孙丽明, 研究员, 2014年3月起, 任中国科学院上海生物化学与细胞生物学研究所课题组长、博士生导师。该实验室主要用分子生物学和生物化学的方法研究细胞坏死信号转导的分子机制以及相关疾病的病理机制。程序性细胞坏死是一种由激酶的激活而引发的细胞死亡方式, 其对于个体发育、集体稳态维持及病理过程等诸多方面都有着重要作用, 主要包括凋亡、坏死和自噬等方式, 其中, 细胞坏死长期以来都被认为是一种被动且不可调控的过程。然而近几年的科学研究表明, 细胞坏死也是受到精密调控的。相对于较为成熟的细胞凋亡领域, 细胞坏死的研究崭新且发展迅速。
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程序性细胞坏死在疾病中的研究进展

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摘要 细胞坏死自从被发现具有严格的“程序性调控”特征以来, 其信号转导机制以及相关疾病机理引起了学术界广泛关注并展开大量研究。程序性细胞坏死(necroptosis)是一种不同于凋亡及传统坏死的新的细胞死亡方式, 主要由肿瘤坏死因子受体(tumor necrosis factor receptor, TNFR)家族或Toll样受体(Toll-like receptor, TLR)家族调控启动。死亡受体被激活之后, 与受体蛋白相互作用的两个蛋白激酶RIP1(receptor interacting protein kinase 1)和RIP3被激活, 进而招募RIP3的底物MLKL(mixed lineage kinase domain-like protein)并催化它发生磷酸化, 磷酸化的MLKL发生寡聚化转位到质膜上, 引起膜通透性的改变, 最终实现坏死的发生。细胞坏死是一种促炎性的程序性细胞死亡方式, 其过程中伴有大量细胞内容物的释放。如损伤相关的模式分子(damage associated molecular patterns, DAMP)的释放, 会激活机体的免疫应答, 因此其广泛参与到各种疾病的病理生理过程中, 包括神经退行性疾病、感染性炎症性疾病、缺血再灌注损伤、肿瘤发生及转移等。细胞坏死抑制剂有望被应用于对这些疾病的干预来改善患者病情及预后。该文将详细阐述程序性细胞坏死在胚胎发育、组织稳态、炎症相关疾病、肿瘤、神经系统相关疾病等疾病中的作用, 并对其在临床治疗中的应用进行回顾及展望。

关键词 程序性细胞坏死; 胚胎发育; 疾病

Necroptosis Related Diseases

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Abstract Necroptosis is a new mode of cell death that different from apoptosis and traditional necrosis,

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it is mainly induced by ligation of tumor necrosis factor receptor (TNFR) family or Toll-like receptor (TLR) family. Once the death receptors are activated, RIP1 and RIP3 would be activated, and then RIP3 recruits and phosphorylates its substrate MLKL. The phosphorylated MLKL releases its auto-inhibition and forms oligomers, which translocate to the membrane compartments, cause membrane leakage. Unlike apoptosis, necroptosis is a form of pro-inflammatory cell death, which releases a large amount of cellular contents, such as DAMP (damage associated molecular patterns), thus necroptosis involves in lots of pathophysiological processes of diseases including neurodegenerative diseases, infectious inflammatory diseases, ischemia-reperfusion injury, tumorigenesis, etc. Necroptosis inhibitors are expected to be used in these diseases to help with improving the patient's conditions. This review elaborates on the role of necroptosis in embryonic development, tissue homeostasis, inflammatory diseases, tumors, neurological diseases and other diseases, aiming to provide prospects for the diagnosis and treatment of necroptosis-related diseases.

Keywords necroptosis; embryonic development; necroptosis-related diseases

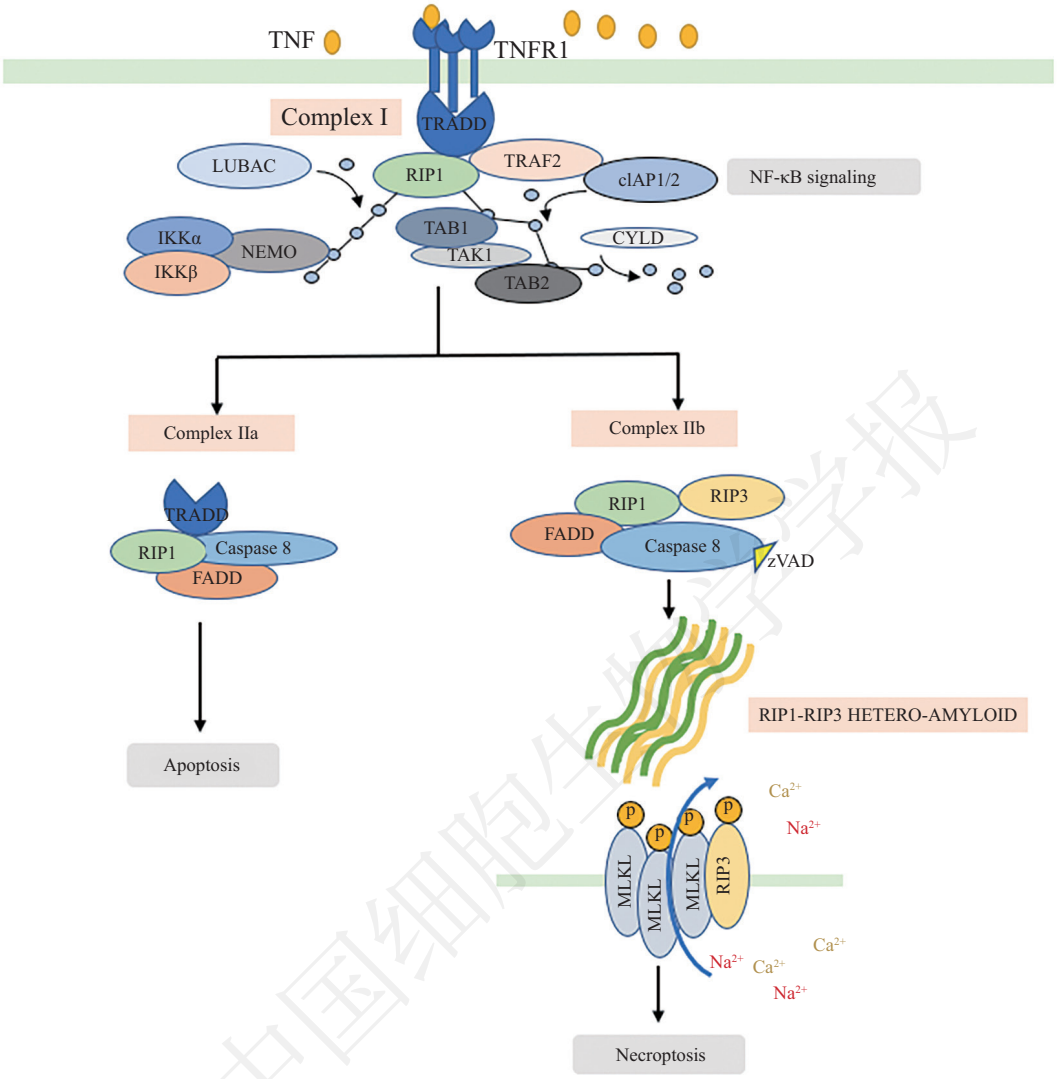
细胞死亡是机体的一项基本生命过程, 对于胚胎发育、成体自稳态维持以及病理过程至关重要。细胞凋亡(apoptosis)是最先被定义的一种程序性细胞死亡方式(programmed cell death, PCD), 它由高度保守的caspases介导发生, 在形态、生化特征上与传统认为的细胞坏死(necrosis)的不可控性相区别[1-4]。然而1988年发现, 诱导apoptosis的TNF在caspases缺失的条件下能够引起细胞坏死的发生[5]; 1996年发现牛痘病毒表达的caspases抑制剂CrmA(cytokine response modifier A)能使宿主猪肾细胞的死亡模式由凋亡转换为坏死[6]; 2005年发现, 此坏死过程能被化学小分子抑制剂Nec-1抑制住。这一系列的发现才使细胞死亡这一领域的研究者认识到当细胞凋亡被抑制之后, 细胞会转而发生另一种同样受到精确调控的死亡方式, 即细胞坏死。Nec-1的发现推动了这一程序性死亡方式被正式命名为程序性细胞坏死(necroptosis)[7]。2008年, RIP1(receptor interacting protein kinase 1)被鉴定为坏死抑制剂Nec-1的靶标[8]。随后, RIP1下游激酶RIP3[9-11]及其底物MLKL[12-13]的发现, 逐步加深了我们对程序性细胞坏死通路的认识。

不同于细胞凋亡, 细胞坏死伴有大量细胞内容物的释放, 暴露的损伤相关的模式分子(damage-associated molecular patterns, DAMP), 如HMGB1(high mobility group box 1)、线粒体DNA、乳酸脱氢酶LDH等, 会激活机体的免疫应答, 引起全身性的炎症反应[14]。目前研究显示, 程序性细胞坏死参与了众多生理病理过程, 如神经退行性疾病的发生发展、炎症性疾病包括病原微生物的感染的免疫激活、缺血性再灌注损伤以及肿瘤的发生和转移等。本文就程序性细胞坏死的分子机制作回顾, 并重点将程序性细胞坏死在相关疾病的作用作一综述。

1 程序性细胞坏死的分子机制

程序性细胞坏死是由TNF、Fas/CD95和TRAIL (TNF-related apoptosis-inducing ligand)等TNF家族细胞因子与膜定位死亡受体结合, 继而激活胞内RIP家族激酶起始的(图1)。此外, LPS(lipopolysaccharide)、病毒DNA和干扰素等均可激活程序性细胞坏死信号通路。对于程序性细胞坏死的分子机制的认识大部分来源于膜受体TNFR1介导的坏死途径的研究。TNFR1与其配体TNF结合后, 其C-端死亡结构域与接头分子TRADD(TNFR1-associated death domain protein)[15]的死亡结构域发生相互作用, 进而招募RIP1[17]、TRAF2/5(TNFR-associated factor 2/5)[18]、cIAP1/2(cellular inhibitor of apoptosis protein 1/2) [19]及线性泛素链组装复合体(the linear ubiquitin chain assembly complex, LUBAC), 形成复合体1(Complex I)。在Complex I中, RIP1快速地被LUBAC和cIAP1/2进行相应的线性和K63连接的多聚泛素化修饰。RIP1作为泛素化修饰的支架蛋白, 募集NEMO或者TAK1, 进而激活NK-κB和MAPK信号通路, 促进炎症的发生和细胞的存活[20]。而后在CYLD(cylindromatosis)[21]对RIP1的去泛素化或者泛素修饰酶A20[22]切割, 以及cIAP1/2被抑制的情况下, Complex I通过细胞内吞到细胞质内形成新的蛋白复合体Complex IIa。Complex IIa的成员包括TRADD、TRAF2/5、RIP1、FADD以及procaspase-8/10。Procaspase-8/10被剪切后激活, 进而将BID剪切成tBID, 激活线粒体凋亡通路。另外,

caspase-8/10也会激活caspase-3/6/7, 引发凋亡[23]。然而, 当RIP3和MLKL的表达水平足够高或caspase-8活性降低或缺失时, TNF刺激会促使RIP1、RIP3、FADD和pro-caspase-8组成的Complex IIb的形成, Complex IIb则会演变形成坏死复合体(necrosome)。在necrosome中, RIP1与RIP3通过它们各自的RHIM(RIP homo-



TNF与TNFR1结合后, 招募TRADD、TRAF2/5、RIP1、LUBAC和cIAP1/2形成复合体I, 其中RIP1被LUBAC和cIAP1/2相应地进行线性和K63连接的多聚泛素化修饰。泛素化修饰的RIP1作为支架蛋白, 募集NEMO或者TAK1, 进而激活NK-κB和MAPK信号通路, 促进炎症的发生和细胞的存活。当RIP1上的多聚泛素链被去泛素化酶CYLD和泛素修饰酶A20切割, 以及cIAP1/2活性被抑制或者表达下调时, TNF刺激会促进由RIP1、TRADD、caspase-8和FADD在细胞内组成的complex IIa的形成, 激活细胞凋亡。然而, 当RIP3和MLKL的表达水平足够高或caspase-8活性降低或缺失时, RIP1会与RIP3、FADD和caspase-8组成复合物IIb(Complex IIb)的形成。其中RIP1与RIP3相结合并激活RIP3的激酶活性, 然后RIP3发生自身磷酸化, 磷酸化的RIP3募集MLKL使其磷酸化, 磷酸化的MLKL发生寡聚并转位至细胞膜上, 使膜通透性发生改变, 从而促进细胞坏死的发生。RIP3的底物MLKL的激活, 标志着坏死复合体(necrosome)的活化。

Upon TNFR1 ligation, TRADD, TRAF2/5, RIP1, LUBAC and cIAP1/2 are recruited to TNFR1 on plasma membrane to form complex I, in which RIP1 is linearly and K63-linked polyubiquitinated by LUBAC and cIAP1/2, respectively. Ubiquitinated RIP1 acts as a scaffold protein and recruits NEMO or TAK1, which in turn activates NK-κB and MAPK signaling pathways, to promote inflammation and cell survival. When the polyubiquitin chain on RIP1 is cleaved by deubiquitinating enzyme CYLD or ubiquitin-modifying enzyme A20, or cIAPs activity is inhibited or down-regulated, TNF stimulation promotes the formation of Complex-IIa that consists of RIP1, FADD, TRADD and caspase 8, which leads to apoptosis. However, when the expression level of RIP3 and MLKL are sufficiently high or the caspase-8 activity is decreased or blocked, TNF promotes the formation of Complex IIb, which consists of RIP1, RIP3, FADD and caspase-8. Once RIP1 binds to RIP3, they form hetero-amyloid signaling complex, called necrosome, which is required for RIP1/RIP3 kinase activation. Then the auto-phosphorylated RIP3 recruits and phosphorylates its substrate MLKL. MLKL forms oligomers and translocates to the membrane compartments, and changes membrane permeability to promote necrotic cell death.

图1 TNFR1介导的程序性细胞死亡信号通路

Fig.1 TNF-signaling mediated cell death pathways

typic interaction motif)相互作用, 形成淀粉样蛋白沉积(amyloid structure)[24-25], 进而RIP3发生自磷酸化(人源RIP3在Ser227位点, 鼠源RIP3在Ser232位点)。磷酸化的RIP3募集底物MLKL并使其磷酸化, 磷酸化的MLKL发生寡聚化, 其N-端螺旋束可结合磷脂酰肌醇磷脂(phosphatidylinositol phosphate lipids, PIPs)和线粒体特异性的心磷脂(cardiolipin, CL), MLKL进而从胞质转位到富含PIPs或CL的质膜上, 使膜完整性受到破坏, 促进细胞坏死的发生[12,26-29]。

2 程序性细胞坏死与胚胎发育和组织稳态

近年来, 程序性细胞坏死参与组织发育的作用及其与细胞凋亡之间的交叉调控得以广泛研究。尽管早已提出凋亡参与发育过程中的形态发生, 如脊椎动物足趾形成期间去除趾间组织, 然而实验证明, 细胞凋亡关键蛋白如caspase-3、caspase-7、apaf-1和Bax/Bak的缺陷并不影响小鼠胚胎发育, 有研究指出, 这与单基因敲除后其他蛋白功能补偿相关[31]。相反, caspase-8及其结合蛋白FADD的缺失会导致胚胎期在约E10.5时致死[33-34]。这一表型的差异一直是无法解释的难题, 直到最近研究表明, caspase-8和FADD在胚胎早期发育期间抑制了RIP1和RIP3的活性, RIP3的缺失完全挽救了caspase-8缺陷小鼠的致死性[35-36], RIP1的缺失恢复了FADD缺陷小鼠胚胎的正常形成[37]。Caspase-8和FADD对程序性细胞坏死的调节作用在组织特异性敲除的小鼠中也被广泛研究。Caspase-8、FADD在小肠上皮细胞中缺失会导致上皮屏障的破坏[38], 在皮肤角质细胞中缺失会导致皮肤炎症[39-40], 这些现象都能够被RIP3全身性或组织特异性缺失所逆转。这表明, caspase-8和FADD对程序性细胞坏死具有抑制作用, 对阻止小肠屏障功能的丢失和皮肤炎症发挥着重要的功能。

程序性细胞坏死对于组织稳态的维持作用更多地集中于免疫相关疾病中。在T细胞克隆性增殖中, caspase-8缺失或者显性失活FADD的过表达, 都能导致T细胞坏死, 同时能被RIP3的缺失和Nec-1所逆转[41-43]。另外, 研究发现, 程序性细胞坏死信号通路上的核心效应蛋白也发挥着独立于程序性细胞坏死的重要功能。*Rip1*全身基因敲除的小鼠出生后不能存活, 通过*Rip1*条件性基因敲除发现依赖于其存活的细胞包括有造血干细胞和小肠细胞, RIP3的缺失能部分回补其表型, 因此RIP1可能通过抑制RIP3来阻止炎症的发生从而调节造血过程[44-45]。在树突状细胞中敲除*Rip1*也能导致系统性炎症、组织硬化以及自身免疫性疾病[46]。RIP1泛素修饰酶A20可负调控胸腺调节性T细胞的发育, 从而调节免疫排斥反应[47]。*Mlkl*与*Rip3*基因的敲除能够挽救caspase-8、FADD缺陷导致的胚胎致死, 但是会发展成严重淋巴结肿大、全身性自身免疫性疾病和血小板减少症, $Casp8^{-/-}Mlkl^{-/-}$和$Fadd^{-/-}Mlkl^{-/-}$比$Casp8^{-/-}Rip3^{-/-}$或$Fadd^{-/-}Rip3^{-/-}$小鼠病情发展得更加快速、严重, 表明RIP3、MLKL可能拥有调节自身免疫耐受的功能[48-49]。除此之外, *Rip3*基因敲除小鼠与WT小鼠相比, 凝血时间延长, 认为RIP3可能参与激活血小板和血栓形成过程[50]。

3 程序性细胞坏死在相关疾病中的作用

3.1 炎症性疾病

细胞死亡和炎症在疾病的病理过程中是不可分割的两个过程, 坏死细胞内容物的释放会引起炎症反应, 而炎症反应又会进一步促进细胞的死亡。越来越多的证据表明, RIP1和RIP3除了作为程序性细胞坏死的核心效应蛋白以外, 还直接参与了炎症反应。TNF/zVAD刺激巨噬细胞产生细胞因子KC依赖于RIP1的激酶活性[51]。在DC细胞中特异性敲除caspase-8导致全身性自身免疫性疾病, 以及DC细胞对于TLR配体的超高反应性, 并伴有大量促炎细胞因子的分泌, 包括TNF、IL-1β、IL-6、IL-12, 其中RIP1介导了这些细胞因子的分泌[52]。在模拟人类疾病的动物模型中, RIP1抑制剂Nec-1的治疗效果可能是出于减少炎症的发生而不是促进细胞的存活。另外, 也有研究表明, RIP3参与了一个独特的NLRP3炎性体信号途径。在cIAP1/2或者XIAP活性缺失的条件下, 巨噬细胞或者DC细胞中TLR4的激活能促发炎性体的形成和IL-1β的产生, 这个过程依赖于RIP3的激酶活性[53-54]。接下来主要讨论程序性细胞坏死在多种炎症性疾病动物模型中的病理机制。

3.1.1 *败血症*(sepsis) 败血症是指由感染引起的全身炎症反应综合征(systemic inflammatory response syndrome, SIRS), 临床主要表现为炎症反应和多器官衰竭。目前研究显示, RIP1/RIP3依赖的程序性细胞坏死与TNF诱导的SIRS致死性相关, Nec-1的预处理以及RIP3的缺失, 均能降低细胞对炎性细胞因子的应答, 提高小鼠的存活率[55-56]。另外, Nec-1对于

阻止小鼠致死的作用是呈Nec-1剂量依赖性的, 因此, Nec-1的药物动力学对于我们在动物模型的观察结果上尤为重要[57]。同时在小鼠体内RIP1激酶活性失活对于TNF诱导的SIRS产生了保护性的效果[58]。

3.1.2 急性胰腺炎(acute pancreatitis) 急性胰腺炎是由多种病因导致胰酶在胰腺内被激活后引起胰腺组织自身消化、水肿、出血甚至坏死的炎症反应。胰腺细胞有凋亡和坏死两种死亡方式, 但是胰腺炎的严重程度与坏死直接相关[59]。在胰腺炎的动物模型中, 可以通过体内激活caspases使胰腺细胞主要发生凋亡, 从而降低胰腺炎的严重程度[60]。同时, 在野生型的胰腺炎小鼠中, RIP3的表达水平上升。*Rip3*和*Mlkl*基因敲除的小鼠与野生型小鼠相比, 蛙皮素诱导的胰腺炎程度降低, 胰腺细胞死亡减少, 血清中淀粉酶水平下降[9,61]。因而证实了程序性细胞坏死参与胰腺炎的病理发展。然而研究显示, Nec-1并没有对胰腺炎的损伤起保护作用, 这可能与Nec-1的药物动力学有关。

3.1.3 炎症性肠病(inflammatory bowel disease, IBD) 炎症性肠病为回肠、直肠、结肠相关的一种特发性肠道炎症性疾病。临床表现为腹泻、腹痛, 甚至血便。主要包括两种类型: 溃疡性结肠炎(ulcerative colitis)和克罗恩病(Crohn' disease)。研究表明, 在小鼠肠上皮细胞中条件性敲除pro-caspases-8, 能导致自发性回肠炎的发生, 并伴有潘氏细胞的死亡, 以及对葡聚糖硫酸钠诱导的结肠炎易感性增加[62]。对体外培养的肠道类器官进行TNF处理诱导细胞死亡, 能够检测到RIP3表达水平上升, 并且能被Nec-1抑制住。在患有克罗恩病的病人末端回肠中可直接检测到RIP3表达水平上升, 因此, 程序性细胞坏死在IBD的发病机制中发挥了潜在作用。同时FADD的缺失也导致了自发IBD样疾病, 表明凋亡蛋白对于程序性细胞坏死具有抑制功能[38]。最近研究也证实了RIP1作为生存因子通过防止肠道细胞凋亡和微生物浸润及继发的炎症来维持肠上皮的完整性。

3.1.4 肺炎(pneumonia) Choi教授实验室[63]发现, 线粒体自噬依赖的细胞坏死促进了慢性阻塞性肺病的病理发展。程序性细胞坏死还被发现参与了特发性肺纤维化的过程。程序性细胞坏死介导了城市大气中的颗粒物质对呼吸道上皮的损伤[64]。另外, 吸烟也被发现是通过程序性细胞坏死促进呼吸道支气管上皮的一种损伤[65]。

3.2 缺血再灌注损伤(ischemia-reperfusion injury, IR)

缺血所引起的组织损伤是许多致死性疾病的主要原因, 诸如冠状动脉硬化导致的心肌梗死、脑中风等, 然而对组织造成损伤的主要因素, 不是缺血本身, 而是恢复血液供应后, 过量的自由基攻击这部分重新获得血液供应的组织内的细胞造成的, 因此这种损伤被称作组织缺血再灌注损伤。目前是通过结扎器官的终动脉来建立IR的动物模型实现对IR的体内研究。有关程序性细胞坏死与IR的关系, 最早是由2005年时人们发现Nec-1能减小缺血性脑损伤的梗死面积而确立的。近年来除了脑损伤还建立了大量的其他器官的IR动物模型, 包括视网膜、心脏、肾脏、肝脏。同样地, Nec-1能减少心肌的梗死面积和炎症[66-67], 以及视网膜[68]、肾脏[69]、肝脏[70]、新生儿低氧缺血脑[71-72]的组织损伤; 而且还检测到Nec-1在缺血的心肌和新生儿低氧缺血的脑中能抑制RIP3的上调, 以及RIP1、MLKL坏死复合体的形成及磷酸化。同时在*Rip3*基因敲除的小鼠中发现, 其肾缺血再灌注损伤程度降低, 且心肌梗死再灌注的重塑过程中肥大和炎症程度降低。因此, 程序性细胞坏死参与了组织缺血再灌注损伤的发生发展。

3.3 肾脏疾病(kidney disease)

程序性细胞坏死广泛参与多种肾脏疾病中。早期Okusa实验室[73]发现, 在急性肾损伤中同时存在凋亡和坏死两种死亡方式。Monte教授实验室[74]发现, 在顺铂诱导的HK-2人肾脏近曲小管上皮细胞损伤中, 加入凋亡抑制剂, 能使肾细胞发生程序性细胞坏死, 且能被Nec-1抑制住。Han教授实验室[75]通过小鼠*Rip3*、*Mlkl*基因敲除, 再次证实了程序性细胞坏死参与顺铂诱导的肾损伤。Ren教授实验室[76]发现, 在双侧输尿管阻塞的小鼠模型中, Nec-1能降低肾脏炎症及纤维化程度。Krautwald教授实验室[77]发现在急性肾衰竭中, 程序性细胞坏死和铁死亡是两种交替的死亡方式, 共同促进肾脏的病理发展。这些研究表明, 程序性细胞坏死的抑制剂可应用于肾脏疾病的治疗中。

3.4 心血管疾病(cardiovascular diseases)

动脉粥样硬化(atherosclerosis, AS)是冠心病、脑梗死、外周血管病的主要原因。早期的研究观察到, 在动脉粥样硬化斑块中存在坏死的巨噬细胞, 直到2013年Han教授实验室[78]展开了程序性细胞坏死与动脉粥样硬化的研究。他们通过在*Ldlr*$^{-/-}$或者

Apoe$^{-/-}$的AS动物模型中, 同时敲除*Rip3*, 发现*Rip3*的缺失对于早起AS没有影响, 但AS晚期病变明显降低。Martinet教授实验室[79]发现, caspase-3缺失能够促进*Apoe*$^{-/-}$的小鼠动脉粥样硬化斑块中内皮细胞和巨噬细胞的坏死, 以及斑块的变大。随后Rayner[80]和Martinet教授实验室[81]提出靶向程序性细胞坏死来预防、干预和治疗动脉粥样硬化疾病。同时, 程序性细胞坏死也参与了腹主动脉瘤的发生。Liu教授实验室[82-83]发现, RIP3通过诱导血管平滑肌细胞坏死和炎症来促进腹主动脉瘤的发生, 并且Nec-1的使用能减轻疾病的进展。另外, Jiang教授实验室[84]提出在急性心肌炎中, 程序性细胞坏死可能是心肌细胞死亡的新型机制。总之, 程序性细胞坏死可能在心血管疾病治疗中成为一个新的重要靶点[85]。

3.5 肝脏疾病

Dimanche-Boitrel教授实验室[86]发现, 在ConA诱导的急性肝炎中, RIP3表达上调, 且Nec-1的使用能降低肝损伤。Trautwein教授实验室[87]发现, 在肝细胞中特异性敲除caspase-8, 能增加ConA诱导的肝损伤。Wirtz教授实验室[88]发现, 在ConA诱导的肝炎动物模型中, MLKL介导了肝细胞不依赖于RIP3的程序性细胞坏死过程。在对乙酰氨基酚诱导的肝炎模型中, Nec-1预处理与后处理以及*Rip3*基因敲除, 都可降低肝损伤[89-90]。在酒精性肝病以及酒精灌胃的小鼠中, 检测到RIP3表达升高, 同时caspase抑制剂并不能阻止小鼠的肝损伤[91-92]。除此之外, *Rip3*基因敲除对于酒精诱导的肝损伤起到保护作用, 但Nec-1的预处理并没有任何影响, 这可能是由于RIP1独立的程序性细胞坏死参与了肝损伤, 也可能与Nec-1的药物动力学相关[93]。非酒精性脂肪性肝炎主要是脂肪的积累或者胰岛素耐受导致的, 它主要与炎症和凋亡导致的肝损伤相联系。然而最近研究显示, poly(I:C)刺激能够诱导肝细胞坏死, 伴有RIP3表达升高; 而且在脂肪性肝炎小鼠模型中, RIP3表达上调[94-95]。Nagy教授实验室[96]发现, RIP3的缺失加重了高脂饮食诱导的肝损伤。

3.6 病原微生物感染(pathogenic microbial infection)

在微生物感染宿主细胞后, 宿主细胞的死亡利于对病原菌及时有效的清除。但是在某些条件下宿主免疫细胞过度的死亡将导致免疫缺陷的发生, 如HIV感染将导致机体CD4$^+$ T细胞大量的死亡。目前研究显示程序性细胞坏死广泛参与了病毒、细菌及寄生虫感染的宿主细胞的死亡过程。

3.6.1 病毒感染(viral infection) 早期研究表明, 牛痘病毒能编码caspase抑制剂B13R/Spi2, 阻止宿主细胞发生凋亡, 但另一方面使细胞向RIP1/RIP3依赖的细胞坏死发展变得更敏感[10]。一旦感染后, 能够在肝细胞中检测到RIP1/RIP3复合体的形成。*Rip3*基因缺失或者RIP1$^{D138N/D138N}$小鼠与WT小鼠相比, 组织损伤和炎性程度都较轻, 但是病毒大量复制, 小鼠致死率增加[58]。该研究显示, 程序性细胞坏死在病毒感染中发挥了先天免疫调控的功能, 对机体产生保护作用。但是病毒介导的宿主细胞程序性细胞坏死的过度发生可能对机体产生致死的效果, 如HIV-1感染的CD4$^+$ T细胞坏死速率较高, 对TNF诱导的细胞死亡高度敏感[97]。同时, 也有大量的研究表明, 其他的病毒感染, 如I型单纯疱疹病毒(HSV-1)[98]、西尼罗河病毒(WNV)[99]、柯萨奇B组病毒(CVB)[100]、呼吸道肠道病毒原型株T3D[101], 也能导致程序性细胞坏死的发生。Nec-1的预处理能减轻这些病毒感染诱导的细胞死亡。但并不是所有的病毒感染都会促进宿主细胞坏死。有一些病毒如巨细胞病毒(murine cytomegalovirus, MCMV)会抑制细胞坏死, 使其能有效地增殖和扩散。MCMV编码的vIRA不仅是凋亡抑制剂, 同时也是坏死抑制剂, 它能阻止病毒感染的细胞的死亡[103]。将vIRA的RHIM结构域突变后, RIP3缺失能阻止MCMV感染诱导的细胞坏死。最近研究显示, DNA感受器DAI也通过RHIM结构域与RIP3相互作用, 介导MCMV诱导的坏死[104]。另外, 马的2型疱疹病毒E8和人的疱疹病毒K13也具有抑制死亡受体介导的坏死的功能[105]。

3.6.2 细菌感染(bacterial infections) 程序性细胞坏死也参与细菌的感染过程。研究显示, 致病性大肠杆菌EPEC能表达致病效应蛋白NleB1, 它能修饰含有DD死亡结构域的蛋白如RIP1和FADD的精氨酸残基, 从而阻止凋亡和程序性细胞坏死的发生, 而NleB1缺失的EPEC不能成功定植于宿主细胞[107]。RIP3的缺失, 尤其是联合FADD或者caspase-8的缺失, 将促进对于Yersinia细菌的易感性[109]。然而金黄色葡萄球菌毒素诱导的肺上皮细胞RIP3依赖的细胞坏死却是致死性的[110]。

3.6.3 寄生虫感染(parasitic infection) 利什曼病是由利什曼原虫引起的人畜共患病, 可引起人类皮肤及内脏黑热病。研究显示, 在利什曼原虫感染的巨

噬细胞中, 程序性细胞坏死信号通路被激活, 从而控制利什曼原虫的复制[111]。

3.7 肿瘤(tumor)

细胞死亡与增殖在多细胞生物中维持着动态的平衡。过度的细胞死亡将导致炎性、退行性疾病的发生, 而细胞过度的增殖则促进肿瘤的形成, 对机体正常稳态的维持都产生着不利的影响。近年来随着我们对程序性细胞坏死的认识不断加深, 我们猜想, 激活肿瘤细胞中程序性细胞坏死信号通路可能会促进肿瘤细胞的死亡, 达到治疗癌症的效果。于是, 开启了研究程序性细胞坏死与肿瘤关系的新领域。

3.7.1 *肿瘤发生及转移* 程序性细胞坏死在肿瘤细胞中扮演着错综复杂的角色, 有研究显示, 程序性细胞坏死抑制肿瘤的发生, 相反也有研究表明, 程序性细胞坏死促进肿瘤的发生及转移(表1)。通常一些癌细胞系缺失程序性细胞坏死信号途径, 在原发人类肿瘤如乳腺癌[112]、结肠癌[113]、急性髓系白血病[114]、多种黑色素瘤细胞中检测到RIP3缺失或者表达下调。Jost教授实验室[115]发现, 在FLT3-ITD急性髓系白血病动物模型中, RIP3信号途径是抑制肿瘤发生的关键机制, 其中白血病起始细胞对于RIP3依赖的细胞死亡高度敏感, RIP3缺失将加速白血病的发生及小鼠的死亡, RIP3缺失将促进白血病前造血干细胞和祖细胞的存活以及抑制白血病起始细胞的分化。Luedde教授实验室[116]发现, 在肝细胞特异性敲除*Tak1*的肝肿瘤模型中, 激活RIP3将抑制肿瘤的生长。同时, 在结肠癌病人以及患有IBD的肿瘤患者中, RIP3水平下降[117]。RIP3缺失的小鼠对于结肠炎相关的结肠癌拥有很高的易感性[117]。相似地, 在生存率比较低的胰腺癌[118]、结肠癌[119]、宫颈鳞癌患者中[120], MLKL水平较低。因此认为, RIP3和MLKL可能是两个关键的肿瘤抑制蛋白。对于RIP3和MLKL抑制肿瘤的机制, 有研究显示, 在多种不同的乳腺癌亚型中, MLKL的表达与肿瘤细胞微环境中B细胞、NK细胞和T细胞的存在呈正相关, 而RIP3的表达只在$HER2^+$的乳腺癌中与淋巴细胞的存在呈正相关。这可能是RIP3/MLKL信号途径影响了肿瘤的免疫原性, 从而调控了肿瘤微环境。另外在多种癌细胞系中, 发现RIP3高度甲基化。

不同的是, Miller教授实验室[121]发现, 在人的胰腺导管上皮癌中, RIP3/MLKL高表达。RIP3/MLKL通过产生了CXCL1和Mincle诱导的肿瘤免疫微环境从而促进胰腺癌的发生。另外, Li教授实验室[112]发现, 在一些乳腺癌细胞系中, *Rip3*基因敲除或者MLKL抑制剂NSA的使用均会降低这些癌细胞在体内的成瘤率。同时还发现, 在一些人的食管癌及结肠癌中, 高表达的磷酸化的MLKL水平与病人的低存活率相关。在炎症相关结直肠癌中, RIP1抑制剂Nec-1的使用抑制了肿瘤的生长[122]。

程序性细胞坏死除了在肿瘤发生中有重要作用, 还有促进肿瘤细胞转移的功能。Strilic和Offermanns教授实验室[123]发现, 肿瘤细胞上的淀粉样前体蛋白与内皮细胞上的死亡受体DR6相互作用诱导内皮细胞的坏死从而促进肿瘤的转移, 其中使用Nec-1或者在内皮细胞中特异性敲除RIP3都将减少内皮细胞的坏死、肿瘤细胞的渗出及转移。然而Wong教授实验室[124]发现, RIP1/RIP3增加血管的通透性促进肿瘤细胞的渗出是不依赖于它的促坏死功能的, RIP3激酶活性丢失和MLKL的敲除并不影响肿瘤细胞肺转移的效率。他们发现, RIP3缺失的内皮细胞对一些渗透因子的反应性降低, 如血管内皮生长因子, 导致血管通透性降低。最近的研究报道, 死亡细胞的微环境会影响肝脏肿瘤生成的亚型; 凋亡性的微环境会促使肝细胞向肝细胞癌转化; 坏死性的微环境会促使肝细胞向肝内胆管癌转化[125]。

3.7.2 *抗肿瘤治疗*(anti-tumor therapy) 一般认为, 激活肿瘤细胞程序性细胞坏死对肿瘤治疗有两个优势: 一是可以克服caspase-8缺失的肿瘤细胞的凋亡抵抗, 二是坏死的肿瘤细胞具有免疫原性和促进炎症的性质, 会诱导机体产生抗肿瘤免疫。研究显示, 坏死的纤维瘤mL929细胞不能诱导巨噬细胞促炎因子的释放[126], 黑色素瘤细胞mB16F10的坏死不能诱导树突状细胞DC的成熟[127], 而坏死的hC4-I宫颈癌细胞、mCT26结肠癌细胞、mNIH 3T3小鼠胚胎成纤维细胞系等能促进DC细胞的激活。也有研究报道, 程序性细胞坏死的发生发挥着抑制炎症的功能, 认为TNF本身具有高度促炎的功能, TNF刺激会导致大量细胞因子和趋化因子的产生, 而快速的细胞坏死使得减慢和减少这些炎性因子的释放[128]。而在mCT26、hHT29等肿瘤动物模型中, 基于四环素诱导的基因表达系统特异性诱导肿瘤细胞程序性细胞坏死的发生, 能有效减少肿瘤

表1 程序性细胞坏死在肿瘤发生发展及转移中的作用

Table 1 The role of RIP1, RIP3 and MLKL in tumorigenesis and metastasis

小鼠肿瘤模型 Mouse tumor model		细胞坏死相关基因期对肿瘤生长的影响 Necroptosis gene and effects on tumor growth/volume						参考文献 References
		Rip1	Effect	*Rip3*	Effect	*Mlkl*	Effect	
Mouse genetic models	TAK1/Caspase-8^{LPC-KO} mice	/	/	KO	Inhibit	/	/	[116]
	Pancreatic ductal adenocarcinoma by expressing Kras	Inhibitor Nec-1	Inhibit	KO	Inhibit	/	/	[118,121]
	RIP3$^{-/-}$*FADD*$^{-/-}$ *MLKL*$^{-/-}$*FADD*$^{-/-}$	/	/	KO	Promote	KO	Promote	[48-49]
	FLT3- ITD- driven myeloproliferative disorder	/	/	KO	Promote	/	/	[115]
Chemically induced mouse models	Colitis associated colorectal cancer	Inhibitor Nec-1	Inhibit	KO	Promote	/	/	[117,119,122]
Xenograft mouse models	MDA-MB-231, 4T1	KO in the tumor cells	Inhibit	KO in the tumor cells	Inhibit	KO in the tumor cells	Inhibit	[112]
	B16, LLC1	Inhibitor Nec-1	Inhibit	KO in the endothelial cells	Inhibit	/	/	[123-124]
	DAI-3b	/	/	Overexpression of RIP3 in the tumor cells	Inhibit	/	/	[114]
	CT26	/	/	Overexpression of RIP3 in the tumor cells	Inhibit	/	/	[113]

的体积[130]。另外, Vandenabeele教授实验室[131]发现, 将坏死的肿瘤细胞注射入机体内, 能有效地激活抗肿瘤免疫反应, 检测到细胞毒T细胞的浸润以及肿瘤抗原的再刺激特异性IFNγ的产生。因此, 坏死的肿瘤细胞或许能够作为肿瘤疫苗应用于抗肿瘤治疗中。

3.8 神经系统相关疾病

3.8.1 阿尔兹海默症(Alzheimer's disease, AD) 阿尔兹海默症是以学习和记忆障碍为特征的神经退行性疾病。这种认知障碍是由渐进性神经元和突触丢失引起的脑萎缩。目前将AD的经典标志如淀粉样蛋白-β(Aβ)的聚集和tau的超磷酸化作为治疗的重要靶点。

Kim教授实验室[132]发现, Nec-1可直接靶向Aβ和tau蛋白, 缓解脑细胞死亡并改善AD模型中的认知障碍。在APP与PS1双转基因小鼠的皮质和海马体中, Nec-1处理后降低了Aβ寡聚体, 斑块和过磷酸化tau蛋白的水平, 但不影响Aβ的产生。可改善小鼠学习和记忆障碍。Oddo教授实验室[133]发现, 程序性细胞坏死在死后的人类AD脑中处于被激活的状态, 与Braak阶段呈正相关, 与脑重量和认知评分呈负相关, 在AD的小鼠模型中降低程序性细胞坏死能够减少神经细胞损失。Rip1调控的基因组与多个AD独立的转录组特征重叠, 表明RIP1活性与AD中大部分转录组变化相关联。

3.8.2 帕金森综合征(Parkinson's disease, PD) 帕金森综合征(Parkinson's disease, PD)是一种常见的神经退行性疾病, 特征为黑质中多巴胺能神经元的丢失和α-突触核蛋白在神经元中的积累。 Cheng教授实验室[134]发现, Nec-1可以抑制坏死并给予多巴胺能神经元保护。Broccoli教授实验室[135]发现, 线粒体功能障碍引起程序性细胞坏死并导致神经元细胞缺失。抑制程序性细胞坏死可有效地抑制小鼠中由线粒体功能障碍引起的多巴胺能神经元的死亡。

3.8.3 肌萎缩性脊髓侧索硬化(amyotrophic lateral sclerosis, ALS) 肌萎缩性脊髓侧索硬化是一种难以治愈的成人发作性麻痹症, 特征为脊髓、皮质和脑干中的运动神经元变性, 导致肌肉萎缩和瘫痪。Przed-

borski教授实验室[136]发现, 程序性细胞坏死是散发性和家族性肌萎缩性侧索硬化中神经退变的关键机制。散发性和家族性ALS中星形胶质细胞以程序性细胞坏死依赖性方式引起神经元死亡。使用Nec-1可减轻ALS小鼠模型中运动神经元损失。Yuan教授实验室[137-138]发现, RIP1可调节程序性细胞坏死与炎症之间的关系。RIP1为ALS中轴突病理学的常见介质, 阻断RIP1可能作为治疗ALS的有效干预措施。

3.8.3 多发性硬化症(multiple sclerosis, MS) 多发性硬化症是中枢神经系统中的一种慢性炎症性脱髓鞘疾病, 有较高的残疾和死亡率。Yuan教授实验室[139-140]发现, MS小鼠模型中RIP1、RIP3和MLKL特异性高表达, 并且可在MS患者皮层损伤的病理样品中介导少突胶质细胞变性或死亡。

3.8.4 脊髓损伤(spinal cord injury, SCI) 脊髓损伤是一种缺乏有效治疗方法的常见严重创伤。其病理特征是病变区域的逐渐扩大, 通常会形成腔并伴有反应性星形细胞胶质化和慢性炎症。Feng教授实验室[141]发现, Nec-1可通过抑制RIP1-RIP3-MLKL蛋白复合物的募集来抑制坏死, 并通过激活B淋巴细胞瘤-2基因(B cell lymphoma-2, *Bcl-2*)的同时抑制caspase3来抑制细胞凋亡。SCI大鼠模型的行为学表现证实了Nec-1对脊髓损伤后的生理功能具有改善和保护作用。Wang教授实验室[142-143]发现在SCI后, 在腔周围的反应性星形胶质细胞发生了程序性细胞坏死, 而不是凋亡和自噬。通过细胞表面Toll样受体(Toll-like receptor, TLR)信号传导, M1小胶质细胞与巨噬细胞诱导星形胶质细胞发生坏死。抑制RIP1或耗尽RIP3不仅可显著性减少星形胶质细胞死亡, 还可以保护星形胶质细胞的神经营养功能。

3.8.5 中风(stroke) 中风目前没有有效的治疗方法。大脑出血后由于原发性和继发性损伤会导致对神经元的不可逆损伤, 从而导致残疾或死亡。Ratan教授实验室[144]发现, 铁死亡和程序性细胞坏死的化学抑制剂可抑制血红蛋白和氯化血红素诱导的神经毒性。铁死亡和程序性细胞坏死抑制剂各自能抑制超过80%的细胞死亡。Dhandapani教授实验室[145-146]发现, 在脑出血小鼠模型中Nec-1具有显著减少血肿体积与神经元细胞死亡的作用, 并且可以降低无功能星形胶质细胞的增殖和神经血管的损伤。

3.9 对神经细胞的作用

神经发生是一个动态过程, 神经祖细胞、干细胞的分化与增殖和程序性死亡信号密切相关(表2)。正确理解程序性死亡信号如何影响神经发生是研究大脑健康和相关疾病的重要基础。Ahmadian教授实验室[147]发现, 程序性细胞坏死在海马体中活跃的时间更长, 大脑皮层区域中程序性细胞坏死调控因子较少。通过观察海马体和额皮质中进一步效应, 发现程序性细胞坏死对额皮质的影响较小。因此, 与大脑的其他区域相比, 海马体更容易发生程序性细胞坏死。 He教授实验室[148]发现了程序性细胞坏死在肿瘤坏死因子TNF诱导下产生对神经系统的毒性。在脑室内注射TNF后, 小鼠海马体中程序性细胞坏死被激活。RIP3缺失可减弱TNF引起的海马神

表2 程序性细胞坏死在神经系统相关疾病中的作用

Table 2 The role of necroptosis in neurological related diseases

疾病 Disease	程序性坏死的作用 The role of necroptosis	参考文献 References
Alzheimer's disease, AD	Nec-1 directly targets Aβ and tau proteins, alleviates brain cell death and ameliorates cognitive impairment in AD models	[132-133]
Parkinson's disease, PD	Nec-1 can elevate the viability of cells and protect dopaminergic neurons	[134-135]
Amyotrophic lateral sclerosis, ALS	RIP1 knockdown or treatment with MLKL inhibitor NSA can promote survival of adjacent motor neurons	[136-138]
Multiple sclerosis, MS	Necroptosis mediates oligodendrocyte degeneration and inhibition of RIP1 protects oligodendrocyte against cell death	[139-140,151]
Spinal cord injury, SCI	Inhibition of RIP1 or RIP3 reduces astrocyte cell death and Nec-1 enhances neuron viability	[141-143]
Stroke	Nec-1 reduces hematoma volume and limits neuron cell death significantly and improves neuron behavior	[144-146]
Hypoxia ischemia, HI	Inhibition of MLKL alleviates brain damage induced by hypoxia ischemia	[152-153]
Traumatic brain injury, TBI	RIP1-RIP3-MLKL mediated necroptosis occurs after TBI	[154-155]

经元损失。Chua教授实验室[149]发现, Nec-1可抑制程序性细胞坏死, 对海马系细胞HT22中谷氨酸诱导的氧化作用具有保护作用。Wang教授实验室[150]在最近的研究中发现在坐骨神经损伤后, MLKL被诱导靶向施万细胞的髓鞘膜以促进髓鞘分解。MLKL在破坏髓鞘的功能时需要磷酸化丝氨酸441位点, 这与RIP3激酶诱导坏死性信号不同。在施万细胞(Schwann cell)中特异性敲除*Mlkl*的小鼠显示髓鞘的破坏被延迟。MLKL缺失会减少损伤后的神经再生, 而MLKL过度表达则会加速髓鞘分解并促进轴突再生。此外, Wang教授实验室[151]还发现, 在使用实验性自身免疫脑脊髓炎和化学诱导的小鼠脱髓鞘模型研究中枢神经系统脱髓鞘过程中, MLKL在中枢神经系统中以不依赖于necroptosis的方式促进脱髓鞘。RIP1激酶抑制剂在免疫诱导的脱髓鞘模型中可阻断多发性硬化症的进展。

3.10 在其他疾病中的作用

程序性细胞坏死在许多其他人类疾病与动物模型中也有重要作用。戈谢病 (Gaucher's disease, GD)是由葡糖脑苷脂酶基因突变引起的遗传性代谢疾病, 是最常见的溶酶体贮积病。Futerman教授实验室[152]发现, 调节RIP3通路能显著改善GD小鼠模型中的神经系统病变, 且RIP3缺陷可提升GD小鼠的生存和运动协调能力, 对脑和肝损伤产生保护作用。

Mu教授实验室[153]发现, 抑制*Mlkl*可减少RIP1-RIP3-MLKL相互作用从而减少神经元死亡。寡聚化MLKL向神经元的膜转运导致细胞膜损伤可能是神经细胞坏死的新机制。在新生大鼠中抑制*Mlkl*可减轻缺氧缺血引起的脑损伤。这些发现表明, 靶向MLKL可能作为大脑发育时发生脑损伤的治疗方法。Jiang教授实验室[154-155]发现, 在实验性创伤性脑损伤(traumatic brain injury, TBI)后会发生程序性细胞坏死, 其通过治疗性低温可以靶向TBI后的程序性细胞坏死来保护受损的中枢神经系统, 使其免受组织损伤和炎症反应。此外, Jevnikar和Zhang教授实验室[156]发现, RIP1和RIP3通过程序性细胞坏死促进了心脏同种异体移植物的存活, 延长了移植物的存活时间, 并指出了移植前在供体移植物中靶向RIP1或RIP3可能是器官移植中的重要治疗策略。

Wang教授实验室[157]发现, 野生型小鼠睾丸的精原干细胞中RIP3发生磷酸化。*Rip3*和*Mlkl*敲除小鼠在进入老年后其雄性生殖器官仍保持年轻的形态和功能。当年轻野生型小鼠的睾丸被给予局部坏死刺激时, 它们的生殖器官显示加速老化。但给它们摄入RIP1抑制剂后, 则阻止了其生殖器官老化迹象的出现。表明雄性小鼠睾丸中的程序性细胞坏死可促进其生殖系统的衰老。此外, 使用二氧化硅纳米颗粒可诱导精原细胞产生RIP1依赖的凋亡和坏死[158]。Rodrigues教授实验室[159]发现, 在人类原发性胆汁胆管炎患者的肝脏样本中RIP3表达增加及MLKL发生磷酸化, 表明样本中的程序性细胞坏死被激活。在小鼠胆总管结扎实验中也发现了程序性细胞坏死的明显标志, 并伴随进行性胆管增生、多灶性坏死、纤维化和炎症等多种症状。此项研究表明, 靶向程序性细胞坏死可能是急性胆汁淤积的治疗策略。

4 总结及展望

程序性细胞坏死是一种caspase非依赖性的细胞死亡形式, 主要由肿瘤坏死因子受体家族与细胞表面Toll样受体家族启动, 由RIP1、RIP3、PARP-1、ROS、Ca^{2+}及多种酶参与程序性细胞坏死的传递和执行。在没有凋亡信号传导的情况下, RIP1作为细胞程序性细胞坏死的开关, 由RIP1和RIP3通过磷酸化激活MLKL, 形成RIP1-RIP3-MLKL复合体启动细胞程序性细胞坏死。此外, 细胞内ROS、Ca^{2+}、NO等物质的增加都能引起细胞发生程序性细胞坏死。程序性细胞坏死在如神经退行性疾病、心脏和肾脏的缺血再灌注损伤、炎症、败血症、多种实体器官损伤等多种疾病中有重要的病理或生理学意义。越来越多的证据表明, 程序性细胞坏死信号抑制与上调可影响疾病的发生与发展, 在多种疾病动物模型中RIP1抑制剂Nec-1的使用被证明可影响缺血再灌注损伤、神经退行性疾病和炎症等多种疾病过程。表明了程序性细胞坏死可作为多种疾病潜在的治疗靶点。可以预见对程序性细胞坏死分子机制及其抑制机制的深入研究, 对研究不同疾病的发生机制具有重要意义。进一步对抗坏死性小分子抑制剂的研究对寻找预防和治疗多种疾病的新靶点和相关药物都具有临床意义。

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