细胞焦亡的机制和功能

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摘要 细胞焦亡(pyroptosis)是一种高度促炎性的细胞程序性死亡,最早是在受细菌感染或者细菌毒素处理后的巨噬细胞中观察到的,很长一段时间被误认为是一种巨噬细胞特异的、依赖于能够切割白介素1 β 的促炎性蛋白酶caspase-1的细胞死亡.后续的研究发现,胞浆内模式识别受体识别病原体来源的模式分子或者机体本身来源的危险信号分子形成炎症小体(inflammasomes),招募和激活caspase-1导致细胞焦亡;鼠的caspase-11和人的caspase-4/5直接作为模式识别受体识别细菌脂多糖类脂A组装的炎症小体也导致细胞焦亡,这一发现颠覆了传统炎症小体的概念.与caspase-1不同,caspase-11/4/5不能切割白介素且引起的细胞焦亡在非单核细胞中也普遍存在.最新的研究发现,caspase-1以及caspase-11/4/5都能切割共同的底物gasdermin D(GSDMD)导致裂解性细胞死亡.GSDMD属于一类具有膜打孔活性的gasdermin家族蛋白成员,细胞焦亡也被重新定义为gasdermin介导的程序性坏死样细胞死亡,开创了细胞焦亡研究的新领域.本文回顾了细胞焦亡研究的历史以及细胞焦亡概念的进化过程,总结了caspase-1和caspase-11/4/5上游目前已知的天然免疫通路,讨论了关于细胞焦亡的研究进展尤其是GSDMD以及其他gasdermin家族细胞焦亡执行蛋白的功能和作用机制,以及细胞焦亡和相关蛋白在对抗感染以及人的自身炎症性疾病过程中的作用.

关键词 天然免疫, 炎症小体, caspase, 细胞焦亡, gasdermin, 膜穿孔蛋白, 炎症性疾病

1 细胞焦亡概念的提出

细胞死亡是动物发育、维持组织平衡以及衰老过程中的一个正常现象^[1].细胞死亡可能是被特定的信号事件驱动的程序性过程,也可能是由“意外”伤害造成的偶发事件.自然发生的或者程序性的细胞死亡在调节细胞数目、促进形态发生、去除有害或者异常的

细胞以及清除已经执行完功能的细胞等方面起重要的作用.

目前,人们已经对细胞凋亡以及两种程序性的细胞坏死——坏死性凋亡(necroptosis)和细胞焦亡——的机制了解得比较清楚^[2,3].由于细胞凋亡和细胞焦亡都是由一类称之为caspases的蛋白酶参与的,并且很长时间以来,细胞焦亡虽然形态上更像坏死,但都被错误

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地认为是单核细胞中发生的一种特殊的细胞凋亡。所以尽管本文主要介绍的是关于细胞焦亡的机制和功能,但还是有必要先了解一下细胞凋亡。

细胞凋亡(apoptosis),是一种受遗传基因调控的细胞自杀机制,在发育和维持组织平衡中起关键作用。直至20世纪90年代末,人们一度简单地认为,只有细胞凋亡才是受特定程序控制的,所以细胞凋亡的概念也就被错误地等同于程序性细胞死亡(programmed cell death, PCD),以区分于“意外”细胞死亡(也就是当时的细胞坏死的概念),并且两种死亡方式在形态上也很容易区分^[4]。“Apoptosis”这个名字来自希腊语(在希腊语中的意思是花瓣从花上或者树叶从树上落下来),最早由Kerr等人^[5]提出,用来描述正常细胞程序性死亡和体内某些病理过程相关的形态学改变模式。这些变化包括细胞收缩并且失去与邻近细胞的接触、细胞质中大量液泡形成、细胞质膜以及核膜起泡、染色体凝集等。

细胞凋亡的核心是一类和线虫(*Caenorhabditis elegans*)细胞凋亡基因编码的CED-3(cell death mutant 3)^[6-8]同源的蛋白酶,即caspases家族蛋白酶。当ced-3基因被克隆出来后,研究人员发现它编码的蛋白和哺乳动物的白介素-1转化酶(interleukin-1 converting enzyme, ICE)同源^[9],所以最初也被叫作CED-3/ICE家族蛋白。随后,一系列序列同源蛋白陆续被鉴定出来^[10-28],考虑到该家族蛋白数量的激增和命名上的混乱,这些具有天冬氨酸特异性的半胱氨酸蛋白酶被统一命名为caspase-1~caspase-10^[29]。另外,这个家族还有两个鼠源caspases(最开始认为没有人源蛋白对应物),被叫作caspase-11和caspase-12^[30,31],以及后来发现的牛源caspase-13(最开始被错误地认为是人源)^[32,33]和人源的caspase-14^[34-36]。选择“caspase”作为所有家庭成员的词根名,是基于这些酶的两种催化特性:“c”反映的是半胱氨酸(cysteine)蛋白酶的催化机制,“aspase”指的是它们在天冬氨酸残基后切割的能力,也是这类蛋白酶不同于其他蛋白酶的催化特性。现在已经很清楚,caspases家族蛋白酶在哺乳动物细胞凋亡和部分细胞焦亡中发挥关键的生物学作用。

caspase-1是caspase家族中第一个被发现的成员,1989年首次被报道,最初被命名为ICE(EC 3.4.22.36)^[37,38],是因为它负责切割白介素1 β (interleukin-1 β , IL-1 β)^[39,40]。caspase-1还能切割白介素18(IL-

18)使其成为成熟形式的IL-18从而发挥免疫调节功能^[41,42]。此外,早期曾有研究报道了白介素33(IL-33)也可以被caspase-1切割并激活^[43],但该观点一直未得到确认。caspase-1蛋白是以45 kD的前体形式合成的,活化过程中蛋白水解为22 kD(p20)和10 kD(p10)两个亚基^[40],其三维结构的X射线晶体学分析表明,成熟的caspase-1是由两个p20和两个p10亚基组成的四聚体^[44,45]。在细胞中过表达ICE能够导致细胞死亡^[46],其他很多CED-3/ICE家族蛋白在不同细胞中的过表达也观察到了细胞死亡,当时都认为是细胞凋亡。但是这其中只有caspase-3(Yama)和caspase-7(ICE-LAP3)的蛋白水解能够被凋亡刺激激活^[16,47,48]。随着研究的深入,细胞凋亡的确切机制越来越明晰^[49-51]。启动型caspases(caspase-8, -9, -10)接受细胞内部和外部的凋亡信号从而激活执行caspases(caspase-3, -6, -7),导致众多底物的切割和细胞凋亡。而caspase-1是否引起细胞凋亡却备受争议,主要有以下4个方面的证据不支持:(i)有些细胞能够稳定地分泌成熟形式的IL-1 β ,但并不显示出经典的凋亡特征。(ii)caspase-1敲除的小鼠虽然不能产生活性形式的IL-1 β ,却没有表现出明显的细胞死亡缺陷表型,对凋亡信号敏感,发育正常,在涉及细胞凋亡的正常生理过程中没有严重缺陷^[52,53]。(iii)在一个体外凋亡模型中,执行凋亡的酶活能切割PARP(poly(ADP-ribose)polymerase),但是不能切割IL-1 β ,而纯化的ICE活性正好相反^[54]。(iv)细胞凋亡是一种免疫沉默性质的细胞死亡^[55],形成的凋亡小体在质膜完整性丢失之前会被周围的吞噬细胞(比如巨噬细胞)吞噬^[56],而IL-1 β 是一种主要由活化的单核细胞和巨噬细胞分泌的多向性促炎因子,其加工成熟以及分泌和炎症密切相关,因此caspase-1介导的细胞死亡和炎症反应密切相关。

与此同时,有研究报道了兼性胞内病原菌志贺氏菌(*Shigella*)和沙门氏菌(*Salmonella*)能够诱导巨噬细胞凋亡^[57,58],并且志贺氏菌诱导的巨噬细胞死亡能导致成熟形式的IL-1释放^[59],是特异依赖caspase-1的细胞死亡^[60-63]。除沙门氏菌和志贺氏菌外,许多其他的病原菌和病毒感染也会引起类似的细胞死亡^[64-68]。caspase-1激活触发巨噬细胞非特异性膜泄漏,其快速死亡的生化过程与冷缺血、缺氧、线粒体受损和ATP耗尽等引起的坏死最为相似^[69-71],但是依赖于caspase-1的这种细胞毒性清楚地将其与任何常见的细胞

意外死亡形式区分开来^[72-74]。并且沙门氏菌诱导的来源于caspase-1敲除小鼠的巨噬细胞的死亡减少, 不可能由于这些细胞而不能发生凋亡, 因为小鼠发育正常, 这些细胞发生凋亡的能力也是正常的。但是有意思的是, 因为小鼠不能产生活性形式的IL-1 β 和IL-18等细胞因子, 所以不再对细菌脂多糖(lipopolysaccharide, LPS)引起的内毒素休克敏感^[52,53]。此外, caspase-1的激活也与很多非感染性炎症疾病有关^[75-78], 这表明在细胞死亡中发生的、由caspase-1介导的事件的发生可能具有广泛的生物学意义。Cookson和Brennan^[79]认为, 这种依赖于caspase-1的细胞死亡显然是和细胞凋亡限制炎症反应的性质相悖, 不同于经典的细胞凋亡和细胞坏死, 于是提出了一种新的名称——“pyroptosis”, 该名称来源于希腊语的词根“pyro”意思是发热或者发烧, 用来描述这种炎症相关的、程序性的细胞死亡^[80], 中文翻译为焦亡。然而, 令人遗憾的是, 长期以来由于人们对caspase蛋白活化诱导细胞凋亡的根深蒂固的认识, 细胞焦亡这种区别于凋亡的死亡方式一直未得到普遍接受, 也没有得到主流学者的认同。

作为巨噬细胞和单核细胞产生的一种促炎因子, IL-1 β 在机体应对感染、损伤和免疫挑战而产生的全身和局部反应中起作用, 是导致慢性和急性炎症的主要原因^[81]。作为一种内源性热原, 它也是发热反应的关键因素^[82]。IL-1 β 的切割成熟需要caspase-1的活化, 那caspase-1是怎么被激活的呢? 目前已经比较清楚的是, 胞质中的受体识别病原或者细胞内源的危险信号, 导致受体活化、寡聚和进一步招募接头蛋白ASC (apoptosis-associated speck-like protein containing a CARD)^[83], 它由PYD(pyrin N-terminal homology domain)^[84-88]和CARD(caspase recruitment domain)^[89]两个结构域组成, 能够连接上游受体和下游caspase-1^[90,91], caspase-1的聚集导致其自切产生活性形式的caspase-1, 从而启动下游一系列反应, 包括IL-1 β 和IL-18的切割以及裂解形式的细胞焦亡的发生^[92-94]。本文详细介绍一下是什么样的胞内受体启动了这个过程。

2 模式识别受体和炎症小体

宿主利用免疫系统防御病原微生物的入侵。在脊椎动物中, 免疫系统通过天然免疫和适应性免疫共同保护机体免受病原感染。虽然这两种系统都依赖于特

异受体对“非我”分子模式的识别, 但适应性和天然免疫系统利用不同的机制最终消除携带这些模式分子的病原体。适应性免疫系统依赖于体细胞基因的重排, 以产生具有随机特异性的数百万抗原受体, 这些受体在造血系的两个细胞群(B细胞和T细胞)上表达。而天然免疫系统使用了一系列由多种细胞群(如和病原接触的巨噬细胞、中性粒细胞和上皮细胞)表达的、种系编码的受体与病原体接触。天然免疫是抵御病原体的第一道防线, 感染后可迅速激活, 最开始被认为是一种非特异性免疫反应, 靠巨噬细胞和白细胞吞噬并消化微生物及外来物质。然而, 天然免疫本质上具有特异性, 也能区分病原体和自身。20世纪80年代末, Janeway^[95]提出天然免疫系统可以识别病原体特有的、进化上保守的关键模式分子, 称为病原体相关分子模式(pathogen-associated molecular patterns, PAMPs)。这些模式分子对微生物生存至关重要, 因此它们的结构发生变化的可能性很小, 因为任何大的变化对微生物都是不利的。这些分子包括细菌的结构成分, 如脂多糖和肽聚糖(peptidoglycan, PGN), 或病毒的核酸分子等。Janeway^[95]预测宿主生物具有一系列识别这些PAMPs的受体, 称为模式识别受体(pattern recognition receptors, PRRs), PRR识别PAMP触发一系列宿主防御机制, 比如补体激活、吞噬作用和促炎基因的表达。天然免疫系统的激活还可以诱导细胞产生细胞因子以及共刺激因子, 帮助激活获得性免疫系统^[96]。对天然免疫系统和适应性免疫系统作用机制和分子组成的比较表明, 天然免疫系统在进化过程中早于适应性免疫系统。对脊椎动物、昆虫、蠕虫甚至植物的天然防御机制的分析揭示了不同物种之间在结构和功能上的一些显著的保守元素, 因此, Janeway和Medzhitov^[97]将天然免疫描述为“一种古老的宿主防御系统”。

作为关键的天然免疫受体, Toll样受体家族蛋白(Toll-like receptors, TLRs)是研究最早的一类模式识别受体, 在宿主抵御真菌、细菌和病毒中发挥关键作用。TLR信号系统除了介导天然免疫反应外, 还能刺激脊椎动物的适应性免疫系统的激活, 并最终消除入侵的病原^[98]。TLR蛋白作为一类跨膜蛋白, 主要负责监视细胞外和内膜环境中的“非我”成分。哺乳动物的TLRs在胞外区含有多个富含亮氨酸的重复序列(leucine-rich repeats, LRRs)负责识别PAMPs, 位于胞内介导蛋白-蛋白相互作用的TIR(Toll/IL-1 receptor)结构域负责

信号传递. 植物天然免疫系统抗病R蛋白是TLRs的同源蛋白, 其中最大的一类是由N端TIR、中间NBD(nucleotide-binding domain)和C端LRRs结构域组成的^[99]. 根据TLRs和植物中R蛋白的二级结构特征, 哺乳动物中一类Apaf-1(apoptotic protease activating factor-1, 线虫中的细胞凋亡基因编码的CED-4在哺乳动物细胞中的同源蛋白)相关的蛋白家族被鉴定出来(NACHT(domain present in neuronal apoptosis inhibitory protein (NAIP), CIITA, HET-E and TP1)家族)^[100,101], 包括能够激活NF- κ B信号通路的NOD1(CARD4)和NOD2(CARD15)^[102~104]以及能激活caspase-1的Ipaf(ICE-protease-activating factor, 后被重新命名为NLRC4)^[105~107]. 进一步研究表明, NOD1能响应病原菌LPS的刺激, 类似植物R蛋白对病原微生物组分的响应, 因此, 推测这类家族蛋白是动物中各类病原组分的胞内受体, 在免疫信号通路中起重要作用^[108~110]. 尽管后续用超纯LPS以及合成的配体做进一步研究发现, NOD1和NOD2响应的是不同的单体PGN片段: NOD1被存在于革兰氏阴性菌和某些革兰氏阳性菌的细胞壁的PGN片段DAP(γ -D-glutamyl-meso-diaminopimelic acid)激活^[111,112], 而NOD2识别的是革兰氏阴性菌和阳性菌中都存在的PGN片段MDP(muramyl dipeptide)^[113,114], 但是它们作为胞内受体的假说得到了印证. 与功能相一致的是, 两种慢性炎症性疾病克罗恩病(Crohn's disease)和布劳氏综合征(Blau syndrome)与NOD2基因突变有关^[115~117]. NACHT家族蛋白除了都含有保守的NTPase和LRRs结构域外, 往往在N端含有Pyrin或者CARD结构域^[118]. 基于对含有CARD结构域蛋白的生化特征的理解(介导CARD-CARD相互作用)^[119], 2002年, Tschopp教授研究组^[120]类比cytochrome-c, Apaf-1和caspase-9形成的凋亡小体(apoptosome)提出了炎症小体(inflammasome)的概念, 认为这个由NACHT家族成员NALP1、接头蛋白ASC和caspase-1组成的蛋白复合物介导了caspase-1的激活和IL-1 β 的切割. 炎症小体这一概念的提出极大地推动了人们对炎症性caspase介导的细胞焦亡的理解. 在过去的十几年间, 这个领域的研究取得了长足的进展, 多个不同的炎症小体被鉴定出来. NACHT蛋白家族作为一类新的胞内PRR, 被命名为NLRs(NACHT-LRRs)^[121~123]. 由于该家族蛋白数量较多, 研究过程中的命名比较混乱, 科学家们于2008年对该家族成员进行了统一命名^[124], 本文后面部分谈到的NLRs都将采

用统一命名后的名称.

NLRP1(以前也被称作NALP1, CARD7, NAC, DEFCAP)^[125,126], 是第一个被发现能形成炎症小体复合物的NLR蛋白^[120], NLRP1的突变和许多白癜风相关的自身炎症性疾病^[127]、自身免疫性肠病乳糜泻(ceeliac disease, CD)^[128]以及自身免疫疾病系统性红斑狼疮(systemic lupus erythematosus, SLE)^[129]等疾病相关. 人类有一个NLRP1基因, 而C57BL/6小鼠基因组编码3个同源基因, *Nlrp1a*, *1b*和*1c*^[130]. 除了NBD和LRR结构域之外, NLRP1在C末端还有另外两个结构域: FIIND(function to find domain)和CARD结构域^[131], 小鼠NLRP1s与人源蛋白的不同之处在于缺失N端的PYD结构域^[132]. FIIND结构域在结构上类似于ZU5-UPA(as initially found in ZO-1 and UNC5-conserved in UNC5, PIDD and ankirins)蛋白自水解结构域^[133], 可以发生翻译后自切割, 这也是NLRP1活化所必需的^[134]. 蛋白水解后, NLRP1的N端和C端产物仍然能结合在一起处于一种自抑制状态, 而单独的C端片段便能够募集ASC激活caspase-1^[135]. 小鼠或大鼠巨噬细胞的NLRP1B可以特异性地响应炭疽芽孢杆菌致死毒素(lethal toxin, LT)并引发强烈的caspase-1激活^[130], 但只有某些小鼠和大鼠品系的NLRP1B变体对LT刺激有反应^[136,137]. 比如, 来自BALB/c和129/Sv小鼠的巨噬细胞对LT诱导的caspase-1激活敏感, 而C57BL/6菌株的巨噬细胞则不敏感. 有研究表明, LT的活性亚基炭疽致死因子(lethal factor, LF)可以直接在Lys-44位后切割NLRP1B, 并且这种切割是NLRP1B炎症小体激活所必需的^[138], 但是来自LT敏感和不敏感小鼠品系的NLRP1B都可以被LF切割, 这表明切割本身不足以激活下游炎症反应^[137]. 人为在Lys-44位后切割NLRP1B也可以导致炎症小体活化, 说明NLRP1B感知的是LF的蛋白酶活性^[139]. 另外, 有研究表明, 一种存在于真核和原核细胞中的蛋白质降解系统——N端规则蛋白降解系统, 可能在LT诱导的巨噬细胞死亡中起作用^[140], 并且LT诱导的NLRP1B炎症小体的激活可以被26S蛋白酶体抑制剂MG132和lactacystin特异性阻断^[141]. N端规则蛋白降解系统通过UBR家族E3泛素连接酶识别多肽或蛋白质的某些特定N-末端残基然后对其进行泛素化修饰最终导致蛋白降解, 并且作为N端降解途径抑制剂的氨基酸衍生物可以保护小鼠巨噬细胞免于LT引发的细胞焦亡^[142]. 近期3项独立研究表明, 经过FIIND结构域

自切割和被LF切割后的NLRP1B的N端部分通过N端规则蛋白途径降解, 从而释放出NLRP1B的C端部分, 激活下游信号通路和炎症小体的组装^[143~145], 从而将之前的各种线索串联起来, 揭示了一种独特的天然免疫小体激活模式。人的NLRP1对炭疽致死毒素不敏感, 其N端的PYD结构域介导蛋白的自抑制, 该结构域的一些突变能够引起几种皮肤相关的自身炎症性疾病^[146], 并且NLRP1是人类表皮主要的炎症小体感受器, 角质细胞的NLRP1可以感受UVB(ultraviolet radiation b)和其他危险信号^[147]。另外, 有研究报道, NLRP1炎症小体能够响应弓形虫的感染^[148~150]以及胞壁酰二肽(muramyl dipeptide, MDP)的刺激^[151,152], 也能够被二肽基肽酶DPP8/DPP9(dipeptidyl peptidase 8/9)的抑制剂激活^[153,154], 但具体的激活机制还不明确。

NLRC4(最初称为Ipaf)炎症小体是第一个研究得比较清楚的炎症小体, 识别3种胞质内的PAMP: 鞭毛素蛋白、三型分泌系统基座蛋白(T3SS rod protein)和针状孔道蛋白(needle protein)。关于NLRC4炎症小体的研究最早得益于细菌遗传学家们发现, NLR蛋白NAIP5(Birc1e)能够限制机会性人类病原菌嗜肺军团菌(*Legionella pneumophila*)在鼠的巨噬细胞中的增殖^[155,156]。随后的研究表明, 巨噬细胞对嗜肺军团菌增殖的限制是通过识别其鞭毛素蛋白(flagellin)实现的^[157~159]。鞭毛素蛋白是细菌鞭毛的单体亚基, 鞭毛和病原菌分泌毒性效应蛋白的T3SS具有很高的序列和结构同源性。同一时期, 有研究组报道了NLRC4参与细菌感染激活caspase-1的信号通路, 在鼠识别沙门氏菌感染中发挥重要作用^[160]。两个研究组同时发现, 沙门氏菌鞭毛突变体不能激活巨噬细胞死亡, 鞭毛素蛋白通过NLRC4依赖的方式激活caspase-1^[161,162], 但不能表达鞭毛素蛋白的鼠伤寒沙门氏菌(*Salmonella typhimurium*)仍引发NLRC4通路的激活, 这表明NLRC4能够响应另外的刺激^[162]。NLRC4能够识别细菌保守的T3SS基座蛋白的发现一定程度上解释了这一现象^[163]。彼时, 该领域的一个基本问题是NLRC4炎症小体如何响应序列差异性如此大的细菌配体。本研究组^[164]和Vance研究组^[165]的两项独立研究发现, NLR家族的NAIPs蛋白作为不同PAMP的直接受体, 决定了NLRC4炎症小体对不同配体反应的特异性, 回答了该领域的诸多未解之谜。C57BL/6小鼠有7个NAIPs, 其中NAIP5和NAIP6与鞭毛素蛋白直接结合, NAIP2结合

T3SS基座蛋白, 并且本研究组^[166,167]还进一步发现, 鼠的NAIP1结合一个以前未知的细菌PAMP——T3SS针状孔道蛋白, 而人类编码的唯一的hNAIP1只响应T3SS针状孔道蛋白的刺激, 推测T3SS的保守蛋白对宿主来说更加特异的PAMP, 因为它只存在于病原菌中而鞭毛素蛋白在病原菌和非病原菌中都存在。NLRC4缺失CARD结构域的单体结构显示出一种自抑制状态^[168], 有研究报道NLRC4的533位磷酸化对其激活至关重要^[169], 晶体结构中也观察到了磷酸化的存在, 但是关于磷酸化对NLRC4炎症小体的重要性目前还有争议, 需要进一步研究。结构研究显示, NAIP不是通过LRR结构域而是通过NBD结构域结合配体^[170], 配体的结合激活了NAIP使其招募NLRC4, 形成一个炎症小体中心, 其中NLRC4发生一个大约90°的旋转从而有利于进一步招募NLRC4聚合形成轮状的炎症小体复合物^[171,172]。人为改造过的、能组成型激活NLRC4炎症小体的病原菌突变体感染结果表明, NLRC4依赖的细胞焦亡导致病原菌的快速清除, 不依赖于促炎细胞因子IL-1 β 和IL-18。细胞焦亡发生时, 中性粒细胞及吞噬细胞通过产生活性氧(reactive oxygen species, ROS)杀死细菌^[173,174]。病原菌感染小鼠的体内模型实验表明, NAIP/NLRC4炎症小体的激活是鼠的肠黏膜上皮细胞限制细菌增殖的主要方式^[175]。在NLRC4炎症小体和疾病关系方面, 有两项研究表明, NLRC4突变能引起罕见的称为SCAN4(syndrome of enterocolitis and autoinflammation associated with mutation in NLRC4)^[176]和MAS(macrophage activation syndrome)^[177]的自身炎症性疾病。

NLRPP3(亦称NALP3, cryopyrin, Pypaf1)是该领域最受关注的NLR蛋白, *NLRP3*基因的突变会引起一些自身炎症性疾病, 如家族性寒冷型自身炎症性综合征(familial cold autoinflammatory syndrome, FCAS)、Muckle-Wells综合征(Muckle-Wells syndrome, MWS)和新生儿发病的多系统炎症性疾病(neonatal-onset multi-system inflammatory disorder, NOMID), 这3种临床特征重叠的综合征统称为隐热蛋白相关周期综合征(cryopyrin-associated periodic syndrome, CAPS)^[178~180]。自NLRC3炎症小体被发现以来^[181], 各种各样激活该炎症小体的刺激物被鉴定出来^[182]。Dixit研究组^[183]最早发现, 长期以来人们看到的LPS和ATP处理过的细胞激活caspase-1并分泌IL-1 β 依赖NLRC3炎症小体的激

活。NLRP3响应的刺激物数量惊人, 包括细菌MDP^[184]、细菌RNA和一些抗病毒药物^[185]、细菌毒素^[183,186]、尿酸结晶^[187]、可吸入颗粒物(石棉、铝盐和二氧化硅颗粒)^[188,189]、阿尔兹海默症患者大脑中的纤维化 β 样淀粉状蛋白(amyloid- β)^[190~192]以及糖尿病患者体内的长链饱和脂肪酸^[193], 还有一些病毒^[194~197]、细菌^[198~201]、真菌^[202~205]和寄生虫^[206~208]等。和其他NLRs相比, NLRP3在细胞静息状态下的表达量太低不足以激活炎症小体的组装, 因此需要一个两步的激活机制, 先是通过TLRs的激动剂如肿瘤坏死因子 α (tumor necrosis factor α , TNF- α)或者IL-1 β 激活NF- κ B通路依赖的NLRP3和pro-IL-1 β 的表达, 然后再被各种刺激物激活^[209,210]。这些刺激物不仅有外源性刺激信号, 也有机体内源产生的“危险信号”, 表明机体不仅可以区分“自我”和“非我”, 而且可以识别机体不良状态下产生的信号从而激活机体的天然免疫系统^[211]。鉴于NLRP3炎症小体的激活物如此之多, NLRP3不太可能直接结合这些PAMPs, 而是响应一个由这些激活物引起的某种共同的细胞自身的变化。经过多年的研究, 研究者们提出了各种各样的激活模型^[212], 包括氧化的线粒体DNA的释放^[213]、活性氧的产生和线粒体功能障碍^[214~216]、溶酶体不稳定^[217]、胞内钙离子水平的变换^[218]以及细胞膜上孔道的形成和钾离子的外流等^[219~222]。目前, 大家普遍接受的观点是钾离子外流导致NLRP3的激活^[223]。研究表明, NLRP3的刺激物大都导致胞内钾离子水平的降低, 但是NLRP3是直接感受钾离子水平的降低或者有另外一个事件和胞内钾离子浓度相关, 目前还没有定论。随着CRISPR-Cas9(clustered regularly interspaced short palindromic repeats-CRISPR-associated proteins 9)技术的应用, NLRP3炎症小体的研究也获得了进展, 3个独立的研究组发现了一个参与有丝分裂纺锤体形成和中心体分离的蛋白NEK7(NIMA-related kinase 7)在NLRP3炎症小体的激活中必不可少, NEK7和NLRP3直接相互作用, 并且NEK7作用于钾离子外流的下游^[224~226]。另有研究表明, 细胞内高尔基体反面网状结构解体形成的富含PI4P的囊泡会直接结合NLRP3导致其激活^[227]。但是截至目前, 关于NLRP3炎症小体的激活机制仍然有很多谜团亟待解决^[93]。

Pyrin蛋白是在寻找造成家族性地中海热(familial Mediterranean fever, FMF)的病因时发现的^[228,229], 是

由MEFV基因编码的非典型NLR蛋白, 由一个PYD、两个B-boxes和一个coiled-coil结构域组成。人的Pyrin比鼠的Pyrin多了一个C端的B30.2结构域(也被称为SPRY/PRY结构域), 正是B30.2结构域的突变导致FMF自身炎症性疾病的发生^[230], 另外一个使Pyrin丧失结合14-3-3蛋白能力的磷酸化位点突变也能引起一种皮肤相关的自身炎症性疾病(pyrim-associated auto-inflammation with neutrophilic dermatosis, PAAND)^[231], 这两个突变都会导致严重的炎症反应。单核细胞中MEFV基因可以被促炎因子如干扰素 γ (interferon γ , IFN- γ)、TNF- α 和LPS诱导表达, 表明Pyrin可能在炎症信号通路中起作用^[232]。随后的研究表明, Pyrin能够结合炎症小体的配体蛋白ASC^[233,234], 且Pyrin过表达形成一个依赖于ASC的、能激活caspase-1的复合物, 暗示Pyrin可能形成炎症小体^[235,236]。另外, 和显性遗传自身炎症性疾病PAPA综合征(pyogenic arthritis, pyoderma gangrenosum, acne, PAPA syndrome)相关的PSTPIP1(proline serine threonine phosphatase-interacting protein)能够结合Pyrin并激活其募集ASC形成复合物的能力^[237,238]。2014年, 本研究组^[239]首次鉴定出Pyrin炎症小体并证明其感受的是Rho家族蛋白的修饰和失活, 并且发现Pyrin炎症小体在洋葱伯克霍尔德菌(*B. cenocepacia*)感染导致的肺部炎症中起重要作用, 这些修饰包括来自艰难梭菌(*C. difficile*)毒素TcdB的糖基化修饰、肉毒梭菌(*C. botulinum*)C3毒素的ADP-核糖基化修饰、洋葱伯克霍尔德菌的脱氨化修饰、Fic-结构域效应蛋白的腺苷酰化修饰和一些病毒的修饰。另外, 鼠疫杆菌中保守的效应蛋白YopE和YopT也可以通过失活RhoA激活Pyrin炎症小体^[240]。有趣的是, Pyrin蛋白不能直接结合修饰过的Rho蛋白, 也并不直接识别危险信号的受体, 其很可能识别的是宿主的某些信号变化^[241]。目前的研究表明, RhoA的失活导致Pyrin去磷酸化, 释放结合的14-3-3蛋白, 此时的Pyrin处于活化状态从而激活下游炎症小体通路^[242,243], 但是RhoA的失活如何导致Pyrin去磷酸化还不明晰。另外, 影响RhoA正常转录后修饰的甲羟戊酸/胆固醇合成通路上的关键激酶MVK的缺失造成的甲羟戊酸激酶缺乏症(mevalonate kinase deficiency MKD/hyperimmunoglobulinemia D syndrome, HIDS)^[244,245]和Pyrin炎症小体的激活有关。尤其值得一提的是, 历史上鼠疫在欧洲的大暴发开始于中东和地中海地区, 也

是FMF流行的地区, 该地区人群中高达1/3的人携带Pyrin突变, 突变种类多于50种。鼠疫杆菌除了分泌激活Pyrin炎症小体的效应蛋白外, 还编码抑制Pyrin激活的效应蛋白YopM, 尽管难以考证, 但是研究者推测, 这些FMF相关的突变改变了机体对鼠疫杆菌的敏感性, 作为一种正向选择压力帮助突变人群在鼠疫暴发时期存活下来^[246]。

另一个研究清楚的能形成炎症小体的蛋白AIM2 (absent in melanoma 2) 不属于NLR家族, 而是属于一类含有HIN200结构域(hemopoietic expression, interferon-inducibility, nuclear localization and characteristic 200 amino-acid domains)的ALR(AIM2-like receptor)模式识别受体家族蛋白, N端含有PYD结构域, C端含有负责结合多聚核苷酸的HIN200结构域^[247,248], 其编码基因最早作为IFN诱导表达基因家族被鉴定出来^[249,250]。Tschopp研究组^[251]根据微生物和宿主的DNA能够以依赖于ASC, 但是不依赖于NLRP3、TLRs和干扰素信号通路的方式激活caspase-1的发现, 预测有一个能够感受DNA的胞内受体。随后几个不同的研究组报道了AIM2炎症小体的存在^[252-254], AIM2直接作为受体识别DNA激活下游的ASC和caspase-1导致细胞焦亡和细胞因子的分泌, AIM2既能结合病毒的dsDNA(double-stranded DNA), 也能结合细菌来源的dsDNA。利用Aim2敲除小鼠的实验证实, AIM2在协助宿主抵抗DNA病毒感染和胞内病原菌感染中起重要作用^[255-257], 但是像土拉热弗朗西斯菌(*Francisella tularensis*)和李斯特菌(*Listeria monocytogenes*)感染, 需要细菌的裂解才能激活AIM2^[257,258]。一些人类疾病和AIM2的表达水平相关, 比如牛皮癣、腹主动脉瘤、系统性红斑狼疮和AIM2高表达有关^[259-261], 而AIM2表达水平降低与前列腺癌和结直肠癌的发展相关^[262,263], 并且Aim2的缺失导致结肠癌发展进程加快^[264,265]。这些研究说明, AIM2除了在宿主抵抗感染中起作用外, 也在癌症的发展过程中起重要作用。

NLRP6(PYPAF5)通过和ASC相互作用激活caspase-1和NF- κ B信号通路^[266], 并且在体外能和ASC及caspase-1形成一个大的复合物^[267]。最新的研究表明, 其PYD结构域本身就可以寡聚并招募ASC^[268], 但是其上游的刺激物还不清楚。NLRP6主要在肝、肾、肺和肠道表达, 在维持肠道菌群平衡和细胞因子IL-18产生中具有重要作用^[269,270]。NLRP6炎症小体的缺失会造

成肠道菌群组成的改变、自发的肠道增生以及增加肠道对化学诱导结肠炎和结肠炎诱导癌症的敏感性^[271], 并且上皮细胞中微生物群(microbiota)相关的代谢物可以调节NLRP6的激活^[267]。有研究报道, NLRP6在骨髓来源的巨噬细胞中抑制免疫信号通路和宿主对病原的防御^[272], 能够感受病毒RNA调节肠道的抗病毒免疫通路^[273], 但是关于NLRP6炎症小体的功能和作用机制仍然有争议^[274]。另一个能形成炎症小体的NLRP7(PYPAF3)蛋白研究也比较少, 有研究报道, NLRP7炎症小体的激活信号是病原体酰化的脂肽, 能够以ASC依赖的方式激活caspase-1^[275]。有趣的是, 除了促炎作用, NLRP7还具有抑制炎症的特性, 可能是通过与抑制NF- κ B信号转导的蛋白相互作用以及抑制pro-caspase-1和pro-IL-1 β 的切割起到抗炎作用^[276]。罕见的NLRP7双等位基因功能缺失突变与复发性葡萄胎有关^[277,278]。目前报道可以和ASC相互作用并能激活caspase-1和IL-1 β 切割的NLRs还有NLRP12(PYPAF7)^[279-281], 主要表达在树突状细胞和中性粒细胞中, 在巨噬细胞中检测不到^[282]。NLRP12基因突变和一些家族性自身炎症性疾病相关, 可以用IL-1的抗体来治疗相关疾病^[283,284]。NLRP12能够维持肠道菌群和避免结肠癌发生^[285], 在防御耶尔森氏鼠疫杆菌(*Yersinia*)^[286]、疟原虫感染(*Plasmodium*)^[287]以及在病毒的感染中起作用^[288,289], 也有研究报道, Nlrp12^{-/-}小鼠显著增强宿主对沙门氏菌^[290]和布鲁士杆菌(*Brucella*)^[291]的炎症反应。关于这几种炎症小体的激活机制还需要进一步研究。

3 LPS激活的非经典炎症小体

宿主的天然免疫系统能够识别细菌的各种PAMPs, 包括细菌的DNA、鞭毛、TTSS的基座蛋白和针状孔道蛋白以及各种毒素等, 激活不同的炎症小体和炎症性caspase-1。作为革兰氏阴性菌细胞壁最主要成分、在人的败血性休克中起关键作用、对天然免疫系统有深刻的影响的LPS, 即俗称的内毒素^[292], 它是怎么被宿主识别的呢? LPS由高度多变的寡聚多糖(O抗原)、一个核心多糖和一个疏水的葡萄糖胺连接的一类脂A(lipid A)组成^[293], 虽然lipid A的两个葡萄糖胺能够连接的脂肪链的数目是可变的, 但是大多数革兰氏阴性菌的lipid A有6条脂肪链, 因此被称为“六酰化”的lipid A。众

所周知, TLR4是LPS的胞外受体^[294], Beutler和Hoffmann因为鉴定LPS的模式识别受体TLR4及相关工作获得了2011年的诺贝尔生理学或医学奖^[295,296]. LPS利用“六酰化”的lipid A结构结合并激活TLR4^[297]. 为了逃避TLR4介导的天然免疫监视, 某些细菌, 如幽门螺旋杆菌(*Helicobacter pylori*)通过独特的酶巧妙地修饰它们的lipid A结构^[298-300]. 那LPS的胞内受体是什么? 这个问题一直是天然免疫领域的科学家们非常关心的.

其实, 早在caspase-1发现之初, 人们利用向小鼠注射大剂量LPS(通常大于50 mg/kg)导致小鼠败血症的模式发现, *Casp1*^{-/-}小鼠对致死剂量LPS处理不敏感^[52,53]. 随后有研究发现, *Casp11*^{-/-}小鼠也可以抵抗LPS引起的内毒素休克, 并且LPS处理能够强烈刺激caspase-11的表达^[301]. 另外, *Il1b*^{-/-}和*Il1b*^{-/-}/*Il18*^{-/-}小鼠仍然能够响应致死剂量的LPS刺激, 说明细胞因子IL-1 β 和IL-18在内毒素引起的小鼠败血症模型中并不是必需的^[302,303]. 后续又有研究报道, *Casp12*^{-/-}小鼠也能抵抗LPS引起的内毒素休克^[304]. 有意思的是, 在两种独立敲除*Casp1*的小鼠中都没有检测到caspase-11的表达^[305], 但是这个发现并没有引起大家的注意. 直到2011年, Dixit研究组^[306]发现, 129/Sv小鼠的*Casp11*基因由于7号外显子上5 bp的缺失产生了一个移码突变造成*Casp11*翻译提前终止, 因此不表达有功能的caspase-11, 而*Casp1*和*Casp11*基因在基因组上的位置非常近, 同源重组不能分离, 所以利用129/Sv种系小鼠的胚胎干细胞构建的*Casp1*^{-/-}小鼠也不表达有功能的caspase-11, 相当于*Casp1*^{-/-}和*Casp11*^{-/-}双敲除小鼠. 这同时也解释了一些其他的基因敲除导致小鼠不发生败血症的表型亦是由于小鼠中的*Casp11*突变, 如*cIAP*, *Casp3*, *Casp12*等基因敲除小鼠^[307,308]. 鼠的caspase-11在人中的同源蛋白是caspase-4^[12,17,18]和caspase-5^[12,27], 而大部分人的*CASP12*基因存在一个移码突变, 导致转录提前终止, 翻译出没有活性的截短体蛋白^[309]. 哺乳动物的*CASP4*, *CASP5*, *CASP11*, *CASP12*和*CASP13*基因与染色体上的*CASP1*基因形成一个簇, 编码的蛋白被归为caspase的一个亚家族, 称为“炎性caspase”^[310]. 它们在人类基因组中的排列为*CASP1*, *CASP5*, *CASP4*和*CASP12*, 在小鼠中为*Casp1*, *Casp11*(*Casp4*)和*Casp12*, 在牛中是*Casp1*, *Casp13*(*Casp5*)和类*Casp12*; 相比之下, 在鸟类、两栖类和鱼类中, 除了*Casp1*基因外, 没有与之密切相关的其他基

因, 基因组数据库搜索结果表明, 鸭嘴兽和负鼠的*Casp1*和类*Casp1*基因簇也定位于基因组的同一区域. 因此, 研究者们认为这是由于哺乳动物进化早期阶段的基因扩增造成的^[311].

本文之前提到的炎症小体的组装都会激活caspase-1, 鉴于*Casp11*^{-/-}小鼠能响应ATP和尿酸盐引起的caspase-1经典炎症小体激活并正常分泌IL-1 β , 一系列刺激信号包括霍乱毒素B(cholera toxin B, CTB)、大肠杆菌(*E. coli*)、柠檬酸杆菌(*C. rodentium*)和霍乱弧菌(*Vibrio cholerae*)感染都可以引发caspase-11激活导致细胞焦亡, 因此, Dixit教授^[306]认为, caspase-11介导一种非经典炎症小体(non-canonical inflammasome)的形成以保护小鼠免受致死剂量的LPS引起的死亡以及体外巨噬细胞焦亡, 在LPS诱导的小鼠败血症中发挥重要作用. 而且越来越多的证据表明, 其他的革兰氏阴性菌感染(如嗜肺军团菌、沙门氏菌、柠檬酸杆菌、志贺氏菌和伯克霍尔德菌等)能激活caspase-11, 而革兰氏阳性菌感染则不能^[312-317]. 随后的研究表明, 胞质内的LPS是caspase-11通路的激活剂, 类似于TLR4的激活, 只有“六酰化”的lipid A能够激活caspase-11, 而“四酰化”的lipid A不能, 如幽门螺旋杆菌(*Helicobacter pylori*)和弗朗西斯菌(*Francisella*)的四条脂肪链的lipid A^[318,319]. 另外, 当胞内生存的病原菌, 如沙门氏杆菌感染细胞时, 受干扰素诱导表达的GBP(guanylate-binding proteins)蛋白可以被招募到细菌所在的膜泡, 促进膜泡的裂解和LPS的释放^[320,321]; 志贺氏痢疾杆菌感染过程中, GBP蛋白会聚集到细菌周围并导致细菌发生不正常的形变^[322], 但GBP具体的作用机制还不明晰^[323,324].

已知的所有caspase的激活都需要一个大的复合物激活平台, 比如分别激活caspase-1, caspase-9, caspase-2和caspase-8的炎症小体、凋亡小体、PIDDosome^[325]和死亡诱导信号复合体(death inducing signaling complex, DISC), 因此, 大家普遍认为存在一个caspase-11上游的蛋白直接感受胞质内的LPS并组装非经典炎症小体从而激活caspase-11. 然而本研究组^[326-328]打破思维定式, 证明caspase-11本身能直接作为LPS的受体, 颠覆了大家对于caspase激活的教条式认知, 阐明了一种全新的天然模式受体识别模型. 该研究表明, 不仅caspase-11能直接结合LPS引起随后的寡聚和激活, caspase-4, -5作为caspase-11在人中的同源蛋白也能够直接结合LPS, 而介导经典炎症小体的caspase-1不能

结合LPS. 结合依赖于lipid A保守的“四酰化”结构和caspase-11的CARD结构域上的一些碱性残基, “四酰化”的lipid A尽管也结合caspase-11但是不能激活. 但有研究报道, 来自新凶手弗朗西斯菌(*Francisella novicida*)“四酰化”的LPS, 甚至人工合成的“四酰化”酯lipid Iva也能激活人的caspase-4, 这说明人体具有比鼠更广泛的系统应对感染^[329].

正常情况下caspase-11的表达量很低, 但是可以被LPS或者干扰素显著诱导表达, 因为在*Casp11*基因启动子附近存在多个NF- κ B和STATs结合位点(分别是TLR和IFN γ 下游的转录因子)^[330], 因此LPS可以通过激活TLR4诱导*Casp11*表达. 研究人员通过利用TLR3的配体诱导caspase-11的表达, 然后注射高剂量LPS引发败血症的模型发现, 这种情况下*Tlr4*敲除小鼠和野生型小鼠一样也会因为败血症死亡, 而*Casp11*敲除小鼠可以抵抗败血症引发的死亡. 这说明TLR4的主要作用是诱导caspase-11的表达, 而caspase-11结合胞内LPS的通路在小鼠败血症模型中发挥更重要的作用^[319], 这就为败血症的治疗提供了新的思路. 像caspase-11一样, 人的caspase-5的表达也需要诱导, 而人的caspase-4的表达范围更广且组成型表达在表皮细胞和角质化细胞中, 不仅局限于单核细胞^[326,331,332], 这也说明细胞焦亡不只发生在单核细胞中, 在多种类型的细胞中都能发生. 随着对小鼠研究的深入, 研究人员发现, 小鼠的肠道表皮细胞也可以识别胞内的LPS^[333], 并且*Casp11*^{-/-}小鼠对葡聚糖硫酸钠(dextran sulfate sodium, DSS)诱导的结肠炎高度敏感^[334], 说明与人类细胞相似, 任何有足够caspase-11表达的细胞都可响应细胞内的LPS, caspase-11介导的细胞焦亡对小鼠肠道表皮细胞具有保护作用^[335].

另一个有意思的现象是, 虽然caspase-11被激活引起细胞焦亡是一个不依赖于caspase-1的过程, 且不能切割pro-IL-1 β 和pro-IL-18, 但是caspase-11也能以caspase-1, NLRP3和ASC依赖的方式导致IL-1 β 和IL-18的加工成熟^[306,316]. 为什么caspase-11的激活会引发NLRP3炎症小体的激活呢? 要回答这个问题, 需要进一步了解细胞焦亡的机制.

4 gasdermin介导的细胞焦亡

随着非经典炎症小体的发现和激活机制的明了,

研究者们发现不只caspase-1激活能导致细胞焦亡, 炎症性caspase的激活都能导致细胞焦亡, 那炎症性caspase的激活如何导致细胞焦亡呢? 这个问题在很长时间内被人们忽视了, 究其原因, 还是由于人们过于强调caspase蛋白在细胞死亡(凋亡)中的作用, 一直误认为caspase活化是细胞死亡的核心和执行步骤, 这样的观点使得人们误认为caspase-1下游可能有很多蛋白被切割和失去功能, 不会存在单个或少数能决定细胞焦亡的因子. 现在看来, 这种观点显然是非常片面的, 也是不正确的. 2015年, 两项独立的研究最早鉴定出来同一个蛋白gasdermin D(GSDMD), Dixit研究组^[336]通过对化学诱变的小鼠突变体进行筛选, 发现GSDMD在LPS诱导的非典型炎症小体的激活中起关键作用, 而本研究组^[337]利用当时刚刚建立的CRISPR/Cas9基因编辑技术, 在细胞系中分别对caspase-11和caspase-1诱导的细胞焦亡通路进行了全基因组筛选, 发现GSDMD是所有炎症性caspase的底物, 是细胞焦亡的真正执行者, 这两项工作首次揭开了细胞焦亡分子机制的神秘面纱.

GSDMD是一个由480个氨基酸组成的蛋白, 由N端和C端两个结构域组成. 全长的GSDMD处于一种自抑制状态, 活化的caspase-1/4/5/11有效地切割GSDMD的N端和C端结构域中间的连接区域. 人为地将GSDMD的切割位点变为caspase-3识别序列, 就可以将TNF- α 诱导的细胞凋亡转变为焦亡, 说明GSDMD的切割本身足以激活细胞焦亡, 另外也说明细胞焦亡先于凋亡发生^[337]. GSDMD的N端对其诱导焦亡至关重要, 在真核细胞和原核细胞中只表达N端结构域蛋白足以引起细胞焦亡和对细菌的毒性^[336,337]. 重组的N端蛋白可以结合真核细胞膜上的磷脂酰肌醇和原核细胞膜上的心磷脂进而发生寡聚, 这个过程起始于真核细胞内部, 因为它需要结合分布在质膜内部的磷脂酰肌醇^[338,339]. 寡聚后的GSDMD-N端蛋白能够在膜上形成分子孔道, 直接破坏细胞膜, 造成脂质体的严重渗漏以及生物膜的裂解, 类似于细菌的打孔毒素^[338-341]. Gasdermin家族成员GSDMA3晶体结构和冷冻电子显微结构显示, 其N端以大约27重对称寡聚形成孔道, 孔道直径在18 nm左右^[339,342]. 高分辨率原子力显微镜(atomic force microscopy, AFM)分析揭示了GSDMD-N端蛋白插膜、聚合和组装成孔道的动态过程^[343]. 孔道的形成破坏了细胞内外的渗透压, 造成细胞涨大和裂解, 这是

典型细胞焦亡的生化特征。孔道介导细胞因子的释放, 所以*Gsdmd*^{-/-}能够抑制IL-1 β 的分泌但是不影响其被caspase-1切割, 原因是*Gsdmd*的缺失无法形成孔道, 而孔道形成对细胞膜的破坏作用又可以间接活化NLRP3炎症小体^[337,344], 这也解释了为什么caspase-11的激活会引发NLRP3炎症小体的激活。

Gasdermin的名字来源于小鼠的胃肠道和皮肤上皮细胞中高度表达GSDMD的同源基因*Gsdma1*^[345]。*GSDMD*和*Gsdma1*编码的蛋白同属于gasdermin家族, 该家族在人中编码了GSDMA, GSDMB, GSDMC, GSDMD, GSDME(DFNA5)和DFNB59(pejvakin), 小鼠中没有GSDMB, 但是有3个GSDMA(GSDMA1-3)和4个GSDMC(GSDMC1-4), 不同家族成员的表达有不同的组织特异性^[346-348], 这也表明焦亡不止发生在巨噬细胞和单核细胞中。除了DFNB59只含有N端结构域外, 其他成员的N端和C端结合形成自抑制状态, 并且N端结构域都具有膜打孔的活性, 过表达都能在细胞膜上打孔导致细胞发生焦亡^[339]。此外, gasdermin家族蛋白GSDME的N端和C端结构域之间包含一个经典的caspase-3切割位点, 可以被caspase-3切割活化进而诱发细胞焦亡^[349,350]。由于细胞癌化过程中表观遗传的重编程作用, GSDME的表达在绝大部分癌细胞中都被沉默了, 但在小肠和其他许多正常组织和细胞中有较高的表达。因此, 临床上广泛使用的导致DNA损伤的抗肿瘤化疗药物在通过线粒体通路激活caspase-3后, 一般导致癌细胞发生凋亡, 但在表达GSDME的正常组织中则会诱导非癌化的细胞发生GSDME介导的细胞焦亡, 而细胞焦亡会导致组织水平的炎性损伤, 这

正是化疗药物产生毒副作用的一个重要原因^[350]。鉴于gasdermin家族蛋白在介导细胞焦亡中的作用, 以及细胞焦亡不一定依赖于炎症性caspase的特性, 本研究组^[2]将细胞焦亡的概念重新定义为由gasdermin介导的细胞程序性死亡, 开辟了一个全新的细胞死亡和炎症免疫研究的新领域。下面表格(表1)系统比较了细胞凋亡和细胞焦亡的一些典型特征供读者参考, 并进一步总结了目前关于炎症小体通路激活及细胞焦亡的研究进展(图1)。

除了GSDMD和GSDME之外, 目前关于其他gasdermin家族成员的生理功能还知之甚少, 只有一些来自人和小鼠的遗传突变提供了一些信息。*GSDMA*在胃中表达, 但是在大多数胃癌细胞中被沉默^[351]。在小鼠中, *Gsdma3*的突变导致表皮增生、过度角化以及毛发脱落^[352-354], 但是*Gsdma3*缺失的小鼠毛发发育正常^[355,356], 这说明小鼠脱毛的症状是由于*Gsdma3*的功能获得型突变(gain-of-function)导致的。分析*Gsdma3*的突变发现, 其中两个突变导致翻译提前终止而缺失了C端结构域, 另外有7个突变位于两个结构域相互作用的区域^[337,339]。与突变蛋白导致组成型的细胞焦亡一致, *Gsdma3*突变小鼠的皮肤有严重的炎症反应, 皮肤毛囊干细胞逐渐丢失^[357,358]。大规模单核苷酸多态性分析表明, 人的*GSDMB*基因和儿童期发生的哮喘关系密切^[359-362], 另外有研究报道, GSDMB和GADMC在癌细胞的增殖中有作用^[363,364]。GSDME(DFNA5)和DFNB59的突变造成非综合性的耳聋^[365]。所有*GSDME*的突变都造成8号外显子的跳读产生一个截短体蛋白^[366]。*GSDME*导致的耳聋是进行性的, 5~15岁之

表 1 细胞凋亡和细胞焦亡的典型特征比较
Table 1 Comparison of the typical characteristics of apoptosis and pyroptosis

细胞凋亡	细胞焦亡
非炎症性的	炎症性的
caspase-3/6/7/8/9/10参与	caspase-1/4/5/11参与
维持细胞质膜完整性	丧失细胞质膜的完整性, 细胞膜上有gasdermin介导的孔道形成
细胞皱缩, 胞浆浓缩, 胞膜内陷凋亡小体形成	细胞吹泡、涨大、破裂, 细胞内容物释放
DNA断裂形成ladder, PARP被切割	对于caspase活化导致的焦亡, 有可能发生DNA断裂, 但PARP不被切割; gasdermin孔道本身不会导致DNA断裂和PARP的切割
染色体凝集, 细胞核凝集	对于caspase活化导致的焦亡, 有可能发生染色体凝集, 但细胞核完整; gasdermin孔道本身不会影响染色体和细胞膜结构
磷脂酰丝氨酸外翻, Annexin V染色阳性	磷脂酰丝氨酸不外翻, 但由于gasdermin孔道可以允许蛋白分子进入胞内, 所以Annexin V染色也呈阳性

图1 炎症小体通路和gasdermin介导的细胞焦亡

Figure 1 Inflammasomes pathway and gasdermin-mediated pyroptosis

间是高频发生的年纪. 与GSDME突变蛋白造成细胞焦亡这一获得功能突变一致的是, *Gsdme*敲除小鼠没有耳聋的症状, 但是*Gsdme*缺失小鼠的耳蜗外毛细胞数目不正常^[367]. *Gsdme*受p53的转录调节, 暗示其功能可能和压力下细胞反应有关^[368], *Gsdme*还被认为是一个抑癌基因, 在许多正常组织中高表达, 但其启动子区域的表观遗传修饰导致它在大多数癌细胞中不表达^[350,369,370]. DFNB59是唯一一个缺失C端结构域的gasdermin家族蛋白, 该蛋白是否具有膜打孔和执行细胞焦亡的功能目前还不明晰. 与GSDME不同, DFNB59导致耳聋的突变是常染色体隐性遗传, 造成的耳聋既有进行式的, 也有非进行式的, 听觉神经病变和耳蜗外毛细胞缺失都有可能其导致耳聋的原因^[371~374]. DFNB59突变基因敲入小鼠显示出不正常的听觉脑干反应, 表明神经元伴随听觉通路功能障碍, 并且DFNA5基因敲除也不能恢复受损的听力, 说明两者通过不同的机制导致耳聋^[375]. DFNB59功能还和过氧化物酶体有关, 它能调节氧化应激诱导的过氧化物酶体增殖. DFNB59缺失小鼠的过氧化物酶体功

能异常, 小鼠的耳蜗外毛细胞和听力神经元因为氧化应激和抗氧化防御系统受损而变得对声音高度敏感^[376]. 综上, 关于这些gasdermin家族蛋白的功能还有待进一步研究.

Gasdermin家族成员的表达有不同的组织特异性^[377], 推测其上游可能不局限于炎症小体和炎症性caspase的激活. 最近有几篇研究报道caspase-8可以切割并激活GSDMD引起细胞死亡^[378~380], 嗜中性粒细胞分泌的蛋白酶也可以激活GSDMD^[381,382], 所以不同的gasdermin家族蛋白在不同组织中的活化方式、激活水平以及作为细胞死亡执行者的生理学功能将是一个热点问题. 细胞焦亡的典型特征是细胞膜的起泡、膨胀直至细胞破裂, 这是在体外培养环境中观察到的一个现象, 在体内的真实情况如何, 是一个非常有趣并且重要的问题. 不同gasdermin介导的细胞焦亡引起的炎症水平的高低和个体的健康以及疾病的发生、发展密切相关. 随着这方面研究的深入, gasdermin家族蛋白作为抗炎症治疗的新靶点越来越受到广泛的关注.

5 细胞焦亡和自身炎症性疾病

在过去的二十几年中,人们对炎症小体激活及细胞焦亡机制有了本质的认识,裂解性细胞死亡导致的炎症是机体应对感染和组织损伤的生理反应,是用来清除病原以及诱导组织修复的过程.可控的炎症反应在清除异物后或者组织损伤被修复后就会慢慢消退,但是危险信号不能被有效地清除就会造成持续的炎症或者对组织的损伤,导致严重的炎症性疾病的发生.自身炎症性疾病的发生,有些是由于危险信号在体内积累导致的炎症小体通路的持续性激活,或者是由于天然免疫受体或相关分子的获得性突变造成的,本文以4种常见的疾病为例探讨NLRP3炎症小体和疾病的关系.

痛风(gout)是一种反复发作的炎症性疾病,属于关节炎的一种.造成痛风的本质原因是体内尿酸水平升高导致的尿酸盐在关节和肾脏部位的沉积.可溶的尿酸盐其实并无大碍,但其在血液中浓度的急剧增加会造成其化学形式转变为单尿酸盐晶体(monosodium urate, MSU).临床上,高尿酸血导致急性痛风的反复发作,患者出现发烧、发冷、红斑以及关节肿胀伴随的局部疼痛.大量中性粒细胞的积累导致关节的炎症,通常发生的部位包括大脚趾、脚踝和手肘.如果未经处理,急性痛风导致的持续性晶体沉积和严重炎症反应会造成不可逆转的关节损伤、严重的僵硬、肿胀甚至残疾^[383].尽管100多年前人们就知道MSU会导致痛风,但始终也没有搞清楚原因. Tschopp教授研究组发现,MSU通过激活NLRP3炎症小体诱发IL-1 β 介导的炎症反应^[187],并且通过抑制IL-1 β 可以有效地治疗痛风,表明炎症小体在疾病进展中起重要作用^[384,385].除了抑制IL-1 β 以及通过抑制嘌呤合成途径中的限速酶降低尿酸水平以外,大多数治疗痛风的方案关注的是症状而非病因,也是受限于目前为止关于MSU如何诱导炎症小体组装的机制还不明晰,因此鉴定出NLRP3信号通路的内源调节途径对于治疗痛风非常必要.

心血管疾病是全球头号死因.动脉粥样硬化(atherosclerosis, AS)是心脑血管疾病的主要病理基础,即随着脂肪和胆固醇的沉积,动脉逐渐变窄甚至被堵塞. AS是一种脂肪诱导的炎症状态,具有炎症性疾病的重要特征,招募巨噬细胞到动脉壁胆固醇沉积部位.巨噬细胞吸收过量的胆固醇存储在细胞中成为所谓的

脂质泡沫细胞.泡沫细胞内结晶胆固醇的形成导致细胞破裂,造成胆固醇的进一步沉积并招募免疫细胞以及进一步形成动脉粥样硬化斑块.当这些斑块变得不稳定并破裂就会导致血管堵塞,出现临床上的心肌梗塞或者中风的表現.有研究表明,巨噬细胞能够通过细胞表面的TLRs识别氧化形式的低密度脂蛋白(oxidized low-density lipoproteins, oxLDL)和胆固醇晶体,激活巨噬细胞中IL-1 α 和IL-1 β 的产生. NLRP3炎症小体在疾病进程中起主要作用,其中IL-1 β 的分泌依赖于NLRP3^[386],但IL-1 α 的分泌不依赖^[387].有临床研究表明,通过IL-1 β 的单克隆抗体抑制炎症反应可以有效降低心血管疾病的复发率^[388,389]. oxLDL是个有趣的分子,它能引发NF- κ B依赖的NLRP3和IL-1 β 的表达,并且其在巨噬细胞内的积累导致可溶性促动脉粥样硬化配体向颗粒形式的胆固醇晶体的转化,并激活炎症小体^[390],是一个将AS和NLRP3炎症小体联系起来的关键分子.

阿尔兹海默症(Alzheimer's disease, AD)是一种渐进性老年痴呆症,目前还没有治愈的方法,主要表现为记忆丧失、认知障碍以及行为的改变.尽管目前AD的病因还不明晰,但是疾病的进展程度和 β -淀粉样蛋白(β -amyloid, A β)在脑中的沉积有关.另外,感染、神经元应激和炎症因子的大量产生都可能会导致疾病的进展.有研究表明, NLRP3炎症小体可以识别A β 从而在这个过程中起重要作用^[191].通过观察具有轻微认知障碍和轻度AD患者的脑部组织发现了激活形式的caspase-1,表明炎症小体的激活可能是疾病发展早期的重要步骤.在小鼠模型中缺失Nlrp3和Caspase-1能在很大程度上避免小鼠发展出与AD相关的学习能力异常和严重病理状态^[391].有趣的是,最近的一项研究表明,即使在没有A β 沉积的小鼠脑中, Nlrp3的缺失也会保护小鼠免于老龄化相关的炎症和认知障碍^[392].因此, NLRP3依赖的炎症反应和A β 相关的神经退行性疾病密切相关.

二型糖尿病(type 2 diabetes, T2D)是世界范围内最常见的人类疾病之一,发病率呈增加趋势.肥胖引起的胰岛素抵抗和胰岛 β 细胞丧失是二型糖尿病的典型特征,外周胰岛素抵抗导致大量的高血糖通过胰腺 β 细胞增加胰岛素输出得到补偿,局部的炎症反应和葡萄糖的毒性作用导致胰腺 β 细胞的大量消失和胰岛素分泌逐渐减少,促进从肥胖和胰岛素抵抗发展成

T2D. 尽管目前T2D病因还不明晰, 但是NLRP3炎症小体的激活在其中起作用, 因为编码炎症小体组分的NLRP3, ASC和caspase-1基因的敲除增加了小鼠胰岛素的敏感性^[193,393,394]. 目前认为, NLRP3对肥胖的脂肪组织中过量或者不正常脂质的识别能够引发系统的炎症反应, IL-1 β , TNF- α 和IL-6的产生促进外周细胞对胰岛素的抵抗. 因为炎症反应产生的IL-1 β 对胰岛细胞来说是有毒的, 因此这种局部产生的IL-1 β 可能导致二型糖尿病患者中常见的胰腺 β 细胞的丧失和胰岛素抵抗. 炎症小体在T2D中起作用的另一个例证是, 一种促进胰岛素分泌的药物glyburide能够抑制NLRP3介导的IL-1 β 的分泌^[395]. 此外, 在二型糖尿病患者中, 高胰岛素血症伴有IAPP激素(islet amyloid polypeptide, 也被称为amylin)的增加, 导致IAPP淀粉样蛋白的形成并沉积在胰岛间质. 对表达人IAPP基因的转基因小鼠的研究表明, IAPP能够通过内吞和溶酶

体裂解激活巨噬细胞中的NLRP3炎症小体^[396], 胰岛中巨噬细胞表达更多的IL-1 β . 因此, 抑制炎症小体和IL-1 β 产生可能会是未来一个有前景的治疗二型糖尿病的策略.

鉴于NLRP3炎症小体介导的炎症反应在这些疾病中的作用, 对NLRP3炎症小体信号通路的进一步研究, 将有助于人们理解这些疾病的致病机制并寻找治疗方案. 目前来看, 抑制炎症小体的激活或者抑制细胞焦亡和炎症因子的释放可能会是有效的治疗策略, 比如开发特异抑制炎症小体激活或细胞焦亡的小分子药物等, 但关于炎症小体如何激活的机制还有很多问题尚不明晰, 必然还有一段很长的路要走. 另外, 之前关于炎症小体和细胞焦亡的研究主要以体外实验和小动物模型为主, 预计接下来的研究重点将逐步以炎症小体功能改变对复杂人类疾病的影响为中心, 最终服务于人类健康.

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Molecular mechanism and immunological function of pyroptosis

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Pyroptosis is a form of programmed cell death that is highly proinflammatory. Pyroptosis was originally observed in macrophages in response to bacterial infection or bacterial toxin stimulation. This cell death, then misclassified as a type of apoptosis specifically in macrophages, requires caspase-1 that also processes interleukin-1 β mainly expressed in monocytic cells. Caspase-1 is activated by canonical inflammasomes, a major cytosolic innate immune surveillance mechanism that can detect various pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). Studies on innate immunity to cytosolic lipopolysaccharide (LPS) have established that murine caspase-11 and its human counterparts caspase-4/5 can also mediate pyroptosis upon directly binding to the lipid A moiety of LPS. Caspase-11/4/5 do not process interleukin and caspase-11/4/5-mediated pyroptosis is prevalent in non-monocytic cells. Very recent studies have identified gasdermin D (GSDMD) as the pyroptotic substrate for both caspase-1 and caspase-11/4/5. GSDMD represents a large gasdermin family that shares membrane pore-forming activity, rendering a new concept of pyroptosis as gasdermin-mediated programmed necrotic cell death. In this article, we review the concept evolvement of pyroptotic cell death from a historic perspective, covering the innate immune pathways upstream of caspase-1 and caspase-11/4/5. We also discuss the recent advances on pyroptosis, particularly mechanistic and functional understandings of GSDMD and the gasdermin family of pyroptosis executor proteins.

innate immunity, inflammasome, caspase, pyroptosis, gasdermin, pore-forming protein, inflammatory disease

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