泛素化调控抗病毒天然免疫的研究进展

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摘要 天然免疫是宿主防御病原微生物入侵的第一道防线, 其活化主要通过天然免疫细胞上的模式识别受体(pattern recognition receptors, PRRs)识别病原微生物上相对保守的相关分子模式(pathogen-associated molecular patterns, PAMPs). 病毒相关的核酸成分可以被机体Toll样受体(Toll-like receptors, TLRs)、维甲酸诱导基因 I 受体(RIG-I-like receptors, RLRs)以及胞浆DNA受体(cytoplasmic DNA sensors)等识别, 通过一系列复杂的细胞信号通路诱导 I 型干扰素(type I interferon)及炎症因子的表达, 从而激发机体抗病毒反应. 泛素化修饰是细胞内广泛存在的蛋白质翻译后修饰方式, 在宿主防御病原微生物感染的动态调控过程中发挥着重要的作用. 已有大量文献报道, 天然免疫抗病毒信号通路中的多个关键接头分子可发生泛素化修饰, 进而调控机体抗病毒免疫应答反应. 本文综述了泛素化修饰在抗病毒天然免疫中的作用及其调控机制.

关键词 天然免疫, 抗病毒, 泛素化修饰

病毒感染等疾病对于人类而言仍旧是严重的威胁, 天然免疫系统在机体对抗病毒感染过程中发挥着重要的作用. 天然免疫细胞可以通过多种模式识别受体(pattern recognition receptors, PRRs)识别病毒的核酸成分(RNA或DNA), 招募相应的接头分子并激活下游的激酶分子, 启动下游信号通路活化, 激活NF- κ B及IRF3/7等转录因子, 诱导 I 型干扰素及炎症因子的表达. I 型干扰素会进一步活化JAK-STAT等信号通路, 诱导大量抗病毒基因的生成, 最终抑制病毒的复制, 引发机体抗病毒天然免疫反应^[1,2].

1 天然免疫抗病毒信号通路

迄今为止, 至少有三种主要的模式识别受体可以识别病毒的核酸成分: Toll样受体(toll-like receptors, TLRs)、维甲酸诱导基因 I 受体(RIG-I like receptors, RLRs)以及胞浆DNA受体^[1,3](图1).

1.1 TLR介导的天然免疫抗病毒信号通路

TLR3, TLR7, TLR8和TLR9分布在天然免疫细胞内体上, 可识别吞噬到内吞体中的相应病毒核酸成分.

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图1 泛素化修饰对抗病毒天然免疫信号通路的调节

红色分子是本实验室发现的E3泛素连接酶

其中, TLR9识别非甲基化的CpG DNA, TLR3识别双链RNA, TLR7/8识别单链RNA^[4].

TLRs介导的信号通路包括MyD88(myeloid differentiation primary response gene 88)依赖的信号通路和TRIF(TIR-domain containing adapter-inducing interferon- β)依赖的信号通路^[5]. 其中TLR3所介导的信号通路依赖TRIF衔接蛋白, 而TLR7/8/9依赖MyD88衔接蛋白. 衔接蛋白TRIF募集下游的TRAF3(TNF receptor-associated factor 3), 进而激活TBK1(TANK-binding kinase)和IKK ϵ (inhibitor- κ B kinase ϵ), 促进IRF3/IRF7所介导的I型干扰素的产生^[6,7]. 另一方面, MyD88招募下游的IRAKs(IL-1R-associated serine/threonine kinases), IRAKs将信号传递至TRAF6(tumor necrosis factor receptor-associated factor 6), 募集并活化下游的TAK1(TGF β -activated kinase 1)和IKK $\alpha/\beta/\gamma$, 促进NF- κ B介导的炎症因子的表达^[8].

1.2 RLRs介导的天然免疫抗病毒信号通路

维甲酸诱导基因I受体(RIG-I-like receptors,

RLRs)是识别胞浆游离RNA分子的主要受体, 其家族有三个成员组成: RIG-I (retinoic acid-inducible gene I, 也称为DDX58), MDA5(melanoma differentiation associated gene 5)和LGP2(laboratory of genetics and physiology-2)^[9,10].

RIG-I和MDA5由N端的两个CARD结构域(caspase-recruitment domains, CARDs)、含DEXD/H结构域的RNA解旋酶及C末端的RD结构域(repressor domain, RD)构成, LGP2则缺乏N端CARD结构域^[11,12]. 目前研究认为, MDA5倾向于识别大于2 kbp的双链RNA(dsRNA), RIG-I则优先识别更短一些的dsRNA和5'端含有三磷酸基团的RNA(5'-triphosphorylated RNA, pppRNA), LGP2促进病毒RNA与配体RIG-I或MDA5结合和识别^[13].

在细胞浆中, 游离的模式识别受体RIG-I, MDA5通过自身的C端结构域(carboxyl terminal domain, CTD)与病毒的dsRNA相互结合, 自身构象发生变化, 进而其CARD结构域与同样含有CARD结构域的线粒体抗病毒信号蛋白(mitochondrial antiviral sig-

naling protein, MAVS)相结合^[14,15]. MAVS被RIG- I 或MDA5募集后, 可以进一步活化IKK相关激酶TBK1/IKK ϵ , 被TBK1/IKK ϵ 磷酸化的IRF3/IRF7形成同源或异源二聚体, 进入细胞核结合到 β 干扰素(interferon- β , IFN- β)等的启动子区, 促进 I 型干扰素的生成^[16,17].

1.3 胞浆DNA受体介导的天然免疫抗病毒信号通路

目前发现的DNA受体包括RNA聚合酶III(RNA Polymerase III), DNA结合蛋白(DNA-dependent activator of IRFs, DAI), 干扰素诱导蛋白16(IFN- γ -inducible protein16, IFI16/p204), DDX41(DEAD-box helicase 41), LSm14 A(mRNA processing body assembly factor)和cGAS(cyclic GMP-AMP (cGAMP) synthase)^[18]. 其中, 人们普遍认为cGAS是一种“通用”DNA受体, 可以在大多数类型细胞中发挥作用并且识别不同类型的dsDNA. DNA受体cGAS等识别dsDNA后, 可催化合成内源性第二信使环化二核苷酸cGAMP(2'-3' cyclic AMP-GMP)^[19].

cGAMP可以被下游重要的接头蛋白STING(stimulator of interferon genes, 也称为MITA, MEM173)所识别, STING发生活化, 进一步使TBK1, IRF3发生磷酸化, 最终促进 I 型干扰素的产生^[18,19].

2 蛋白质泛素化修饰调控天然免疫信号转导

泛素化修饰作为一种重要的蛋白质翻译后修饰方式, 在天然免疫信号转导中发挥重要作用. 蛋白质的泛素化修饰由三种泛素相关酶共同参与完成: E1泛素激活酶(ubiquitin-activating enzyme)、E2泛素结合酶(ubiquitin-conjugating enzyme)和E3泛素连接酶(ubiquitin-protein ligase). E3泛素连接酶是蛋白质泛素化修饰途径中最重要的酶, 是将泛素分子精确偶联到特定蛋白分子的关键因素^[20]. 在人类细胞中存在700多种E3泛素连接酶, 目前发现的E3泛素连接酶主要有四大类: HECT结构域家族(homologous to E6-associated protein C-terminus)、RBR蛋白家族(RING-in-between RING)、RING(really interesting new gene)结构域家族和U-box蛋白家族(UFD2 homology)^[21]. 蛋白质的泛素化修饰是可逆的过程, 泛素链可在去泛素化酶(deubi-

quitinating enzymes, DUBs)的作用下被水解, 动态调控机体泛素化修饰过程^[22].

根据泛素化链的长度不同, 泛素化修饰可分为单泛素化(mono-ubiquitination)、多泛素化(multiple-ubiquitination)和多聚泛素化(poly-ubiquitination)^[23~25]. 由于泛素分子本身包含7个赖氨酸残基(K6, K11, K27, K29, K33, K48和K63), 因此底物蛋白可以被至少7种多聚泛素链型的非线性泛素化修饰, 其中K48及K63位泛素化修饰是研究最为广泛的, 其他形式的泛素化修饰及其对蛋白功能的调控目前正逐渐成为国际研究热点问题^[26]. 不同形式的泛素化发挥的作用也不尽相同, 如发生K48位泛素化修饰的蛋白质将通过26S蛋白酶体途径或者溶酶体途径被迅速降解, 而K63位泛素化修饰主要调控信号转导、蛋白质活性以及DNA修复等功能^[27].

2.1 泛素化修饰调控TLRs介导的天然免疫信号转导

在TLRs信号通路中, 多个E3泛素连接酶被报道可以通过K48位泛素化修饰的形式调控TLRs本身或者下游重要接头分子如MyD88和TRIF等蛋白的表达. 文章报道, E3泛素连接酶Triad3A促进TLR9等多种Toll样受体发生K48位泛素化修饰, 进而导致这些蛋白通过蛋白酶体途径降解, 最终抑制了信号通路的传递^[28]. E3泛素连接酶Smurf1/2和Nrdp1, 可以结合MyD88, 并通过K48位泛素化修饰, 导致其降解. 另外, Nrdp1对于TLRs信号通路的调控存在另外一种机制, 即通过对TBK1进行K63位泛素化修饰, 激活IRF3介导的信号通路^[29,30]. E3泛素连接酶WWP2和TRIM38可以促进TRIF发生K48位泛素化修饰, 负向调控TLR3介导的IFN- β 表达, WWP2敲基因小鼠(*Mus musculus*)对poly(I:C)(polyinosinic:polycytidylic acid)的易感性相对野生型小鼠明显提高, 死亡率显著增加^[31,32].

TRAFs, NEMO(NF- κ B-essential modulator)和TBK1等多个TLRs下游信号分子可以发生K63泛素化修饰. TRAF6具有E3泛素连接酶活性, 一方面可以进行自身K63位泛素化修饰, 并对下游靶蛋白进行泛素化; 另一方面, TRAF6可以合成非锚定的K63位泛素链, 并招募和活化下游的TAK1和IKK, 最终促进多种炎症因子的产生. 同样有报道称, TRAF3自身发生K63位泛素化修饰后可以作为一个“脚手架”招募下游

NEMO-TBK1/IKK ϵ 复合体的形成, 活化天然免疫信号转导^[33,34].

此外, NEMO作为IKK α /IKK β 复合体和TBK1/IKK ϵ 激酶复合体的同源结合伴侣, 可以被催化发生K27, K29及线性泛素化修饰, 进而调控NF- κ B和IRF3的磷酸化及入核^[35-37]. 天然免疫抗病毒的核心激酶TBK1可以被E3泛素连接酶RNF128进行K63位泛素化修饰, 导致其激酶活性的活化, 并最终促进I型干扰素的产生^[38]. 相反, Triad3A和DTX4(protein deltex-4), TRIP(TRAf-interacting protein)可以通过促进TRAF3和TBK1发生K48位泛素化修饰, 导致二者在蛋白酶体中发生降解, 最终抑制I型干扰素的产生^[39-41]. 转录因子IRF3可以被TRIM26, Pin1, RBCK1, Ro52和RAUL等多个E3泛素连接酶进行K48位泛素化修饰及降解, 最终负向调控机体天然免疫抗病毒反应^[42-47](图1).

2.2 泛素化修饰调控RLRs介导的天然免疫信号转导

蛋白质的泛素化修饰在RLRs信号通路中, 同样发挥着非常重要的作用. 多项研究表明, RIG-I会被RNF135, TRIM4和MEX3C进行K63泛素化修饰, 分别缺失这三个E3泛素连接酶后, 病毒介导的I型干扰素的产生受到明显抑制^[48-50]. TRIM25可以催化形成游离的K63泛素链, 该泛素链以非锚定的形式与RIG-I结合, 进而引起RLRs信号通路的活化^[51]. 同样有报道称, E3泛素连接酶TRIM25可对RIG-I的第172位赖氨酸进行K63位泛素化修饰, 促进RIG-I与下游接头蛋白MAVS的结合, 最终促进I型干扰素的产生^[52]. 由此可见, TRIM25在RLR信号通路调控过程中起到关键、复杂的作用. Inn等人^[53]发现, 泛素连接酶HOIL-1L(heme-oxidized IRP2 ubiquitin ligase 1L)和HOIP(HOIL-1-interacting protein)可以组成线性泛素化装配复合体LUBAC(linear ubiquitin chain assembly complex), 可以对RIG-I进行线性泛素化修饰并导致其降解; 同时, LUBAC可以与TRIM25竞争性结合RIG-I, 进而抑制RLRs所介导的信号通路.

Paz课题组^[54]研究发现, 使用RNA病毒感染巨噬细胞后, 线粒体蛋白MAVS的第500位赖氨酸发生了K63位泛素化修饰, 而促进MAVS进行K63位泛素化的E3连接酶多年来却一直未被发现. 直到2017年, 本研

究团队^[55]发现E3泛素TRIM31可以对MAVS上的第10, 311和461位点的赖氨酸进行K63位泛素化修饰, 进而促进MAVS蛋白多聚体的形成, 促进I型干扰素的分泌, 最终抑制病毒的复制; 体内实验进一步证实TRIM31缺陷小鼠更易被RNA病毒(VSV或SeV)所感染. TRIM31是第一个被发现可调控MAVS进行K63连接形式泛素化修饰的E3连接酶.

信号通路中受体或者接头蛋白的含量需要被严格控制, 以避免信号通路的过度活化. K48位的泛素化修饰可以导致目标靶蛋白的降解, 因此K48位泛素化修饰在RLRs信号通路中也发挥着十分重要的作用. RIG-I, MDA5和MAVS均被报道都可以发生K48位的泛素化修饰. 其中RNF125, RNF122和CHIP, 可以促进RIG-I的K48位泛素化修饰. E3泛素连接酶TRIM13通过对MDA5进行K48位的泛素化修饰, 导致MDA5在蛋白酶中降解, 最终抑制病毒介导的天然免疫反应^[56-59]. TRIM40被报道可以与RIG-I和MDA5结合, 分别促进它们的K27和K48位的泛素化修饰, 促进RIG-I和MDA5的蛋白酶体途径降解, 抑制信号转导^[60].

研究发现, RNF5对MAVS的第362和第461位赖氨酸进行K48位泛素化修饰, 进而使之降解, 负向调控IFN- β 的产生^[61]. E3泛素连接酶Smurf1可以被Ndfip1募集到MAVS上, 并对MAVS进行K48位泛素化^[62]; Smurf2作为与Smurf1同家族的蛋白, 同样被报道可以将MAVS进行K48位泛素化, 负向调控IRF3/7的磷酸化及I型干扰素的产生^[63]. 此外MAVS还被报道可被TAX1BP1, MARCH5, PCBP1, PCBP2, TRIM25等多个分子进行K48位泛素化修饰^[64-68](图1).

2.3 泛素化修饰调控cGAS-STING介导的天然免疫信号转导

在cGAS-STING介导的天然免疫抗DNA病毒信号通路中, TRIM家族两个分子TRIM56和TRIM32通过促进STING的K63位泛素化修饰正向调控STING信号通路的活化^[69,70]. TRIM32可以特异性地与STING结合, 并对其第20, 150, 224, 236位赖氨酸进行K63位泛素化修饰, 促进STING与下游信号分子TBK1的结合, 诱导I型干扰素的产生^[70]. 在转化细胞系或者人肺成纤维细胞中, 敲减TRIM56后, poly(I:C)及poly(dA:dT)诱导的I型干扰素受到明显的抑制, 这说明TRIM56在抗RNA和DNA病毒中都发挥着重要的作用. 深入研究发

现, TRIM56可以与STING结合并促进其第150位赖氨酸的K63位泛素化修饰。有趣的是, 科学家发现TRIM56同样可以靶向DNA受体cGAS, 促进其第335位赖氨酸发生单泛素化修饰, 这种修饰可以促进cGAS的二聚化并增强其与DNA的结合能力, 诱导第二信使cGAMP的产生。构建HSV-1感染小鼠模型, 发现TRIM56缺陷小鼠的I型干扰素产生降低, 更易感染HSV-1病毒^[71]。RNF185被报道可以增强cGAS的酶活性, 促进I型干扰素的产生, 其机制依赖于RNF185对cGAS进行K27位的泛素化修饰^[72]。文献报道, TRIM14可以抑制选择性自噬受体p62介导的cGAS降解, 从而促进天然免疫反应。他们发现cGAS的K48位泛素化修饰是p62依赖的选择性自噬降解的识别信号, TRIM14可以招募USP14, 去除cGAS的K48位泛素链, 进而抑制cGAS的降解^[73]。

除了K63位泛素化修饰方式外, STING还存在K48和K27位等形式的泛素化修饰形式。病毒感染机体后, 内质网相关的E3泛素连接酶RNF5可促进STING的第150位赖氨酸发生K48位泛素化修饰, 导致STING通过蛋白酶体途径发生降解, 抑制病毒引起的I型干扰素的产生^[74]。在树突状细胞(dendritic cells, DC)中, HSV-1感染可以诱导E3泛素连接酶TRIM30 α 的表达, TRIM30 α 靶向结合STING, 并促进其第275位的赖氨酸发生K48位泛素化修饰, 引起STING的蛋白酶体途径降解^[75]。TRIM29被报道可以对STING进行K48位泛素化修饰, 促进STING的降解^[76]。

E3泛素连接酶RNF26可以对STING第150位赖氨酸发生K11位泛素化修饰, 竞争性降低RNF5介导的K48位泛素化修饰, 阻碍STING的降解^[77]。位于内质网上的E3泛素连接酶AMFR, 可以介导STING的第137, 150, 224和236位赖氨酸发生K27位泛素化修饰, 这种K27形式的泛素化链可以作为“脚手架”, 募集并活化下游TBK1, 形成更有效的抗病毒免疫反应^[78](图1)。

2.4 E2泛素结合酶和病毒蛋白对抗病毒天然免疫信号转导的调控作用

除了E3泛素连接酶调控天然免疫信号转导之外, 不同的E2泛素结合酶也可以特异调控天然免疫的信号转导。例如, E2泛素结合酶Ubc5在IRF3激活过程中发挥重要的作用^[79]。Ube2D3和Ube2N作为E2泛素结合酶可以影响RIG-I的泛素化, 进而影响RIG-I所介导

的MAVS的多聚化^[80]。泛素结合酶UBE2J1被报道可以负向调节I型干扰素的产生, 促进病毒的复制, 其机制依赖于UBE2J1可以对转录因子IRF3发生泛素化修饰, 并促进IRF3的降解^[81]。

泛素化修饰作为天然免疫信号转导的主要调控机制, 其本身也可以受到病毒蛋白和宿主蛋白等其他因素所调控。肠病毒71型可以抑制机体I型干扰素的产生, 其机制依赖于肠病毒71型可以抑制RIG-I的泛素化水平^[82]。寨卡病毒感染机体后, 诱导NS1蛋白产生, NS1会进一步募集去泛素化酶USP8, USP8会移除天冬氨酸特异性半胱氨酸蛋白酶-1(cysteinyI aspartate specific proteinase-1, caspase-1)上的K11位泛素化链, 抑制caspase-1的降解, 增多的caspase-1会加快对cGAS的切割, 抑制I型干扰素的产生^[83]。Barro等人^[84]研究发现轮状病毒感染机体后, 轮状病毒的基因编码产物NSP1可以与IRF3相互结合, 促进IRF3蛋白酶体途径的降解, 抑制I型干扰素的产生。典型的猪瘟病毒和牛病毒性腹泻病毒编码的病毒蛋白Npro和牛疱疹I型病毒编码的病毒蛋白bICP0, 也可与IRF3相互结合, 促进IRF3蛋白酶体途径的降解, 抑制I型干扰素的产生^[85,86]。UL36是I型单纯疱疹病毒含量最多的外壳蛋白, 文献报道在UL36蛋白的N端包含一个去泛素化酶活性的结构域。机体感染SeV病毒后, UL36蛋白可以对TRAF3进行去泛素化, 抑制对下游TBK1分子的招募, 抑制I型干扰素的产生^[87]。人16型乳头瘤病毒编码的E6癌蛋白, 可以和TRIM25与USP15形成复合体, 促进RIG-I的K48位泛素化修饰, 抑制RIG-I的K63位泛素化修饰, 抑制RIG-I所介导的固有免疫应答^[88]。MLL5是一种重要的表观遗传修饰蛋白, 具有控制细胞周期进程、染色质结构维持和造血等功能, 文献报道MLL5在抗病毒固有免疫中也发挥重要作用, MLL5可以与RIG-I和E3泛素连接酶STUB1相互作用, 促进RIG-I的K48位泛素化修饰, 进而促进RIG-I的降解、抑制I型干扰素的产生^[89]。

3 总结

近年来, 天然免疫在机体感染性疾病、肿瘤、阿尔兹海默症等方面发挥的作用正逐步受到广泛关注, 并成为当今国际免疫学研究领域的热点问题。天然免疫活化不足会导致病原微生物的逃逸, 而天然免疫活

化过度则导致机体感染性休克甚至是肿瘤等发生。因此天然免疫信号转导必须受到严格调控, 才能使机体处于良好的免疫稳态。蛋白质的泛素化修饰是天然免疫抗病毒信号转导调控的重要方式, 近年来在这一领域发表的论文也呈井喷式增长。然而, 随着人类基因组计划的完成和信息生物学的快速发展, 发现在人类基因组中E3泛素连接酶的数目超过700个。但相对于

数目众多的E3泛素连接酶, 只有很小一部分泛素连接酶在天然免疫中的功能得到了阐明。并且目前已经初步探测机体60%以上的蛋白质会发生不同形式的泛素化修饰, 人们已经发现的泛素化调控机制仅仅是冰山一角。因此, 发现新的调控机体天然免疫抗病毒信号转导的E3泛素连接酶及新的调控机制, 并将复杂的蛋白质修饰进行网络化研究, 仍然是亟需解决的问题。

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Regulation of innate antiviral immunity by protein ubiquitination

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Innate immunity is the first line of host defense against invading microbes. Activation of innate immunity primarily relies on the recognition of pathogen-associated molecular patterns (PAMPs) by pattern-recognition receptors (PRRs). Viral nucleic acids are recognized by the retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) and the Toll-like receptors (TLR) as well as the DNA sensor cyclic GMP-AMP (cGAMP) synthase (cGAS), leading to the production of proinflammatory cytokines and type I interferon and elimination of viral infection. Protein ubiquitination has emerged as a crucial mechanism that regulates signal transduction in diverse biological processes. A variety of key molecules in innate antiviral signaling transduction pathways can be ubiquitinated. The current review summarizes our current knowledge on the functions and regulatory mechanisms of protein ubiquitination in innate antiviral immunity with a focus on the E3 ligases.

innate immunity, antiviral immune response, protein ubiquitination

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