

NOD2介导的信号通路及其与自身炎症性疾病关系以及抑制剂研究进展

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摘要: 核苷酸结合寡聚化结构域蛋白2 (nucleotide-binding oligomerization domain containing 2, NOD2) 是一类胞浆内模式识别受体, 其被激活后通过一系列信号级联转导, 诱导炎症因子释放, 从而在固有免疫应答中发挥重要作用。NOD2 信号通路异常涉及多种疾病的发生和发展, 尤其是其基因的遗传多态性与自身炎症性疾病 (autoinflammatory diseases, AIDs) 密切相关。因此, 靶向NOD2通路的抑制剂在炎症免疫性疾病治疗中具有巨大潜力。基于此, 本文对NOD2受体介导的信号转导通路及其调节机制, NOD2与AIDs的关系以及NOD2通路抑制剂研究的最新进展进行综述。

关键词: 核苷酸结合寡聚化结构域蛋白2; 受体相互作用蛋白2; 自身炎症性疾病; 抑制剂; 自噬; 内质网应激
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Research progress of NOD2-mediated signaling pathways and relationship with autoinflammatory diseases and its inhibitors

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Abstract: Nucleotide-binding oligomerization domain containing protein 2 (NOD2) is a member of intracellular pattern recognition receptor. After being activated, it will induce the release of inflammatory factors through a series of signal cascade transduction, thus playing an important role in the innate immune response. The abnormal NOD2 signaling pathway is involved in the occurrence and development of many diseases, especially the single nucleotide polymorphisms (SNPs) of the *NOD2* gene have been identified to be closely associated with autoinflammatory diseases (AIDs). Therefore, inhibitors targeting NOD2 pathway have great potential in the treatment of inflammatory immune diseases. This review presents the recent progress of NOD2 receptor-mediated signal transduction pathways and its regulation mechanisms, the relationship between NOD2 and AIDs, and the inhibitors of NOD2 pathway.

Key words: nucleotide-binding oligomerization domain containing 2; receptor-interacting protein 2; autoinflammatory disease; inhibitor; autophagy; endoplasmic reticulum stress

核苷酸结合寡聚化结构域蛋白2 (nucleotide-binding oligomerization domain containing 2, NOD2), 也称为半

胱天冬酶募集结构域15 (caspase recruitment domain-containing protein 15, CARD15), 是一种细胞内模式识别受体 (pattern recognition receptors, PRRs), 在固有免疫应答中发挥重要作用^[1,2]。自2001年被发现以来^[1], *NOD2*单核苷酸多态性 (single nucleotide polymorphism, SNP) 首先被报道与克罗恩病 (Crohn's disease, CD) 的

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发生密切相关^[3-5]。随着研究的不断深入,发现NOD2在多种炎症免疫性疾病中发挥重要作用^[6]。本文对NOD2受体介导的信号转导通路及其调节机制、NOD2与自身炎症性疾病 (autoinflammatory diseases, AIDs) 的关系以及抑制剂研究的最新进展进行综述。

1 NOD2受体简介

NOD2属于NOD样受体 (NOD-like receptors, NLRs) 家族,其编码基因位于人染色体16q12上,含有12个具有编码功能的外显子^[7]。编码的NOD2蛋白含有1 040个氨基酸,分为3个结构域:碳端为富含亮氨酸重复序列 (leucine-rich repeat, LRR),主要用于配体识别;中间的核苷酸结合结构域 (nucleotide-binding domain, NBD, 也称为NACHT),含有ATP/GTP酶特异性P环 (Walker A基序) 和Mg²⁺结合区 (Walker B基序),与依赖于ATP的自身寡聚化反应相关;氨基端的半胱天冬酶募集结构域 (caspase recruitment domain, CARD) 是信号传递位点,可与效应分子通过蛋白质-蛋白质相互作用将信号往下游转导^[8,9] (图1)。

NLRs包括NLRA、NLRB、NLRC、NLRP和NLRX 5个亚家族。NOD1和NOD2是NLRC亚家族的主要代表,二者的结构基本相同,区别在于NOD1氨基端有一个CARD结构域,而NOD2则含有两个CARD结构域^[2]。此外,NOD1在各种组织细胞中广泛表达,主要识别细菌肽聚糖 (peptide glycan, PGN) 中的 γ -D-谷氨酰基-二氨基庚二酸 (γ -D-glutamyl-meso-diaminopimelic acid, iE-DAP); NOD2主要在单核细胞、巨噬细胞和树突状细胞 (dendritic cells, DCs) 中表达,识别细菌PGN中的胞壁酰二肽 (muramyl dipeptide, MDP)。最新研究报道,N-乙酰氨基葡萄糖激酶 (N-acetylglucos-

amine kinase, NAGK) 磷酸化MDP C6位的羟基产生的6-O-磷酸化-MDP是NOD2识别的关键,在敲除NAGK的巨噬细胞中,NOD2不能识别MDP^[10]。此外,NOD2还可以识别其他类型的配体,如病毒单链RNA (single-stranded ribonucleic acid, ssRNA)^[6,8,11,12],以及像其他NLR一样,对危险信号或损伤相关分子模式 (damage-associated molecular patterns, DAMPs),如ATP或尿酸等作出反应,从而响应内源性应激信号^[13]。

2 NOD2受体介导的信号转导通路

2.1 细菌入侵的信号转导通路

在无配体存在时,LRR结构域折叠到NBD结构域和CARD结构域上,使NOD2处于“自抑制”的无活性单体形式^[14]。NOD2还通过与热休克蛋白 (heat shock protein, HSP) 70或HSP90结合,进一步稳定避免降解^[15]。当LRR结构域识别MDP后,NOD2构象改变,通过NBD结构域和CARD结构域发生自身寡聚化,寡聚化的NOD2通过CARD-CARD同源蛋白相互作用招募受体相互作用蛋白2 (receptor-interacting protein 2, RIP2, 又称RIPK2或RICK,其结构包括CARD结构域、激酶结构域和中央区域),从而形成异质性CARD复合物,RIP2持续结合在CARD复合物上形成了螺旋丝状结构的RIP2聚合体,RIP2聚合体的形成对于NOD2依赖的NF- κ B活化是必需的^[16,17]。随后,E3泛素连接酶使RIP2发生K63泛素化和M1泛素化,K63多聚泛素链招募转化生长因子激活激酶1 (transforming growth factor β -activated kinase 1, TAK1)、TAK结合蛋白 (TAK-binding proteins, TAB) 1和TAB2/3复合体至RIP2的激酶结构域,M1多聚泛素链通过与NF- κ B必需调节蛋白 (NF- κ B essential modulator, NEMO, 又称

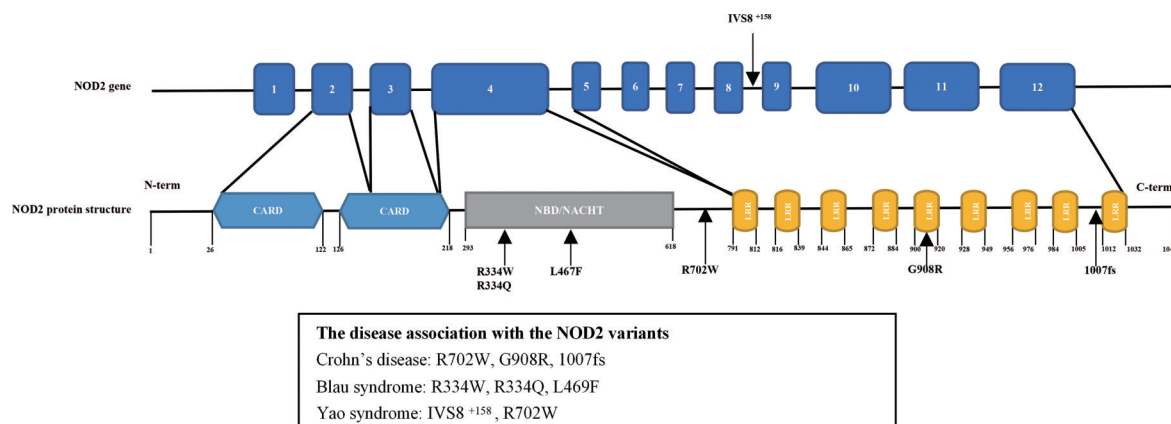


Figure 1 Schematic representation of nucleotide-binding oligomerization domain containing 2 (NOD2) gene and protein structures. The numbers over the diagram are the first and last amino acids of each structural domain or motif. The NOD2 gene mutations are categorically located: NBD/NACHT (R334Q, R334W, L469F), LRRs (G908R and 1007fs), and in between LRRs and NBD (R702W). The NOD2 protein comprises 3 domains: 2 CARDs, NBD, and 9 LRRs. CARD: Caspase recruitment domains; NBD: Nucleotide-binding domain; LRR: Leucine-rich repeat. Data from UniProt, <https://www.uniprot.org/>

IKK γ)的相互作用将IKK复合体募集至RIP2的中央区域。空间的接近促使TAK1磷酸化并活化IKK β , NF- κ B抑制蛋白(inhibitor of NF- κ B, I κ B)被IKK β 磷酸化,继而经泛素化被蛋白酶体降解,释放出的NF- κ B二聚体由胞浆易位到细胞核,诱导目标基因转录和表达^[18]。TAK1亦能够磷酸化MEK4、MEK6和MEK7,继而激活p38、c-Jun氨基末端激酶(c-Jun N-terminal kinase, JNK)和细胞外调节蛋白激酶(extracellular regulated protein kinases, ERK),它们可进一步活化AP-1转录因子^[19-21]。胱天蛋白酶募集域蛋白9(caspase recruitment domain-containing protein 9, CARD9)被报道通过募集到NOD2和RIP2上,在p38和JNK活化中发挥重要作用^[22]。综上,MDP通过NOD2-RIP2-TAK1通路激活NF- κ B和MAPK,从而促进炎症反应发生(图2)。

2.2 病毒入侵的信号转导通路

NOD2通过诱导I型干扰素(interferon, IFN)亦在抗病毒免疫中发挥作用。NOD2被进入细胞内的病毒ssRNA激活后转位至线粒体,通过其CARD和NBD结构域与线粒体抗病毒信号蛋白(mitochondrial antiviral signaling protein, MAVS)相互作用,随后以TNF受体作用因子3(TNF receptor associated factor 3, TRAF3)依赖的方式激活干扰素调节因子3(interferon regulatory factor 3, IRF3),诱导I型IFN产生^[11]。此外,NOD2可感知双链DNA病毒入侵,敲除/过表达NOD2的实验证实巨细胞病毒(cytomegalovirus, CMV)感染细胞后可通过NOD2通路诱导NF- κ B活化及IFN- β 产生,从而限制CMV复制^[23]。MDP可通过TBK1/IRF3/7通路增强CMV诱导的IFN- β 产生^[24](图2)。

2.3 NOD2介导的自噬

NOD2在入侵细菌的自噬反应中发挥重要作用。细菌成分侵入细胞后,NOD2识别MDP而活化,继而将自噬相关16样蛋白1(autophagy-related 16 like 1, ATG16L1)募集到细菌侵入位点的细胞膜上,触发自噬,将胞内细菌隔离到自噬体中,促进其后续清除。突变的NOD2则不能完成上述过程,导致细菌自噬性清除受损^[25]。NOD2诱导的自噬通路不依赖于NF- κ B通路的活化,但是否涉及RIP2尚有争议,有研究证实RIP2参与NOD2介导的自噬^[26],亦有研究发现在RIP2缺失的情况下仍可发生自噬^[25]。NOD2和ATG16L1基因多态性与CD相关,这导致CD中自噬缺陷和细菌清除降低。此外,DCs中的细菌处理及抗原提呈需要NOD2介导的自噬^[26],上述过程在NOD2和ATG16L1突变的CD患者DCs中受阻^[27](图2)。

2.4 NOD2与ERS

2.4.1 NOD2诱导ERS 各种生理或病理因素可干扰

内质网(endoplasmic reticulum, ER)稳态,导致ER应激(endoplasmic reticulum stress, ERS)。ERS可通过ER膜上的蛋白激酶RNA样内质网激酶(protein kinase RNA like ER kinase, PERK)、肌醇需求酶1 α (inositol-requiring enzyme-1 α , IRE1 α)和活化转录因子6(activating transcription factor 6, ATF6)等3个跨膜受体触发未折叠蛋白反应(unfolded protein response, UPR)^[28]。研究发现,在人单核细胞来源的巨噬细胞中,MDP激活NOD2后促使含漆酶结构域1(laccase domain containing-1, LACC1)定位于ER, LACC1可分别与PERK、IRE1 α 和ATF6相互作用并将它们活化,从而促进UPR。而IRE1 α 、PERK和ATF6的活化又可促进NOD2介导的NF- κ B和MAPK活化、细胞因子产生及病原体清除^[29]。

2.4.2 ERS激活NOD2

NOD2在UPR介导的炎症通路中亦发挥重要作用^[28]。活化的IRE1 α 可将肿瘤坏死因子受体相关因子2(tumor necrosis factor receptor associated factor 2, TRAF2)招募至ER膜,通过NF- κ B和JNK途径启动炎症反应^[30]。此外,IRE1 α /TRAF2复合物还可直接活化NOD2,此过程需要RIP2。病原体(流产布鲁氏菌和衣原体)及ERS的化学诱导剂(毒胡萝卜素和二硫苏糖醇)可通过激活上述IRE1 α -TRAF2-NOD2-RIP2通路促进细胞因子IL-6的产生,提示ERS通过不依赖PGN的方式活化NOD2,但具体机制尚不清楚^[31]。然而,另一项研究认为毒胡萝卜素引起的NOD2活化是通过增加胞浆Ca²⁺水平实现的,细胞内Ca²⁺增加导致细胞培养基或血清中含有的微量PGN被内吞,从而活化NOD2;不影响细胞内Ca²⁺水平的ER诱导剂,如衣霉素等,则不能活化NOD2。因此,该研究认为ERS本身不能直接激活NOD2,需要通过PGN依赖的方式^[32]。但该研究并没有评价该微量PGN是否可以充分活化NOD2,因此得出此结论尚需进一步探索。

2.5 NOD2信号通路的调控

2.5.1 NOD2的膜定位

虽然NOD2属于胞浆内受体,但其活化与细胞膜、吞噬体膜和核内体膜的定位有关,推测可能是将NOD2输送到这些配体进入的位点^[33]。NOD2如何定位于细胞膜尚不清楚, LRR结构域对其细胞膜定位是必需的,该结构域的突变导致NOD2定位于胞浆。此外,NOD2的细胞膜定位可能涉及与肌动蛋白细胞骨架的相互作用^[34]。研究发现,NOD2与细胞膜上的波形蛋白共定位;与调节细胞骨架的Rho GTPases相互作用;还能够与肠上皮细胞基底外侧膜上的FRMPD2和Erbin结合,它们分别是NOD2信号通路的正向和负向调节因子。最近发现

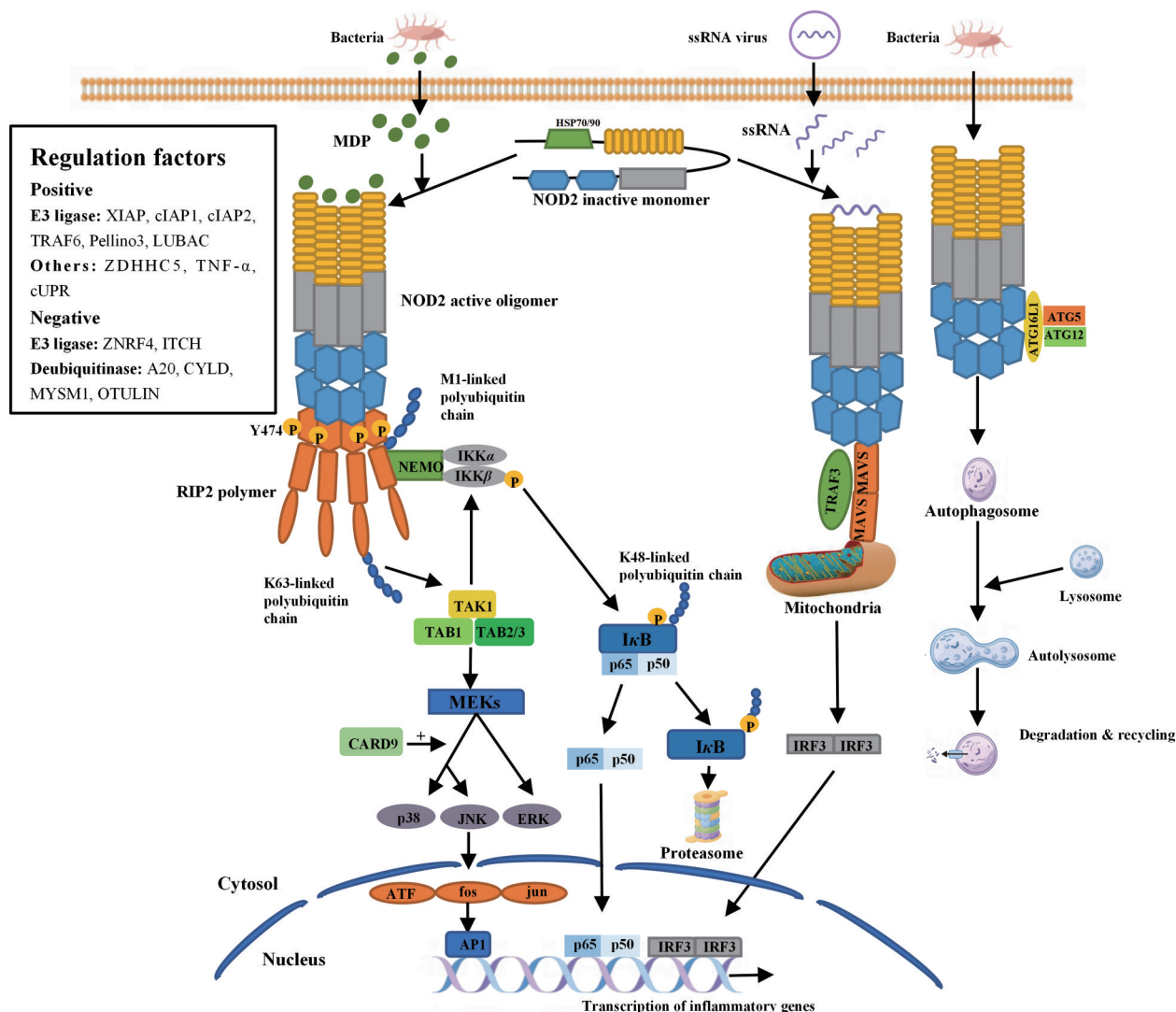


Figure 2 NOD2-mediated antibacterial and antiviral signalling pathways and the regulation. Bacterial MDP is recognized by the cytosolic receptor NOD2. Once activated, NOD2 oligomerises and then recruits RIP2 *via* CARD-CARD interactions, resulting in hetero-CARD complex formation. Cumulative binding of RIP2 to the hetero-CARD complex promotes filament elongation to form the helical assembly. Then RIP2 is polyubiquitinated by E3 ubiquitin ligases, resulting the recruitment of TAK1: TAB complex and the activation of NF- κ B and MAPKs. They translocate to the nucleus and transactivate target genes. In addition, NOD2 is also activated by sensing of virus-derived ssRNA. Binding of NOD2/TRAF3 to MAVS induces activation of IRF3 which induces IFN- β gene expression. CARD9 plays a critical role in NOD2-mediated p38 and JNK activation. At the bacterial entry site on the plasma membrane, NOD2 can recruit the autophagy protein ATG16L1, leading to degradation of intracellular pathogens. The above pathway is regulated either positively or negatively by multiple events, such as membrane localization, phosphorylation and polyubiquitination. MDP: Muramyl dipeptide; RIP2: Receptor-interacting protein 2; TAK1: Transforming growth factor β -activated kinase 1; TAB: TAK-binding protein; NF- κ B: Nuclear factor-kappaB; MAPK: Mitogen-activated protein kinase; ssRNA: Single-stranded ribonucleic acid; TRAF3: TNF receptor associated factor 3; MAVS: Mitochondrial antiviral signaling protein; IRF3: Interferon regulatory factor 3; JNK: c-Jun N-terminal kinase; ERK: Extracellular regulated protein kinases; ATG16L1: Autophagy-related 16 like 1; XIAP: X-linked inhibitor of apoptosis protein; cIAP: Cellular inhibitors of apoptosis proteins; TRAF6: TNF receptor associated factor 6; LUBAC: Linear ubiquitin chain assembly complex; ZDHHC5: Zinc finger DHHC-type palmitoyltransferase 5; cUPR: Cytosolic unfolded protein response; ZNRF4: Zinc and ring finger 4; MYSM1: Myb like, SWIRM and MPN domains 1; OTULIN: OTU deubiquitinase with linear linkage specificity

NOD2的膜定位以及对PGN的免疫应答需要棕榈酰转移酶ZDHHC5介导的NOD2棕榈酰化修饰^[33]。

2.5.2 RIP2的磷酸化 RIP2是NOD2信号通路的重

要成员, 激酶组分析发现了RIP2的多个磷酸化位点, 多位于暴露在外的可变形区域, 包括S168、S176、S363、Y381、S393、Y474、Y520、S527、S529、S531、S539

等^[15]。其中Y474的磷酸化对NOD2信号通路转导具有重要作用,RIP2不仅具有丝氨酸/苏氨酸蛋白激酶活性,还具有酪氨酸激酶活性。MDP激活NOD2后,RIP2的Y474发生自磷酸化,该位点磷酸化被抑制可降低下游NF- κ B活性和细胞因子产生^[35]。

2.5.3 RIP2的泛素化 RIP2泛素化是下游信号传导的关键因素,TAK1、TAB1和TAB2/3复合体的募集以及NF- κ B活化依赖于K209位点的泛素化^[36]。凋亡抑制蛋白(inhibitor of apoptosis proteins, IAPs)家族的XIAP、cIAP1和cIAP2,以及TRAF6、Pellino3是参与RIP2 K63泛素化的E3连接酶,线性泛素链组装复合物(linear ubiquitin chain assembly complex, LUBAC)则介导RIP2的线性泛素化,它们是NOD2信号的正向调节因子^[37]。尤其XIAP对NOD2信号是必需的,XIAP缺失导致RIP2泛素化被抑制、LUBAC募集受阻以及炎症信号减弱和细菌清除不足,XIAP基因突变可引起极早发型炎症性肠病^[38]。

最近亦发现负向调节NOD2-NF- κ B通路的E3连接酶:ZNRK4诱导RIP2的K48泛素化,促进其降解^[39];当RIP2的Y474发生磷酸化及NF- κ B激活后,痒E3泛素蛋白连接酶(itchy E3 ubiquitin ligase, ITCH)泛素化RIP2从而抑制NF- κ B活化和细胞因子产生^[40]。ITCH^{-/-}巨噬细胞因缺乏对NOD2信号的负反馈调节作用,在MDP刺激后显示增加的NF- κ B活性和细胞因子水平。此外,去泛素化酶,包括A20、OTULIN、CYLD和MYSM1,通过介导RIP2的去泛素化负调控NOD2信号转导^[41-43]。

2.5.4 其他调控分子和机制 NOD2等细胞内固有免疫受体被激活后会组装成大的蛋白复合物,称之为信号小体(signalsome)。近期研究发现,NOD2信号小体的组装以及下游NF- κ B通路的活化受到由HRI(heme-regulated inhibitor)-eIF2 α (eukaryotic initiation factor 2 α)-HSPB8构成的细胞内UPR(cytosolic UPR, cUPR)的调控^[44]。此外,TNF- α 被证实是NOD2通路正向调节因子,包括上调MDP诱导的NOD2、RIP2、CXC趋化因子和促炎细胞因子的mRNA和蛋白表达水平,以及MAPK和NF- κ B的活化^[45]。

3 NOD2与AIDs

NOD2信号通路异常涉及多种疾病的发生和发展,包括2型糖尿病、炎症肠病、肿瘤、动脉粥样硬化和哮喘等。而NOD2基因的遗传多态性更是与AIDs密切相关^[46](表1)。AIDs是在1999年被定义的一组由于基因突变致其编码蛋白改变,固有免疫失调,最终导致机体出现全身或器官炎症反应的疾病,其主要特征是反复或持续炎症^[47]。与自身免疫性疾病不同,AIDs缺乏适应性免疫系统的主要致病作用(自身反应性T细胞

或自身抗体产生)^[48]。NOD2相关的AIDs包括Blau综合征(Blau syndrome, BS)、要氏综合征(Yao syndrome, YAOS)和CD。根据遗传方式不同,BS和YAOS属于单基因AIDs,CD属于多基因AIDs。

Table 1 Causal genetic variants of NOD2 that are associated with autoinflammatory diseases (AIDs). Source: <https://infefers.umai-montpellier.fr/web/>. BS: Blau syndrome; YAOS: Yao syndrome; CD: Crohn's disease

AID	Number	Common NOD2 variant
BS	38	R334W, R334Q, E383K, E383G, G464W, L469F, W490L
YAOS	2	IVS8 ⁺¹⁵⁸ , R702W
CD	85	R702W, G908R, 1007fs, R373C, A611A, L348V, A432V

3.1 NOD2与BS

BS是一种罕见的以非干酪样坏死性肉芽肿炎症反应为主要特点的AID,由Blau在1985年首次报道而得名。BS一般在4岁前发病,典型临床症状为皮炎、关节炎和葡萄膜炎三联征,进行性加重,可能导致严重并发症,如关节破坏和失明等^[49,50]。BS呈常染色体显性遗传,偶以散发形式出现,称为早发性结节病(early-onset sarcoidosis, EOS)^[51]。致病基因为NOD2,目前已报道的突变位点均位于NBD结构域的外显子4,包括R334W、R334Q、E383K、E383G、G464W、L469F、W490L、C495Y、H496L、M513R、M513T、R587C、T605N和N670K等^[52],其中R334W和R334Q位点突变最常见,在60%~80%的患者中可以检测到^[53]。上述位点突变导致BS的确切机制尚未完全阐明,细胞转染实验结果表明BS中NOD2基因突变是功能获得性(gain-of-function)的,可能是由于NBD结构域涉及NOD2寡聚化,该部位的突变导致寡聚化阈值降低,在无MDP刺激或轻微刺激时亦可致NF- κ B激活及大量促炎细胞因子释放^[8,54,55]。然而,NOD2^{R314Q}(对应R334Q突变)突变转基因小鼠模型并没有出现BS患者类似的炎症性症状,而且在腹腔注射MDP后,小鼠骨髓来源巨噬细胞(bone marrow-derived macrophage, BMDM)中NF- κ B和MAPK通路的活化以及产生IL-6的水平均较野生型小鼠BMDM明显降低^[7]。最近在HEK293T细胞的转染实验也发现,BS相关的R314W突变使NOD2寡聚化减少,R334W和R334Q突变降低MDP诱导的NOD2-RIP2相互作用、RIP2的磷酸化和泛素化以及NF- κ B活化^[56]。因此,BS中NOD2基因突变的致病机制有待进一步深入探究。

3.2 NOD2与YAOS

YAOS是一种在2011年被首次报道的全身性

AID^[57], 既往称作 NOD2 相关 AID (NOD2-associated autoinflammatory disease, NAID)。主要临床表现包括周期性发热、皮炎、关节炎、关节痛、非特异性胃肠道症状、下身浮肿及浆膜炎。相较 BS, YAOS 均为成年后发病, 散发病例, 女性多见, 几乎所有患者均为内含子 8 的 IVS8⁺¹⁵⁸ 突变, 约 20% 患者同时携带 IVS8⁺¹⁵⁸ 和 R702W (位于 NBD 和 LRR 之间的区域) 单倍体突变, 其他突变位点亦有报道, 但发生率较低^[5,58,59]。由于 YAOS 是一种新近认识的 AID, 关于 NOD2 基因突变在其发病中的确切作用并不十分清楚。仅有的研究发现, 在 IVS8⁺¹⁵⁸NOD2 突变的 YAOS 患者外周血单个核细胞 (peripheral blood mononuclear cell, PBMC) 中, NOD2 转录本内含子 8 的剪接并未受影响, 但 p38 MAPK 活性和 IL-6 分泌水平均较健康对照 (healthy control, HC) PBMC 显著升高, IL-6 拮抗剂托珠单抗 (tocilizumab) 可显著改善患者的临床症状。然而, IVS8⁺¹⁵⁸/R702W 单倍体突变的 YAOS 患者 PBMC 在 MDP 刺激后, p-p65 和 TNF- α 水平较 MDP 刺激的 HC PBMC 是降低的, 提示 YAOS 患者的 R702W 突变与 CD 患者的 R702W 突变类似, 是功能缺失性 (loss-of-function) 突变^[59]。此外, 鉴于 IVS8⁺¹⁵⁸NOD2 突变亦可出现在 HC 中, 推测其可能是疾病的遗传易感因素, 存在未知的致病机制。

3.3 NOD2 与 CD

CD 是一种慢性炎症肉芽肿性胃肠道疾病, 病变呈节段性分布, 多位于回肠末端和邻近结肠, 但也可累及胃肠道各部位, 临床表现为腹痛、腹泻、体重减轻和疲劳。CD 属于复杂遗传性 AID^[60], 发病涉及遗传因素、环境因素、免疫因素和肠道微生物因素等。NOD2 是第 1 个发现与 CD 相关的基因^[3-5], 迄今约 85 个 NOD2 SNP 与 CD 发病相关 (<https://infervers.umai-montpellier.fr/web/>)。其中, 最主要的是 R702W、G908R 和 1007fs, 在高加索人中约占 82%^[61]。研究发现, 具有其中一种杂合突变的个体患 CD 的风险增加 2~4 倍, 而在携带纯合或复合杂合变异的个体中, 这种风险增加 20~40 倍^[8,61]。然而, 这 3 种 SNP 与 CD 的相关性具有地域和种族差异, 在中国、日本和韩国等亚洲国家人群中未发现它们与 CD 发病相关^[62], P268S 可能是与我国 CD 患者具有相关性的 NOD2 SNP, 且与患者的发病年龄、病变部位及严重程度相关^[63]。

对 NOD2 突变与 CD 发病的研究有助于揭示 NOD2 在肠道中的作用以及深入阐明 CD 的病理机制。有关 3 种主要突变 (R702W、G908R 和 1007fs) 与 CD 的关系开展了广泛研究, 目前提出了几种机制: 一方面, 因为它们位于 LRR 结构域内或附近, 所以被认为会影

响 MDP 的识别, 产生功能缺失性表型, 包括减少上皮细胞中 ATG16L1 募集和自噬以及 ROS 的产生, 抑制 Paneth 细胞中 NF- κ B 通路活化和抗菌肽产生等, 从而导致细菌清除受阻和黏膜屏障功能减弱; 另外, NOD2 作为 TLR2 通路的负调控因子, 它的突变减弱了肠道巨噬细胞和 DC 中对 TLR2 的抑制作用, 导致 IL-12 等促炎细胞因子产生增加, TLR2 介导的 Th1 炎症反应失调; 此外, NOD2 与杯状细胞数量和黏液分泌、干细胞存活、肠道微生态等有关, 突变对上述功能的削弱亦可促进 CD 的发生^[6,8]。

4 NOD2 信号通路抑制剂研究进展

调节固有免疫应答靶点是开发自身免疫和慢性炎症性疾病治疗药物的重要策略之一, 尤其 NOD2 可直接激活导致促炎细胞因子产生增加的多条炎症通路, 因此靶向 NOD2 通路的抑制剂在炎症免疫性疾病治疗中具有巨大潜力。

4.1 NOD2 抑制剂

姜黄素和小白菊内酯可以抑制 MDP 诱导的 NOD2 受体寡聚化及随后的信号活化, 而对 RIP2 介导的活化通路无影响, 说明它们在 NOD2 受体层面发挥作用^[64]。含铬化合物 [芳烃 Cr(CO)₃ 配合物] 可以特异性减少 NOD2 介导的 NF- κ B 活化和炎症反应, 而对 TLR2、TLR4 或 TNF- α 介导的 NF- κ B 无影响, 由于此类化合物的数量有限, 尚不能充分探讨结构活性关系 (structure-activity relationship, SAR)^[65]。上述研究均在细胞水平开展, 需要进一步在动物水平评价它们对 NOD2 相关疾病的疗效。

4.2 RIP2 抑制剂

4.2.1 RIP2 激酶活性的抑制剂 RIP2 是 NOD2 信号通路的关键分子, 靶向 RIP2 的抑制剂是研究的重点方向^[66]。RIP2 具有酪氨酸激酶活性, 因此筛选出能够抑制其酪氨酸激酶活性的小分子抑制剂, 包括吉非替尼和厄洛替尼。它们能够抑制 RIP2 Y474 磷酸化以及细胞因子释放, 且此影响不依赖于对 EGFR 的作用^[28], 吉非替尼亦改善 MDP 诱导的小鼠腹膜炎模型^[67]。随后的研究发现 II 型抑制剂对 RIP2 的抑制作用明显强于吉非替尼等 I 型抑制剂, 其中普纳替尼作用最强, 提示 II 型抑制剂可用于治疗 NOD2 通路异常活化导致的相关疾病^[28]。此外, 新型化合物 OD36 和 OD38 在体外生化分析和细胞水平上均可抑制 RIP2 的酪氨酸激酶活性, 同时可在体内动物模型中有效减少 RIP2 介导的作用^[67]。p38 抑制剂 SB203580 亦被报道可以抑制 RIP2 的活性并改善炎症肠病 (inflammatory bowel diseases, IBD) 动物模型^[67,68]。

然而上述抑制剂对 RIP2 的选择特异性不足, 因此

合成了一种高效、选择性 RIP2 激酶抑制剂 GSK583。在人单核细胞中, GSK583 能够显著抑制 MDP 诱导的 TNF- α 产生 (IC_{50} 为 $8 \text{ nmol}\cdot\text{L}^{-1}$), 而对 TLR2、TLR4、TLR7、IL-1R 和 TNFR 介导的细胞因子产生无影响^[69]。此外, GSK583 还能改善静脉注射及腹腔注射 L18-MDP 的小鼠模型, 且在 IBD 患者肠黏膜组织的离体培养实验中, GSK583 以浓度依赖性方式抑制 TNF- α 和 IL-6 的产生^[69]。然而, GSK583 对 hERG 通道的抑制以及不理想的药代动力学特征影响其作为候选药物的开发, 仅作为研究的工具药。对 GSK583 进行结构优化, 消除后口袋苯并咪唑的芳香环, 并在 C7 位引入甲氧基后获得的新化合物与 RIP2 ATP 结合口袋的亲和力以及对 NOD2 通路的抑制作用显著增强, 同时对 hERG 通道的影响明显降低^[70]。

4.2.2 阻碍 RIP2-XIAP 相互作用的抑制剂 近年研究显示, RIP2 的激酶活性对 NOD2 炎症通路传导不是必需的, GSK583、普纳替尼和达沙替尼等激酶抑制剂是通过阻断 RIP2-XIAP 相互作用来抑制 NOD2 信号通路的, 这也解释了激酶抑制剂对 NOD2 通路的抑制强度与其对激酶的抑制活性并不成正比相关^[69,71]。解析 XIAP 和 RIP2 结合位点的研究发现, XIAP 的杆状病毒 IAP 重复序列 (baculoviral IAP repeat, BIR) 2 结构域结合在 RIP2 激酶结构域 N-lobe 的 $\beta 2$ - $\beta 3$ 之间的 loop 上, 该区域接近 ATP 结合口袋。当把 loop 上的 R36 和/或 R41 突变后, 其结合 XIAP BIR2 结构域的能力被明显抑制。而 R36 和 R41 形成的碱性斑块 (basic patch) 位于 GSK583 等激酶抑制剂结合 RIP2 的深口袋的顶部。明确 SAR 后, 开发出 CSLP 系列化合物, 尤其 CSLP37 在 ADPGlo 测定中 IC_{50} 为 $16.3 \text{ nmol}\cdot\text{L}^{-1}$, 且在 NOD2 活化的细胞和动物模型中均显示出显著的抑制作用^[71]。

4.3 IAP 抑制剂

迄今已发现 8 个人类 IAPs 家族蛋白成员, 其中 XIAP、cIAP1 和 cIAP2 可泛素化 RIP2, 它们均含有 3 个 BIR 结构域。拮抗 XIAP、cIAP1 和 cIAP2 可阻碍它们与 RIP2 的相互作用, 因此是抑制 NOD2 通路的另一个策略。IAPs 能够阻断细胞凋亡, 其抑制剂作为抗肿瘤药物被充分研究, 如 LCL161、AT-406、birinapant、APG-1387、ASTX660 和 UC-112 等, 部分已进入以肿瘤为适应症的临床试验阶段。但这些药物均是泛 IAP 抑制剂, 通过与 XIAP、cIAP1 和 cIAP2 的 BIR3 结构域结合或者同时与 XIAP 的 BIR2 和 BIR3 结构域结合, 从而抑制其活性。然而与 cIAP1 和 cIAP2 的 BIR3 结构域结合会促使它们的降解, 并引起因降解导致的非经典 NF- κ B 通路激活, 产生促炎作用^[72,73]。此外, 鉴于 XIAP 对 NOD2 信号转导的必需性, 以及 XIAP 以 BIR2 结构

域与 RIP2 结合, BIR2 选择性 XIAP 抑制剂是阻断 NOD2 信号通路的研究重点。Andrew 课题组^[74,75]报道了一类新型的苯并西平类和苯并二氮杂萘类 BIR2 选择性 IAP 抑制剂。Goncharov 等^[38]在此基础上发现, BIR2 选择性 XIAP 抑制剂 XB2m49、XB2m54、XB2d89 可以拮抗 XIAP 和 RIP2 的相互作用, 并在细胞水平抑制 MDP 或 L18-MDP 诱导的 RIP2 泛素化, NF- κ B、JNK 和 p38 活化以及促炎细胞因子的产生, 而且对细胞活力、cIAP1 稳定性及非经典 NF- κ B 通路无影响。此外, 它们在 NOD2 活化的动物模型中亦发挥显著疗效。提示了 BIR2 选择性 XIAP 抑制剂在 NOD2 相关炎症性疾病中的治疗潜能。

5 问题与展望

NOD2 感知细菌 PGN 和细胞应激信号, 激活多条炎症免疫通路, 是抵抗感染性、炎症性疾病和维持机体健康的重要固有免疫受体。NOD2 基因突变与 BS、YAOS 和 CD 等 3 种 AIDS 密切相关; 而 NOD2 的不恰当激活导致促炎细胞因子增多, 参与多种疾病的发生发展。虽然近年对 NOD2 的研究取得进展, 揭示了一些调节 NOD2 信号通路的机制和分子, 但仍留有很多关键问题尚未解决, 包括配体识别的具体过程、与其他炎症通路的相互作用、信号通路调控的确切机制、突变或功能失调后促进疾病发生的机制、如何安全抑制 NOD2 而不影响机体对病原微生物的防御功能等。上述问题的解决有利于获得更新的研究思路 and 开发更有效的治疗炎症免疫性疾病的方法。

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