

综述



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抗病毒天然免疫通路的双链RNA感受器

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摘要: 天然免疫系统是机体抵抗病原体入侵的第一道防线, 主要通过模式识别受体来识别病原相关分子模式。病毒核酸(包括DNA和RNA)是一类重要的病原相关分子模式, 目前在细胞中已经鉴定出多种感知病毒核酸的模式识别受体, 其中能够感知双链RNA的感受器在宿主防御病毒感染过程中具有至关重要的作用。此外, 除了外源双链RNA分子, 某些病理情况下自身RNA也会成为双链RNA感受器的激活剂, 导致引发自身免疫性疾病。目前已经鉴定出的双链RNA感受器主要包括 Toll 样受体、RIG- I 样受体、NOD样受体、2',5'-寡腺苷酸合成酶样受体和RNA依赖性蛋白激酶, 这些受体在与病毒双链RNA结合后会引发一系列抗病毒反应, 在机体健康与疾病预防中发挥着十分重要的作用。本文主要聚焦几类双链RNA感受器, 并对相关蛋白家族的结构功能、配体种类以及免疫信号转导途径展开介绍。

关键词: 天然免疫; 双链RNA感受器; 模式识别受体; 抗病毒信号传导; 自身免疫疾病

Double-stranded RNA sensors in antiviral innate immunity

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Abstract: Innate immune system is the first line of defense against pathogen, primarily recognizing pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors (PRRs). Viral nucleic acids (including DNA and RNA) are a typical class of PAMPs. Various PRRs that sense nucleic acids have been identified in cells, among which receptors that detect double-stranded RNA (dsRNA) play a crucial role in host defense against viral infections. Furthermore, in addition to exogenous dsRNA molecules, endogenous

收稿日期: 2024-07-15

基金项目: 国家自然科学基金项目(32071283); 科技部重点研发计划项目(2021YFA1300503)

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RNA can also become activators of dsRNA sensors under certain pathological conditions, leading to autoimmune diseases. The identified dsRNA sensors include Toll-like receptors, RIG- I -like receptors, NOD-like receptors, 2',5'-oligoadenylate synthetase-like receptors, and RNA-dependent protein kinases. These sensors, upon binding to viral dsRNA, trigger a series of antiviral immune responses, playing an essential role in maintaining health and preventing diseases. This article focuses on several types of dsRNA sensors, discussing the structure and function of proteins, types of ligands, and immune signaling pathways.

Key Words: innate immune; dsRNA sensors; PRR; antiviral signaling; autoimmune disease

自然界中, 高等生物在与微生物的长期博弈过程中, 逐渐进化出一系列抵抗病原微生物的防御机制, 包括天然免疫(innate immune)和适应性免疫(adaptive immune)两大系统。天然免疫是机体对抗感染和外来物质的第一道防线, 也被称为固有免疫或非特异性免疫, 在入侵病原体接触到机体时, 天然免疫系统通过一系列复杂的反应机制, 迅速做出反应以遏制感染的扩散。哺乳动物的天然免疫系统已经发展出多种模式识别受体(pattern recognition receptors, PRRs), 能够检测细胞内和周围的病原相关分子模式(pathogen associated molecular patterns, PAMPs)和损伤相关分子模式(damage associated molecular patterns, DAMPs)信号。当模式识别受体被激活后, 它们会将信号传递至细胞内部, 激活下游的信号分子, 如干扰素调节因子3(interferon regulatory factor 3, IRF3)、干扰素调节因子7(interferon regulator factor 7, IRF7)和细胞核因子- κ B(nuclear factor kappa B, NF- κ B)等, 最终导致细胞因子、干扰素等的释放, 帮助机体抵御病原微生物的感染(图1)^[1,2]。

病原微生物的核酸作为一类重要的PAMP, 能够被细胞中所感知核酸的特异性受体——核酸感受器所识别, 在天然免疫中起着关键的作用^[3]。这

些核酸感受器中包括重要的双链RNA(dsRNA)识别受体, 如Toll样受体(Toll-like receptors, TLRs)、RIG- I 样受体(RIG- I receptors, RLRs)、NOD样受体(NOD-like receptors, NLRs)、2',5'-寡腺苷酸合成酶样受体(OAS-like receptors, OLRs)和RNA依赖性蛋白激酶R(protein kinase R, PKR)(图2)。在识别外源dsRNA之后, 这些核酸感受器可以启动抗病毒防御, 如促进干扰素(interferon, IFN)和相关促炎细胞因子的产生^[2,4,5]。分泌的干扰素(包括IFN α 和IFN β)激活干扰素刺激基因(interferon-stimulated genes, ISGs)的表达, 帮助机体抵抗病毒感染并形成适应性免疫反应。同时, I型干扰素的产生能够进一步增强机体的免疫反应, 包括抗原呈递细胞的激活、CD8⁺ T细胞的交叉启动以及免疫细胞的招募, 来共同抵抗入侵的病原微生物^[6,7]。

作为机体重要的保护防线, 天然免疫系统需要严格的调控来增强对感染的敏感性, 同时也要防止过度炎症的产生。值得注意的是, 越来越多的证据表明, PRRs的错误激活是导致免疫功能紊乱和自身免疫疾病的主要原因之一^[4,8]。有研究表明, RNA感受器在某些细胞功能紊乱的情况下与内源RNA相互作用, 诸如内源性RNA的长期上

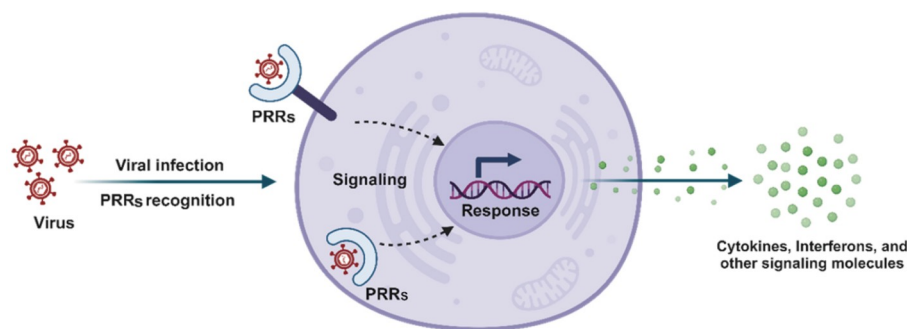


图1 模式识别受体介导的抗病毒天然免疫

调、定位紊乱以及错误加工都会导致免疫信号的激活, 这种不适当激活可导致单基因疾病或复杂的自身免疫性疾病, 如Aicardi-Goutières综合征(Aicardi-Goutières syndrome, AGS)和系统性红斑狼疮(systemic lupus erythematosus, SLE)^[1,9]。因此, 揭示这些dsRNA感受器的作用机制, 不但有助于开发新的抗病毒疗法和疫苗, 还能促进对自身免疫性疾病的理解。本文对细胞中参与抗病毒天然免疫的dsRNA受体蛋白家族进行总结, 包括其结构、配体RNA和信号传递机制等。

1 TLRs

TLRs是最早发现的模式识别受体家族, 可以追溯到果蝇中Toll基因及其蛋白质的鉴定和功能探索, 由于与Toll蛋白的相似性而得名^[10]。TLRs能够识别广泛存在于病原体中的PAMPs, 如细菌的脂多糖和病毒的dsRNA。通过激活TLRs, 宿主细胞可以启动信号传导通路, 诱导促炎细胞因子和I型干扰素的产生, 从而在早期免疫反应中发挥重要作用。TLRs不仅在抗感染免疫中起关键作用, 还参与胚胎发育和免疫调节过程^[11,12]。

TLRs隶属于I型整合跨膜蛋白, 是一个具有700~900个氨基酸的单链型蛋白质, 通常由三个结构域组成: 位于细胞外的N末端结构域(N-terminal domain, NTD)、中间为单螺旋跨膜结构域以及位于胞质内的C末端结构域(C-terminal domain, CTD)^[13,14]。N端包含有19~25个高度保守的亮氨酸重复序列(leucine-rich repeats, LRRs), 这种结构通过特定的排列方式形成一个能够与PAMPs结合的凹槽型结构, 形似马蹄铁。这些LRRs不仅能够增强蛋白质结构的稳定性, 还能识别PAMPs, 在TLRs的配体结合中发挥着关键的作用, 能够通过特定的模式识别来区分不同类型的PAMPs^[15]。中间的跨膜区域起着桥梁作用, 能够连接LRRs和CTD, 跨膜结构域包含一个单独的 α 螺旋结构, 能够将胞外的信号转导至细胞内部。C末端含有一个TIR(Toll/interleukin-1 receptor)结构域, 能够通过同源结构域与各种信号转导适配器相互作用, 如髓样分化因子88(myeloid differentiation factor 88, MyD88)、 β 干扰素TIR结构域衔接蛋白(TIR domain containing adaptor inducing interferon β , TRIF), 从

而启动一系列的下游信号转导^[16,17]。

目前, 在人类中发现了10种TLRs, 其中TLR1、TLR2、TLR4、TLR5、TLR6和TLR10分布于细胞表面, TLR3、TLR7、TLR8、TLR9分布在胞质中, 可分别识别不同的PAMPs^[12,13]。其中, TLR3是一种dsRNA感受器, 它能够对病毒的dsRNA以及人工合成的poly(I:C)做出反应, 以不依赖RNA序列特异性的结合模式在抗病毒信号转导中发挥关键作用, TLR3对dsRNA的识别取决于dsRNA的长度, 最小限度为40~50 bp^[18-22]。研究表明, TLR3缺陷会导致对多种病毒的易感性增加, 包括脊髓灰质炎病毒和I型单纯疱疹病毒(herpes simplex virus type 1, HSV-1)^[23], 人类TLR3的缺陷会导致儿童患HSV-1脑炎^[24]。当细胞处于静息状态时, TLR3以单体和膜受体的形式存在。当TLR3与dsRNA结合后, 会形成由一个dsRNA和两个TLR3分子组成的dsRNA-TLR3信号复合物^[20,21,25]。二聚化的TLR3通过TIR结构域相互作用招募TRIF来触发下游的信号级联反应, 包括肿瘤坏死因子受体相关因子6(tumor necrosis factor receptor-associated factor 6, TRAF6)、TANK结合激酶(TANK-binding kinase, TBK)和激酶IKK ϵ (I- κ B kinase ϵ , IKK ϵ)等^[21,26,27], 导致IRF3和NF- κ B的激活, 最终导致I型干扰素和促炎细胞因子的产生, 增强局部的免疫反应达到抗病毒的效果(图2)。除了TLR3识别dsRNA之外, 人TLR7和TLR8则主要识别ssRNA^[28], 同样也会诱导其二聚化进而与MyD88相互作用, 招募下游白细胞介素-1受体相关激酶4(interleukin-1 receptor associated kinase 4, IRAK4)、IRAK1和TRAF6等, 激活下游信号通路发挥抗病毒效应。此外, 小鼠还表达TLR13, 能够对细菌和病毒的RNA产生反应, 细菌的23S核糖体RNA的特定序列被认为是TLR13的有效激活剂(图2)^[29,30]。

2 RLRs

RLRs几乎存在于所有的哺乳动物中, 它们具有较高的保守性, 且作为胞质内的dsRNA感受器在病毒识别和免疫应答中起着极为关键的作用。在静息状态下RLRs的表达量较低, 病毒感染时其表达会被干扰素上调, 是干扰素刺激基因^[31,32]。

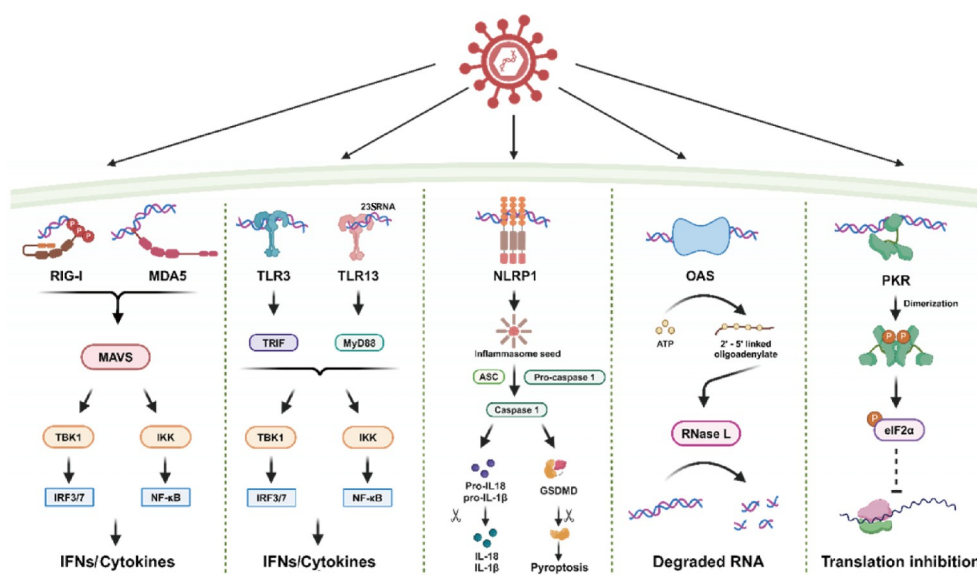


图2 天然免疫中的双链RNA传感器及其信号传导机制

RLRs家族包括三个成员，分别为视黄酸诱导基因蛋白I (retinoic acid-inducible gene I, RIG-I)、黑色素瘤分化相关基因5 (melanoma differentiation-associated protein 5, MDA5) 和遗传学和生理实验室蛋白2 (laboratory of genetics and physiology 2, LGP2)，它们具有类似的结构，均隶属于2型解旋酶超家族成员^[33-35]。RLRs包含特殊的DExD/H-box的RNA解旋酶结构域和C末端结构域。RNA解旋酶结构域能与dsRNA结合并且具有RNA依赖的ATP酶活，C端结构域被称为调节结构域，负责识别并结合病毒RNA^[1,35-37]。有研究表明，RIG-I的C末端结构域为自抑制结构域，可以在没有病毒RNA刺激时处于非活化的状态^[37-39]。MDA5和RIG-I在N端区域均包含两个半胱氨酸酶活化和募集结构域 (caspase activation and recruitment domain, CARD)，该结构域在抗病毒天然免疫应答过程中具有非常重要的信号传递作用^[35]。在识别并结合到病毒的dsRNA之后，RIG-I和MDA5结合到下游同样含有CARD结构域的一个重要接头分子MAVS (mitochondria antiviral signaling protein, 也称IPS-1、VISA、Cardif)^[40-43]，将信号传递给下游的TRAF3、TBK1和IKK ϵ ，进而磷酸化活化IRF3、IRF7使其入核，诱导I型干扰素的产生。除此之外，活化的MAVS还可导致NF- κ B的激活，入核后

促进促炎因子和炎症趋化因子的产生，发挥抗病毒效应(图2)^[33-35,44,45]。由于LGP2缺乏CARD结构域，无法向下游传递信号，近几年的研究表明，LGP2扮演了一个调控者的角色，在RLRs信号通路中发挥正调控或者负调控的功能^[46-48]。

作为dsRNA感受器，MDA5和RIG-I识别不同的dsRNA底物。RIG-I主要识别5'平末端具有三磷酸基团的短dsRNA(5'-PPP)。此外，5'末端具有二磷酸(5'-PP)基团和在2'-O位置未甲基化的5'末端核苷酸的RNA均可以作为RIG-I的有效激动剂^[49-53]。MDA5则倾向于识别较长的dsRNA(>1 kb)，不依赖于dsRNA序列^[54,55]。目前的研究表明，RIG-I和MDA5通过沿着dsRNA形成filament来发挥功能，ATP酶活对于filament的形成具有非常重要的作用^[34,50,51,56-61]。作为一个信号转导的级联反应，该通路能够被多种机制所调控，包括翻译后修饰、蛋白相互作用以及非编码RNA等的调控^[35,62]。有研究表明，E3泛素连接酶Riplet(RING finger protein leading to RIG-I activation)和TRIM65(tripartite motif containing 25)分别在RIG-I和MDA5信号通路中具有不可或缺的功能^[63-67]。此外，短($n \geq 3$)和长($n \geq 8$)的K63连接多聚泛素链(K63-polyUb_n)对于RIG-I和MDA5的CARDs组装也具有重要的作用^[65,68,69]。

3 NLRs

NLRs是另一类定位于细胞质的模式识别受体, 在无脊椎动物和脊椎动物的细胞中都有表达, 目前在人类细胞中共发现22种NLRs^[70,71]。作为一种PRRs, NLRs可识别多种PAMPs, 如细菌的细胞壁成分(肽聚糖、鞭毛蛋白)、微生物分泌毒素、病毒RNA和真菌毒素等。NLRs蛋白的激活会诱导促炎性细胞因子白细胞介素-1(interleukin-1, IL-1)和IL-18的产生, 还会激活丝裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK)诱导细胞焦亡(图2)^[72,73]。

NLRs蛋白家族成员具有相似的结构, 中间为核苷酸结合结构域(central nucleotide-binding oligomerization domain, NACHT), 此结构域负责蛋白质自身的寡聚化并且对ATP依赖的NLRs的激活至关重要; N端为效应结构域, 具有极大的多样性, 此结构域通过与衔接蛋白相互作用向下游信号分子传递信号; C端由富含不同数量的LRRs组成, 参与感知激动剂和配体识别^[70,74,75]。根据N端效应结构域的结构, 可将NLRs分为5个亚家族: NLRA、NLRB、NLRC、NLRP和NLRX^[76-78]。NLRA亚家族N端由酸性反式激活结构域组成, 被认为是主要组织相容性复合体Ⅱ类抗原呈递的转录调节因子^[79]。NLRB亚家族N端由杆状病毒凋亡抑制蛋白重复序列(baculovirus inhibitor of apoptosis repeat, BIR)组成, 参与宿主防御和细胞存活^[77,80]。NLRC亚家族N端由CARD组成, 可与含有CARD结构域的其他蛋白发生相互作用^[76,78]。NLRP亚家族N端由热蛋白结构域(pyrin domain, PYD)组成, 参与炎症小体的组装和激活^[76,78]。NLRX亚家族仅含有一个成员NLRX1(也称为NOD9), 该蛋白质的N端结构域与其他四个亚家族没有显著的同源性, 包含一个线粒体靶向序列相关蛋白, 使蛋白质迁移至线粒体外膜^[81,82]。

NLRP1是首个被发现参与炎症小体和Caspase-1激活的NLRs家族成员, NLRP1的激活会导致IL-1 β 、IL-18的加工和释放以及GSDMD(gasdermin D)所介导的细胞焦亡^[83-85]。有研究表明, NLRP1能够作为一个dsRNA感受器来发挥功能, NLRP1的LRR和NACHT结构域能感知长度>500 bp的

dsRNA, dsRNA结合会诱导ATP水解驱动NLRP1构象变化, 向下游传递信号, 然而其激活的机制细节仍需进一步研究^[86]。目前的研究认为NLRP3能够检测内源性RNA, 细胞产生的内源性dsRNA分子Alu元件能够作为NLRP3的激活剂, 但是其激活的分子机制仍然是未知的^[87]。激活的NLRP3发生寡聚, 随后通过PYD-PYD相互作用激活招募凋亡相关斑点样蛋白(apoptosis-associated speck-like protein containing a CARD, ASC)向下游传递信号^[88,89]。NLRP6也被报道在抗RNA病毒免疫反应中发挥作用^[90]。机制研究表明, NLRP6通过与DHX15(DEAH-box helicase 15)协同作用来识别长dsRNA, 从而通过MAVS诱导干扰素表达并激活ISG来发挥抗病毒作用^[91]。另一项研究表明, NLRP6能够直接与dsRNA结合, 但是无法断定dsRNA是激活NLRP6的直接配体^[92]。此外, 还有研究表明, dsRNA结合会诱导NLRP6的液-液相分离(liquid-liquid phase separation, LLPS), 从而增强炎症小体的信号^[93]。NLRP9b也被鉴定为一种dsRNA感受器, 研究表明, NLRP9b与DHX9(DEXH-box helicase 9)协同作用能够对短dsRNA进行识别, 激活后诱导ASC和caspase的活化向下游传递信号^[92,94]。此外, NLRC2(又称NOD2)也被证明能够感知呼吸道合胞病毒基因组的ssRNA, 随后依赖MAVS激活IRF3抗病毒免疫反应^[95,96]。

4 OLRs

2',5'-寡腺苷酸合成酶(oligoadenylate synthetase, OAS)属于核苷酸转移酶(nucleotidyltransferase, NTase)折叠蛋白的一个高度多样化的大家族, 也是胞质内一种重要的dsRNA感受器, 与环鸟苷酸-腺苷酸合酶(cyclic GMP-AMP synthase, cGAS)具有相同的结构特征, 属于不依赖模版的核苷酸转移酶家族, 通过激活潜伏性核糖核酸酶L(ribonuclease L, RNase L)抑制病毒复制并构建抗病毒免疫反应体系, 在限制病毒感染方面发挥关键作用(图2)^[97,98]。

人类OAS家族由4个亚型组成: OAS1、OAS2、OAS3和OAS样蛋白(oligonucleotide synthase-like protein synthetase, OASL)^[4], OAS1、OAS2、OAS3都具有合成2-5'连接的寡腺苷酸的蛋

白酶活性,能够作为第二信使激活RNase L,而OASL则缺乏这种合成活性^[97,99,100]。OAS1、OAS2、OAS3蛋白彼此具有显著的同源性,分别含有一个、两个、三个串联重复的NTase结构域^[101-104]。尽管OAS2和OAS3含有多个NTase结构域,但是其中只有一个具有催化合成活性,其他则为无活性结构域,这种无活性的结构似乎也具有非常重要的功能^[103,104]。有研究表明,OAS3中第一个无活性结构域能够结合dsRNA,并且推测第二个无活性结构域也能结合dsRNA,从而增强OAS3对dsRNA的亲和力^[105]。其他的研究也表明,OAS3能够比OAS2和OAS1更快地对dsRNA产生应答,并且是病毒入侵期间RNase L的主要上游效应分子,OAS3能够优先识别较长的dsRNA(>50 bp),这也侧面表明无活性结构域发挥着不可或缺的作用^[103,106]。OASL尽管没有催化合成活性,但是研究表明,它也具有抗病毒功能^[107,108]。OASL包含单个无活性的NTase结构域,之后连接着两个串联重复的泛素样结构域^[109,110]。研究表明,OASL能够通过多种机制来调节抗病毒活性,其中最为有趣的是,能够直接结合和激活RIG-I,OASL的泛素样结构域能够取代K63-Ub_n,帮助RIG-I的CARDs形成功能性的四聚体^[107,111]。

结构研究表明,NTase结构域与dsRNA的一侧结合,结合模式与dsRBD相似,dsRNA的结合会诱导其构象发生变化,形成功能性的催化三联体,激活其催化合成的蛋白酶活性,使两个ATP分子能够通过2'-5'键连接,而无活性NTase结构域则缺少催化三联体^[112,113]。2'-5'连接的寡腺苷酸(2-5A_n)是由这种迭代ATP连接产生的,充当第二信使以激活下游效应物RNase L^[102,114]。RNase L是OLRs的下游效应分子,由三个结构域组成:N端的锚蛋白重复结构域、假性激酶结构域和C端的核糖核酸酶结构域,定位于细胞质中,在没有激活的情况下以自抑制的单体形式存在^[114]。2'-5'连接的寡腺苷酸与RNase L结合,活化的RNase L通过降解病毒和细胞的ssRNA发挥抗病毒活性^[106,115]。有研究表明,在病毒感染期间通过RNase L剪切产生的RNA会激活RIG-I,进而诱导I型干扰素和促炎细胞因子的产生发挥抗病毒效应^[116]。

5 蛋白激酶R

蛋白激酶R(protein kinase R, PKR)是一种在细胞内起重要抗病毒作用的酶,是一种dsRNA依赖性的丝氨酸-苏氨酸蛋白激酶,由细胞中的EIF2AK2基因转录表达^[117]。细胞处于静息状态时有低剂量的本底表达,作为一种ISG,干扰素的刺激会诱导PKR的大量表达,表达的PKR在mRNA翻译、调控细胞凋亡和增殖、转录调控等方面发挥着重要的作用^[118]。dsRNA的结合诱导PKR构象发生变化,蛋白二聚并进行自磷酸化,自磷酸化的PKR进一步磷酸化其下游底物蛋白,执行抗病毒功能^[119-121]。

PKR主要由两个功能结构域组成:N端和C端。N端包含两个保守的串联双链RNA结合结构域(double-stranded RNA binding domain, dsRBD),中间由23个氨基酸隔开,主要用于识别和结合病毒感染时产生的dsRNA。C端是激酶结构域(kinase domain, KD),主要负责执行磷酸化功能^[122,123]。dsRBD是一种常见的双链RNA结合蛋白基序,如RNA特异性腺苷脱氨酶(adenosine deaminase action RNA, ADAR)等都具有此结构,具有由 α - β - β - α 构象组成的高度保守的二级结构,能够特异性识别并结合A构象的dsRNA^[124-126]。结构研究表明,dsRBD通过两个相邻小沟的磷酸盐和核糖骨架与dsRNA的一侧结合,以不依赖序列特异性的方式与dsRNA相互作用^[125,127,128]。研究显示,PKR的两个dsRBD在其功能发挥过程中都非常重要。尽管第一个dsRBD被认为是主要负责与短dsRNA结合的结构域,但在与长dsRNA进行高亲和力的相互作用以及dsRNA诱导的激酶结构域激活过程中,两个dsRBD都是必不可少的^[122,129-131]。C端的KD结构域也具有高度的保守性,分为N-lobe和C-lobe两部分,N-lobe具有ATP结合位点,通过将ATP的磷酸基团转移至C-lobe多个位点的苏氨酸处,使PKR完成激活^[120,132]。

早期有研究表明,在没有dsRNA刺激的情况下PKR处于自抑制的状态,dsRNA的结合会诱导PKR自抑制的解除,从而激活其激酶活性。PKR的自抑制主要通过第二个dsRBD与KD结构域的直接相互作用来实现,当dsRBDs结合到病毒感染期间产生的dsRNA时,会释放出KD结构域,使得PKR发生

磷酸化后激活^[133-135]。还有研究表明, 另一种dsRNA结合蛋白PACT能够与PKR的第二个dsRBD相互作用从而导致KD结构域的释放^[136]。然而, 有一些研究对自抑制模型提出了质疑, 指出PKR的二聚化是其激活的关键机制。通过采用生物物理学手段研究发现, 溶液中的PKR处于伸展的单体构象, 这与折叠的自抑制构象不相符^[121,137,138]。dsRBD结构域的删除并不会影响PKR的组成性激活, 用其他能够二聚化的结构域替换dsRBD仍然能够激活PKR^[120]。过量的dsRNA会导致PKR二聚化的程度降低, 也被发现能够降低PKR的活性^[139]。此外, 能够激活的PKR的最小dsRNA长度为33 bp, 正好是容纳两个PKR分子的最短长度, 这也与二聚化激活模型相符^[139]。

当PKR识别并结合dsRNA之后, 会触发自身磷酸化、二聚化, 进入激活状态, 进而磷酸化下游的靶标分子。PKR的主要底物之一是真核翻译起始因子2 α (eukaryotic translation initiation factor 2 α , eIF2 α)^[117,140]。活化的PKR磷酸化eIF2 α 的 α 亚基能够抑制鸟嘌呤核苷酸交换因子eIF2B, 导致eIF2无法转变成能够结合tRNA和核糖体40S亚基的活性构象, 不能有效地参与翻译起始复合物的形成, 从而阻止蛋白质合成(图2)。这种翻译抑制不仅限制了病毒蛋白的合成, 还可以降低宿主细胞蛋白质的合成, 进而引发细胞进入休眠状态, 减少病毒的复制和传播^[141]。除了磷酸化eIF2 α 导致翻译终止之外, PKR后能够磷酸化I κ B, 导致I κ B发生泛素化并被降解, 释放NF- κ B, 促进干扰素产生; 还可以作用于多种促分裂原活化蛋白激酶, 促进细胞分化和发育^[142]; 同时还能够协同鞘氨醇激酶1共同调控细胞在胁迫下的凋亡和存活, 以应对不同程度的胁迫^[143]。

6 其他dsRNA感受器

除了RLR蛋白家族之外, 其他的一些RNA解旋酶也被证明在天然免疫过程中具有非常重要的功能。已有研究表明, 解旋酶ZNF1(zinc finger NFX1-type containing 1)能够在线粒体处与病毒的RNA发生相互作用, 从而促进MAVS介导的I型IFN的产生^[144]。DHX9除了帮助NLRP9b协同识别RNA之外, 有研究还认为, DHX9可以与IR-Alus

相互作用进而与ADAR1协同效应来调控细胞的dsRNA稳态^[145]。DDX60(DEXD/H-box helicase 60)是一种干扰素诱导表达的解旋酶, 能够以组织依赖型的方式协助RLRs对外源dsRNA进行感知, 同时也能够对病毒RNA进行降解, 因此认为DDX60能够以两种不同的方式对病毒入侵进行抵抗^[5]。

hnRNPU(heterogeneous nuclear ribonucleoprotein U)作为一种异质核糖核蛋白, 主要参与RNA加工、剪接和转录调控等功能。然而, 有研究表明, hnRNPU也可以作为核dsRNA感受器, 参与抗病毒免疫反应^[146]。hnRNPU能够识别和结合由HSV-1感染产生的dsRNA, 从而诱导I型IFN的产生。

Dicer是一种关键的RNA酶, 在RNA干扰通路中发挥重要作用。Dicer的主要功能是识别并切割长的dsRNA或前体miRNA, 生成长度为21~25个核苷酸的小RNA分子, 这些小RNA分子在RNAi通路中起作用^[147]。Dicer通过其PAZ结构域识别并结合dsRNA, 有研究表明, Dicer可通过对病毒dsRNA切割来降解病毒RNA, 从而限制病毒复制和传播^[148]; Dicer还可以通过消除RLRs、PKR和OAS的dsRNA配体来抑制dsRNA依赖性的抗病毒免疫。有研究表明, Dicer可以切割Alu RNA并防止Alu RNA过量积累^[149]。

ADAR是一类将dsRNA中的腺苷(A)修饰为肌苷(I)的酶, 主要通过对RNA分子的编辑来调控基因表达和细胞功能。ADAR1已被证明在抗病毒天然免疫中具有重要的作用, ADAR1能够识别和结合病毒感染过程中产生的dsRNA并对其进行A-to-I编辑, 导致病毒RNA的不稳定、功能丧失或产生不正确的蛋白质, 从而限制病毒的复制和传播^[150,151]。ADAR1通过编辑宿主免疫相关基因RNA, 调控天然免疫反应, 如ADAR1可以编辑干扰素诱导基因的mRNA, 调控干扰素的表达和功能, 从而影响抗病毒免疫反应的强度^[152]。此外, ADAR1还能编辑宿主RNA, 调控与免疫反应相关的基因表达, 防止过度的免疫反应, 如ADAR1能够对细胞内的IR-Alu元件进行编辑, 从而抑制Alu元件对MDA5的激活^[153]。

7 dsRNA感受器的过度激活

为了应对入侵机体病毒的复制和传播, 干扰素

的产生需要正确激活dsRNA感受器来触发。先前的研究表明,干扰素信号转导的缺陷显著增加了机体对病毒的易感性,可能会导致较高的致死率;然而持续或者不恰当的干扰素信号激活也会引起自身免疫疾病的发生^[154]。与病毒dsRNA一样,某些条件下细胞产生的内源dsRNA能够被dsRNA感受器识别并激活抗病毒天然免疫信号,而dsRNA感受器的自我激活往往是许多自身免疫性疾病的发病机制。感受器的异常持续激活会导致I型干扰素和其他炎症因子的过度产生,触发慢性炎症和自身免疫反应,导致多系统损伤和功能障碍。

目前认为主要的内源性dsRNA来源为以下几种。(1)转座子激活、某些表观遗传修饰机制的失活以及染色质构象的异变都能够导致转座子的激活:内源性逆转录病毒(endogenous retrovirus, ERV)、长散在核元件(long interspersed nuclear element, LINE)、短散在核元件(short interspersed nuclear element, SINE, 如Alu元件)和长末端重复序列(long terminal repeat, LTR)等;(2)非编码RNA产生:小核RNA(small nuclear RNA, snRNA)可以被RLR和TLR所识别,小核仁RNA(small nucleolar RNA, snoRNA)可以作为PKR的配体;(3)RNA修饰的失调:通过RNA修饰可影响其二级结构并降低其免疫原性,如ADAR1对dsRNA的修饰能够避免其被MDA5、PKR等感受器所识别,m⁶A修饰的缺失能够dsRNA的形成从而激活感受器;(4)RNA未正确加工和降解:加工和降解途径的停止可能导致免疫刺激性dsRNA在细胞内积累,如Dicer功能的缺失导致逆转录元件转录本的积累,SKIV2L的缺陷能够导致RIG-I的激活;线粒体RNA泄露;(5)线粒体dsRNA会受到多核苷酸磷酸化酶(polynucleotide phosphorylase, PNPase)和解旋酶SUV3等的调控,线粒体的失调会导致dsRNA泄漏到细胞中,有研究表明,泄漏的线粒体RNA能够激活RNA感受器TLR7、RIG-I和MDA5^[4,155-159]。

过度激活的I型干扰素反应、内源性dsRNA的累积、dsRNA感受器的基因突变以及机体信号传导和反馈机制的失调都可能导致全身性自身炎症和各种自身免疫疾病的发生,包括AGS、SLE和辛格顿-莫顿综合征(singleton-Merten syndrome,

SMS)等。其中,AGS是一种炎症性疾病,严重影响患者的大脑和皮肤,通常表现为严重的智力障碍、脑白质病变、大脑皮层钙化、冻疮样皮肤病变和肌张力障碍等^[160]。有研究发现,多个基因的单核苷酸多态性(single nucleotide polymorphism, SNP)与AGS密切相关,包括*TREX1*、*SAMHD1*、*ADAR1*、*RNASEH2A*、*RNASEH2B*和*RNASEH2C*等9种不同基因,这些基因的突变使细胞内DNA和RNA的降解和处理异常,导致内源性核酸的积累,导致I型干扰素的持续高水平表达^[161,162]。SLE则是一种慢性、自身免疫性疾病,影响多个器官和系统。SLE的症状多样,病情波动,常见的表现包括皮肤、关节、肾脏、心脏、肺、血液和神经系统的受累^[163]。多种基因与SLE的易感性相关,包括*HLA-DR2*、*HLA-DR3*、*IRF5*、*STAT4*、*TNFAIP3*、*PTPN22*等,这些基因的变异能够影响免疫系统的功能和调节。此外,SLE在某些家族中更为常见,提示遗传因素在SLE发病中起重要作用^[164]。研究表明,IFN是参与炎症通路的重要细胞因子,在SLE中发挥重要的致病作用,其中IFN α 是SLE的重要治疗靶点^[165]。SMS是一种罕见的遗传性疾病,具有高度变异的临床表现,通常包括骨骼异常、牙齿异常和心血管疾病。SMS的发病机制主要涉及遗传突变和免疫系统异常^[166]。研究表明,*IFIH1*基因的突变可以导致MDA5的过度活化,持续激活I型干扰素反应,导致慢性炎症和组织损伤^[167]。在SMS患者中发现了RIG-I的错义突变(E373A),此外,对100例先天性青光眼患者*RIG-I*基因的进一步分析发现了另一种突变(C268F),然而目前关于这些RIG-I突变体的组成性激活和对dsRNA的反应性尚未得到表征^[168]。

8 总结与展望

本文全面回顾了天然免疫系统中识别dsRNA的感受器及其在抗病毒免疫中的作用,主要聚焦于几种重要的dsRNA感受器,包括TLRs、RLRs、NLRs、OLRs和PKR,并介绍了这些蛋白家族的结构功能、配体种类以及信号转导途径。在识别并结合病毒dsRNA后,这些感受器能够启动一系列免疫反应,诱导干扰素和促炎细胞因子的产生,增强机体的抗病毒能力。然而,过度或错误激活

也可能导致免疫功能紊乱和自身免疫疾病的发生, 如系统性红斑狼疮和Aicardi-Goutières综合征。尽管已经有大量的研究增强了我们对dsRNA感受器的理解, 但是仍然有许多问题需要我们去进一步去探索, 如dsRNA感受器如何精确区分自我和非我的RNA; 不同的dsRNA感受器信号通路中已经鉴定出多种调控因子, 这些调控因子如何精确协调信号转导及其在疾病中的潜在功能角色仍然未知; 此外, 病毒如何通过进化出多种策略来逃避宿主的dsRNA感受器识别和免疫反应。理解这些逃避机制有助于开发更有效的抗病毒疗法。

未来的研究可以在以下几个方面进行扩展。

(1)深入理解dsRNA感受器的分子机制。尽管已有许多研究揭示了感受器的基本功能, 但很多RNA底物识别及信号传递过程仍然不清楚, 如RLRs家族感受器的ATP酶活具体执行了什么功能? RLRs调控蛋白具体影响了底物识别还是信号传递? PKR如何在众多“自我”RNA中特异地被病毒RNA所激活等。(2)探索dsRNA感受器在不同疾病中的角色。进一步研究dsRNA感受器在不同病毒感染和自身免疫疾病中的作用, 将有助于针对不同疾病开发特定的治疗策略, 如通过设计RNA适配体可以抑制PKR激活, 在未来有望缓解SLE, 改善自身免疫疾病患者的生活质量。(3)开发新型抗病毒疗法。基于dsRNA感受器的作用机制, 开发能够特异性激活或抑制这些受体的新型抗病毒药物和疫苗, 有望显著提高抗病毒治疗的效果, 并减轻因免疫系统紊乱引发的疾病负担。

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