 pro-IL-1 β 的转录, 完成启动过程^[12, 20]. 激活信号出现后, NLRP3 的 LRR 与其配体结合后 NLRP3 发生结构变化, 暴露出 NACHT 结构域, 通过 ATP 聚合形成高度有序的 NLRP3 蛋白寡聚体, 并募集 ASC 及 Caspase 形成复杂的复合物——炎症小体(inflammasome), 产生活化的 Caspase-1. 具体过程为, ASC 的 PYD 结构域与 NLRP3 中的 PYD 相结合, 而其 CARD 结构域通过同型结构募集 Caspase-1 的 CARD 结构域^[21-22]. Caspase-1 也称 IL-1 β 转化酶, 活化的 Caspase-1 能将细胞内不具活性的 IL-1 β 前体在 116 位天冬氨酸裂解, 形成活化的成熟 IL-1 β 分泌到胞外^[23], 继而诱发炎症级联反应^[24-25] (如图 1 所示).

2 泛素化-NLRP3 的翻译后修饰

NLRP3 炎症小体的激活可通过翻译后修饰 (Post-translation modification, PTM) (如磷酸化^[26]、泛素化^[27-28]、苏莫酰化^[29]、烷基化^[30]和 S-亚硝基化^[31]) 进行严密控制与调节. 其中, 泛素化作为一种多功能 PTM, 在 NLRP3 介导的炎症免疫反应中起着关键的调节作用^[32-33]. 泛素(Ubiquitin, Ub)是一种由 76 个氨基酸组成的蛋白质, 通常通过异肽键与底物蛋白质的赖氨酸残基结合^[34]. 泛素化是指泛素分子在一系列酶的作用下, 对靶蛋白进行特异性修饰的过程. 这些酶包括泛素激活酶(E1)、泛素结合酶(E2)和泛素连接酶(E3)^[35]. 泛素化级联反应开始于 E1

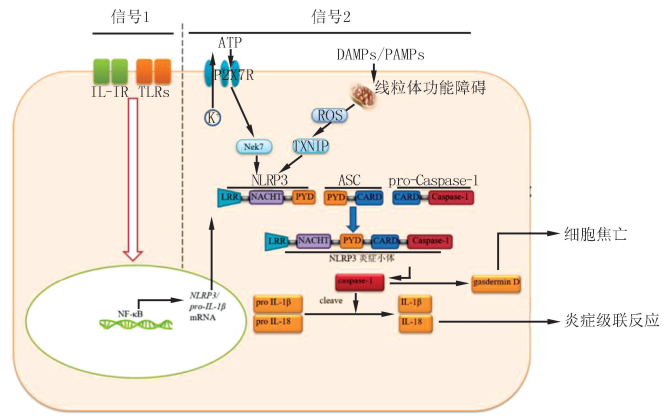


图1 NLRP3炎症小体激活过程

Fig.1 The activation of NLRP3 inflammasome

酶激活 Ub 的 C 端以形成硫酯键. 然后通过反式硫酯化将 Ub 转移到 E2 酶的活性半胱氨酸位点. 最后, Ub 被 E3 连接酶转移到底物蛋白上, 并促进 Ub 的 C 端甘氨酸和底物蛋白的赖氨酸残基之间形成异肽键. 泛素本身包含 7 个赖氨酸残基(K6, K11, K27, K29, K33, K48, K63)和一个蛋氨酸^[34]. 根据泛素的数量和附着类型, 泛素化可以分为以下 3 种类型: 如果泛素化发生在单个残基中, 则称为单泛素化; 如果在同一蛋白质中但在不同位点发生多个泛素化, 则称为多单体泛素化; 当多个泛素化发生在一个位点时, 它被称为聚泛素化.

3 泛素化对 NLRP3 炎症小体的调控

泛素化和去泛素化在 NLRP3 炎症小体的降解或激活中起着至关重要的作用^[3-4]. NLRP3 在静息巨噬细胞中泛素化, 并在启动和激活时去泛素化^[36]. 目前已发现了 2 个 E1 家族, 约 40 个 E2 和 600 个 E3 家族^[37-39].

3.1 NLRP3 的泛素化负性调控

目前发现只有 E3 泛素连接酶通过靶向 NLRP3 本身或炎症小体的其他成分, 如 ASC 和 caspase-1, 实现对炎症小体的负性调控, 其主要机制之一是通过蛋白酶体降解途径来控制 NLRP3 水平而实现. 多个 E3 泛素连接酶(包括 MARCH7, TRIM31, Cbl-b, RNF125, FBXL2 等)已报道作为 NLRP3 炎症小体的内源性负性调节因子. 多巴胺受体 D1 可通过 E3 连接酶 MARCH7 诱导 NLRP3 的泛素化和降解来抑制 NLRP3 炎症小体的激活, 缓解神经毒素诱导的神经炎症、LPS 诱导的全身炎症和单钠尿酸盐晶体诱导的腹膜炎^[40]. TRIM31 可以直接结合 NLRP3, 促进静息和活化巨噬细胞中 NLRP3 K48 聚泛素化和蛋白酶体降解, 从而

抑制 NLRP3 炎症小体的激活;TRIM31 缺乏促进了 NLRP3 炎症小体的激活,增强了 IL-1 β 的分泌,从而加重了体内明矾诱导的腹膜炎^[41].在 NLRP3 炎症小体受刺激后,Cbl-b 通过其泛素相关域与 NLRP3 LRR 域中 K63 连接的泛素链结合.这种结合导致 NLRP3 的 Nucleotide-binding domain(NBD)结构域中 K496 位点的 K48 连接泛素化,并诱导其蛋白酶体降解^[42-43].E3 泛素连接酶阻止 NLRP3 炎症小体激活的其他主要机制是通过将 NLRP3 保持在失活状态,与蛋白酶体降解无关,如 ARIH2,Cullin1, Parkin 等.E3 连接酶 ARIH2 过表达促进 NLRP3 泛素化并抑制 NLRP3 炎症小体激活;相反,使用 CRISPR/Cas9 基因组编辑删除内源性 ARIH2 可抑制 NLRP3 泛素化并促进 NLRP3 炎症小体激活,导致 ASC 寡聚化、pro-IL-1 β 加工和 IL-1 β 生成^[27].在炎症小体启动时,Cullin1 结合并泛素化 NLRP3,使其保持非活动状态.Parkin 诱导泛素修饰酶抗凋亡信号蛋白 20,后者可通过抑制核因子 κ B (Nuclear factor- κ B, NF- κ B)活化来抑制 NLRP3 炎症小体活化,而 Parkin 缺陷细胞 NLRP3 激活增加^[44-45].

3.2 NLRP3 的泛素化正性调控

E3 泛素连接酶还可作为正性调节因子促进炎症小体的激活,如 Pellino2, TRAF6 和 HUWE1.Pellino2 在 NLRP3 调节中起双重作用.LPS 导致 pellino-2 与 NLRP3 的关联并促进 NLRP3 K63 连接的聚泛素化.这种泛素化可能会促进 NLRP3 炎症小体的激活^[46].另一方面,Pellino-2 也可以抑制 NLRP3 炎症小体的激活.在野生型骨髓衍生巨噬细胞中,Pellino-2 泛素化 IL-1R 相关激酶 1 (IRAK1), IRAK1 的泛素化可以抑制 NLRP3 炎症小体的激活^[46].TRAF6 通过其泛素 E3 连接酶活性与 NLRP3 炎症小体的非转录启动相关^[47],可视为 TLR 介导的 NLRP3 炎症小体激活的正性调节因子.HUWE1 与 NLRP3 的相互作用导致 NLRP3 的 K27 连接聚泛素化.这有利于 NLRP3 组装、ASC 斑点形成和 caspase-1 激活,亦可视为 NLRP3 炎症小体的正性调节因子^[48].

3.3 NLRP3 的去泛素化调控

去泛素化酶与 E3 泛素连接酶一起在控制 NLRP3 炎症小体的激活方面发挥着至关重要的作用.去泛素化酶负责通过裂解蛋白质上赖氨酸残基和泛素 C 端甘氨酸之间的异肽键从蛋白质中去除泛素链^[49].目前已经发现的大约 100 种去泛素化酶可分为 7 大类:1)泛素特异性蛋白酶(USP),2)泛素 C 端水解酶(UCH),3)卵巢肿瘤蛋白酶(OTU),4)Machado-约瑟夫病蛋白结构域蛋白酶(MJD),5)单核细胞趋化蛋白诱导蛋白(MCPIP),6)含有 MIU 的新型去泛素化酶家族(MINDY),7)JAMM/MPN 结构域相关金属肽酶(JAMMS).首次发现去泛素化酶可作为 NLRP3 炎症小体的正性调节因子是源于去泛素化酶抑制剂 G5, PR619, bAP15 和 WP1130 显示出阻断 NLRP3 诱导的 caspase-1 激活和 IL-1 β 释放的作用^[3,50].以下去泛素化酶已被证明可以调节 NLRP3 炎症小体的激活.BRCC3 可以直接与 NLRP3 结合并通过其去泛素化有利于 NLRP3 炎症小体的激活;BRCC3 抑制剂 G5 阻断 NLRP3 炎症小体的激活^[4],同样,BRCC3/ABRO1 的缺失会减轻 NLRP3 相关的炎症性疾病,如腹膜炎或败血症^[51].由此验证了 BRCC3 作为 NLRP3 炎症小体的正性调节因子作用.在巨噬细胞暴露于炎症小体激活信号后,USP7 和 USP47 的酶活性增强,表明这些信号在翻译后水平调节去泛素化酶活性,而化学抑制和敲除 USP7 和 USP47 均可通过抑制 ASC 寡聚化和斑点形成而阻止 NLRP3 炎症小体的形成^[50,52].泛素特异性肽酶 1 相关因子 1(UAF1)是 3 种去泛素化酶复合物的组成部分,即 UAF1/USP1, UAF1/USP12 和 USP1/USP46^[53].最近的一项研究表明 UAF1/USP1 可以去除 NLRP3 的 K48 连接的泛素化,阻止 NLRP3 的蛋白酶体降解并增加 NLRP3 mRNA 和蛋白质的表达,从而促进 NLRP3 炎症小体的激活;此外,UAF1/USP12 和 UAF1/USP46 复合物增加 p65 表达,促进 NF- κ B 活化并增加 NLRP3 和 IL-1 β 表达水平;而体内/体外 UAF1 的缺失导致 NLRP3 炎症小体的激活和 IL-1 β 的分泌减少^[54].到目前为止,发现的首个具有炎症小体负性调节能力的去泛素化酶是属于 OTU 蛋白酶家族的 A20.A20 是 NF- κ B 通路的关键负性调节因子,其表达水平通过激活该通路而上调.A20 缺失的巨噬细胞表现出自发的 NLRP3 炎症小体激活,A20 缺失的小鼠会出现自发性侵蚀性多关节炎,类似于患者的类风湿性关节炎^[55-56].最近的一项研究发现,属于 JAMM 家族的信号转导接头分子结合蛋白(STAMPB)亦具有负性调节 NLRP3 炎症小体激活的能力.STAMPB 缺乏会增加 ASC 斑点和活性 Caspase-1 水平,同时 IL-1 β 基因表达和细胞因子水平亦显示增加^[57].

4 NLRP3 泛素化的靶向治疗

NLRP3 炎症小体与炎症性疾病之间的直接联系使其成为突出的治疗靶标^[15].NLRP3 炎症小体被认为是应激和抑郁症之间的桥梁,发挥着将心理应激转化为炎症反应的关键作用,在抑郁症的发病机制中占据着重要地位^[58].NLRP3 炎症小体与阿尔茨海默病^[59]、帕金森病^[60]、动脉粥样硬化、2 型糖尿病、痛风性关节炎等^[19]老年性、神经退行性疾病的发病机制有关(见图 2).NLRP3 炎症小体过度激活导致 IL-1 β 活化可能是阿尔茨海默病、糖尿病、抑郁症等多种炎症疾病的共同致病因素^[61].NLRP3 炎症小体过度激活会导致多发性硬化症^[62]、自身免疫性脑脊髓炎^[63]等多种自身免疫性疾病.NLRP3 炎症小体还与多种癌症(例如,乳腺癌、结肠癌、胃肠道癌、黑色素瘤)有关^[64-65].泛素介导的翻译后修饰激活 NLRP3 炎症小体是寻找控制 NLRP3 炎症小体激活治疗靶点的新兴领域.研究发现,泛素系统可成为治疗不同形式癌症和神经退行性疾病的潜在治疗靶点^[66-67].泛素蛋白酶体系统调节剂/抑制剂(例如,硼替佐米、卡非佐米、伊沙佐米、马里佐米)已成功用于癌症治疗或临床试验^[68-71].最有潜力作为 NLRP3 炎症小体抑制剂的 MCC950,OLT1177,MNS,CY-09 和 BOT4-one 等小分子目前正在进行临床前或临床试验^[30,72-76],其在 NLRP3 炎症小体相关疾病中的治疗效果值得期待.

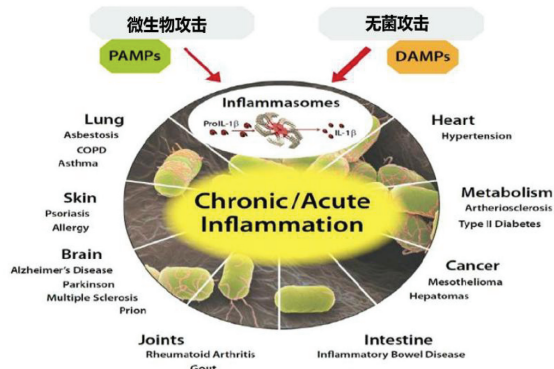


图2 NLRP3炎症小体与多种人类疾病密切相关

Fig.2 NLRP3 inflammasome is closely related to a variety of human diseases

5 结论与展望

NLRP3 炎症小体是迄今为止研究最多最广泛的炎症小体,其激活的分子机制及其与疾病发病机制的关系是新兴的研究热点.激活信号出现后,NLRP3 发生结构变化,并募集 ASC 及 Caspase 形成复杂的复合物——炎症小体,产生活化的 Caspase-1.后者将 IL-1 β 前体转化为成熟的 IL-1,继而诱发炎症级联反应.泛素化和去泛素化在 NLRP3 降解或激活中起着至关重要的作用.目前发现能调控 NLRP3 炎症小体的泛素化酶为 E3 泛素连接酶,其负性调控 NLRP3 炎症小体激活的主要机制为蛋白酶体降解作用从而降低 NLRP3 水平,或者将 NLRP3 保持在失活状态,与蛋白酶体降解无关.E3 泛素连接酶亦可正性调控 NLRP3 炎症小体的激活.去泛素化酶与 E3 泛素连接酶一样,亦存在正/负性调控 NLRP3 炎症小体激活的作用.

抗感染治疗作为防治抑郁症等炎症性疾病的新方向、新角度,NLRP3 炎症小体泛素化调控作为防治炎症性疾病的新靶点,前景广阔.但目前对 NLRP3 炎症小体激活的确切调控机制仍不清楚,难以减少过度的免疫抑制作用增加临床上感染的风险.如何解决副作用和非靶向作用是推广 NLRP3 泛素化靶向治疗的关键问题.

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Research progress of ubiquitination on the regulation of NLRP3 inflammasome

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Abstract: NOD-like receptor family pyrin domain-containing protein 3 (NLRP3) inflammasomes plays a key role in a variety of disease processes because of its activation by many types of pathogens or danger signals. As the core of inflammatory response, NLRP3 inflammasome may provide a new target for the treatment of various inflammatory diseases. As a multifunctional post-translational modification, ubiquitination plays a key regulatory role in the inflammatory immune response mediated by NLRP3. The study elaborated on the composition, structure and function of NLRP3 inflammasome, the ubiquitination process, and the positive and negative regulation of NLRP3 inflammasome by ubiquitination and deubiquitination, and introduced the targeted therapy effects of NLRP3 ubiquitination, which provided new ideas for the treatment of inflammatory diseases.

Keywords: ubiquitination; inflammatory disease; NLRP3 inflammasome