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微囊藻毒素诱导细胞自噬的研究进展

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摘要: 微囊藻毒素是由蓝藻产生的次生代谢产物, 具有很强的肝毒性、肾毒性、生殖毒性及神经毒性等。而自噬作为维持细胞稳态的自我更新机制, 在应对有毒物质胁迫时发挥着重要作用, 最新的研究报道表明微囊藻毒素可诱导细胞自噬。目前, 微囊藻毒素诱导细胞自噬的研究, 主要以肝细胞、肾细胞、性腺细胞及神经细胞为研究对象。本文综述了微囊藻毒素对上述细胞自噬诱导的研究进展, 且从氧化应激途径和内质网应激途径探讨微囊藻毒素诱导细胞自噬的机制, 并对今后研究方向进行了展望, 可为全面剖析微囊藻毒素的毒性机理以及自噬在其中发挥的具体作用提供参考。

关键词: 微囊藻毒素; 自噬; 氧化应激; 内质网应激

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Research Progress on Autophagy Induced by Microcystins

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Abstract: Microcystins, the secondary metabolites produced by cyanobacteria, have strong hepatotoxicity, renal toxicity, reproductive toxicity, neurotoxicity, etc. Autophagy is considered to be a self-renewal mechanism to maintain cell homeostasis, and plays important roles under toxic stress. The latest researches indicate that microcystins can induce the autophagy. At present, the studies on autophagy induced by microcystins mainly focused on hepatocytes, renal cells, gonadal cells and neurocytes, which are summarized in this review. Besides, the mechanisms of autophagy induced by microcystins have been investigated from oxidative stress and endoplasmic reticulum stress signaling pathways. Moreover, the further studies on autophagy induced by microcystins are prospected in the present paper. Overall, this review will provide references for the comprehensive analysis of the toxic mechanisms of microcystins as well as the specific role of autophagy.

Keywords: microcystins; autophagy; oxidative stress; endoplasmic reticulum stress

随着工农业的迅速发展, 由水体富营养化引起的蓝藻水华不仅会恶化水质, 更为严重的是, 蓝藻细

胞死亡后所释放的藻毒素可富集于水生生物体内, 随后通过食物链的传递危害人类健康^[1]。在众多藻

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毒素中,微囊藻毒素(microcystins, MCs)为毒性最强、分布范围最广的毒素之一,其主要由微囊藻属(*Microcystis*)、鱼腥藻属(*Anabaena*)、念珠藻属(*Nostoc*)、束丝藻属(*Aphanizomenon*)以及颤藻属(*Oscillatoria*)等蓝藻产生^[2-3]。MCs进入机体内依靠多种有机阴离子转运多肽(organic anion transporting polypeptides, OATPs)的转运,研究显示,OATPs在肝脏、肾脏、性腺和脑等组织中均存在表达^[4]。因此,MCs能进入肝脏、肾脏、性腺及脑组织中而发挥其肝毒性^[5]、肾毒性^[6]、生殖毒性^[7]及神经毒性^[8]作用。

自噬在所有真核生物的进化上高度保守,是细胞内一种重要的溶酶体依赖性降解机制^[9]。根据内容物质运输到溶酶体途径的不同,细胞自噬可以分为微自噬(microautophagy)、分子伴侣介导的自噬(chaperone mediated autophagy, CMA)和巨自噬(macroautophagy)^[10]。微自噬是溶酶体膜直接内陷吞噬胞质成分,而分子伴侣介导的自噬是在分子伴侣(HSC70)的作用下溶酶体降解含有 KFERQ 序列的可溶性胞质蛋白底物^[11]。巨自噬即通常所说的自噬,是指具有双层膜结构的自噬体(autophosome)包裹着胞浆成分与溶酶体融合形成自噬溶酶体(autopholysome),最终胞浆成分被溶酶体酶降解的过程。基础水平的自噬能清除细胞内衰老细胞器及长寿蛋白以维持细胞稳态^[12]。然而,过低或过高水平的细胞自噬都会导致细胞不可逆死亡即Ⅱ型程序性细胞死亡(programmed cell death Ⅱ, PCD Ⅱ)^[13]。本文就MCs诱导细胞自噬的相关研究进行综述,并总结其可能存在的诱导机制,以期为进一步明确自噬在MCs的细胞毒性中发挥的作用提供理论基础。

1 微囊藻毒素及其毒性机制 (Microcystins and their mechanisms of toxicity)

MCs是一类相对分子质量在800~1100 Da之间的单环七肽化合物,其主要由非核糖体肽合成酶(nonribosomal peptide synthetases, NRPSs)、聚酮合成酶(polyketide synthases, PKSs)和其他杂合酶等多功能蛋白复合体合成^[14]。该化合物的结构通式为环(D-Ala¹-L-X²-D-isoMeAsp³-L-Z⁴-Adda⁵-D-isoGlu⁶-Mdha⁷),其中,2,4号位的X、Z为2种可变的L型氨基酸^[15-16]。在已鉴别的100多种MCs异构体中,MC-LR、MC-RR和MC-YR为分布广泛且毒性较强的3种异构体,其中L、R和Y分别代表亮氨酸、精氨酸和酪氨酸^[17]。MCs理化性质稳定,耐酸碱、紫外线辐射及高温,常规处理方法(如混凝、沉淀、絮凝

和过滤等)很难有效去除水体中的MCs^[18]。因此,MCs对水体环境和人群健康的危害已成为全球关注的重大环境问题。

据报道,MCs的毒性作用机制主要是其Adda基团与丝/苏氨酸蛋白磷酸酶1和2A(PP1和PP2A)催化亚基的半胱氨酸残基共价结合,从而阻止其他底物进入磷酸酶的活性中心^[19-20],进而影响PP2A对细胞内多种反应如细胞增殖和死亡、细胞迁移及细胞信号通路的调控^[21]。MCs还能通过促进活性氧(reactive oxygen species, ROS)的生成或消耗还原型谷胱甘肽(GSH)造成细胞氧化应激、脂质过氧化、DNA氧化损伤和细胞骨架改变等最终引起细胞凋亡^[22-23]。研究表明MC-LR还能通过TLR/MyD88信号通路引起机体炎症^[24]。此外,MC-LR诱发的DNA甲基化作用和miRNA调控基因的改变在癌症的形成、增殖、侵袭、迁移和代谢中发挥重要作用^[25]。尽管国内外许多学者进行了大量关于MCs毒性研究,但仍难以全面揭示其毒性机制,目前有研究指出MCs诱导细胞自噬也可能是MCs的毒性机制之一^[26]。

2 微囊藻毒素诱导细胞自噬的研究 (Studies on autophagy induced by microcystins)

现有报道主要以肝细胞、肾细胞、性腺细胞和神经细胞为研究对象,开展了MCs诱导细胞自噬的研究。下面将主要从MCs对不同细胞自噬的影响以及机制进行综述。

2.1 微囊藻毒素诱导细胞自噬

2.1.1 微囊藻毒素诱导肝细胞自噬

肝脏是MCs作用的靶器官,主要是由于肝脏中存在多种高表达的OATPs,如OATP1 B1、OATP1 B3和OATP1 A2等,MCs在这些OATPs的主动转运作用下进入肝细胞,造成严重的肝损伤^[27]。大量报道表明,MCs可导致哺乳类^[28-29]、鱼类^[30-31]和两栖类^[32]等动物肝脏超微结构和多种酶活性的改变,并诱导肝细胞凋亡或坏死,从而导致机体肝功能障碍。近年来,研究者发现MCs在造成肝细胞损伤的同时启动了细胞自噬。如Dabholkar和Carmichael^[33]发现MC-LR除了可诱导小鼠肝细胞内质网和线粒体肿大、空泡化及脂滴的形成之外,还诱导了含颗粒状物质或碎片的自噬泡的产生,推测MC-LR诱导了肝细胞自噬。Menezes等^[34]将人肝癌(HepG2)细胞暴露于MC-LR(6、12和25 mg·L⁻¹)24 h后,利用透射电镜可观察到MC-LR诱导HepG2细胞自噬体的产生,但数量随着浓度的增加而减少,而高浓度MC-LR诱导

的细胞凋亡及坏死显著增加。吴珍等^[35]在饮用水中添加 MC-LR 对小鼠染毒 3 个月,发现 MC-LR 诱导的小鼠肝脏自噬相关基因 *LC3 α* 转录水平的增加与线粒体膜电位的下降存在相关性,认为在线粒体膜电位消失的同时,细胞启动了自我保护机制。由以上报道可知,MCs 可诱导肝细胞自噬的发生。

2.1.2 微囊藻毒素诱导肾细胞自噬

研究表明,肾脏中也存在多种 OATPs,致使 MCs 容易造成肾脏损伤^[36]。非洲绿猴肾(Vero-E6)细胞经 5、20 和 40 $\text{mg}\cdot\text{L}^{-1}$ MC-LR(LMECYA113 提取物)胁迫 24、48 和 72 h 后,MC-LR 对 Vero-E6 细胞溶酶体、内质网、线粒体和细胞骨架的损伤具有时间-剂量依赖性,低浓度的 MC-LR 能诱导 Vero-E6 细胞内质网和线粒体自噬,而较高浓度则会导致 Vero-E6 细胞凋亡及坏死^[37]。Menezes 等^[34]将 Vero-E6 细胞分别经 6、12、25 和 50 $\text{mg}\cdot\text{L}^{-1}$ MC-LR(LMECYA110 提取物)暴露 24 h,发现低浓度 MC-LR 诱导 Vero-E6 细胞自噬,而高浓度主要导致 Vero-E6 细胞坏死。综上所述,MC-LR 能诱导肾细胞自噬且自噬水平与 MC-LR 的浓度有关。

2.1.3 微囊藻毒素诱导性腺细胞自噬

MC-LR 可作为一种内分泌干扰物造成机体生殖系统功能紊乱而影响动物机体的繁殖、生长和发育^[38],而细胞自噬与 MC-LR 的生殖毒性密切相关。Adegoke 等^[39]报道 MC-LR 可引起牛睾丸支持(Sertoli)细胞线粒体损伤并诱导线粒体自噬。Chen 等^[26]利用不同浓度 MC-LR 对大鼠 Sertoli 细胞染毒后,发现低浓度 MC-LR 能诱导细胞自噬,而高浓度 MC-LR 可通过影响溶酶体的功能而导致自噬体堆积,随后下调 Bcl-2 蛋白表达水平促进 Sertoli 细胞凋亡。另有学者发现,MC-LR 可显著抑制中国仓鼠卵巢(Chinese hamster ovary, CHO)细胞增殖,并诱导 CHO 细胞自噬^[40]。在 Liu 等^[41]的研究中,不同浓度(8.5、17 和 34 $\text{mg}\cdot\text{L}^{-1}$)MC-LR 可促进小鼠卵巢颗粒(KK-1)细胞内 ATG5、ATG12、Beclin1 及 LC3 II/LC3 I 的蛋白表达而诱导细胞自噬。

2.1.4 微囊藻毒素诱导神经细胞自噬

MC-LR 具有神经毒性,可通过血脑屏障进入脑组织^[8]。慢性 MC-LR 暴露可引起小鼠神经细胞凋亡、记忆丧失和学习障碍,从而引起阿尔茨海默病样症状^[42]。研究显示,自噬在神经系统疾病中具有重要的调控作用,而 MC-LR 诱导神经细胞自噬的研究也有相应报道。为确定 MC-LR 诱导的自噬是否

为神经毒性的潜在机制,Yang 等^[43]利用人神经母细胞瘤(SK-N-SH)细胞经 15 $\text{mg}\cdot\text{L}^{-1}$ 和 30 $\text{mg}\cdot\text{L}^{-1}$ MC-LR 处理 48 h 后,发现 MC-LR 可显著抑制 SK-N-SH 细胞活性,自噬相关蛋白 LC3 II/LC3 I 和 p62 表达的升高均呈浓度依赖性,此外,与对照组相比,MC-LR 处理组细胞内游离 Ca^{2+} 浓度显著升高,认为由 MC-LR 引起的细胞存活率降低可能与自噬通量的抑制和细胞内游离 Ca^{2+} 浓度的增加有关。对神经细胞株 PC12 细胞进行 MC-LR(1、25 和 50 $\text{mg}\cdot\text{L}^{-1}$)染毒 24 h 后,发现 MC-LR 处理组细胞内 ROS 水平显著提高,线粒体膜电位显著降低,线粒体自噬相关基因 *PINK1* 和 *Parkin* 表达呈剂量依赖性上升,推测 MC-LR 通过氧化应激激活 *PINK1/Parkin* 线粒体自噬,参与 MC-LR 致 PC12 细胞损伤^[44]。然而,MC-LR 引发的自噬在神经系统中的作用机制还有待深入研究。

2.2 微囊藻毒素诱导细胞自噬的机制

细胞自噬发生过程由多种蛋白参与,首先机体受到刺激后在 ULK1-ATG13-ATG101 和 Beclin1-Vps34 蛋白复合物的作用下自噬体成核延伸,随后在 ATG12-ATG5-ATG16L1 和 ATG8(LC3)-PE 这 2 个泛素结合系统的参与下形成自噬体,最终通过相关蛋白识别机制与溶酶体融合形成自噬溶酶体进行底物降解^[45]。该过程受多条上游信号通路的调控,如哺乳动物雷帕霉素靶蛋白(mammalian target of rapamycin, mTOR)通过 AMPK-mTORC1、PI3K/AKT/mTORC1 和 Ras/ERK/RSK/mTORC1 等信号通路调控自噬相关蛋白 ULK1 复合物,从而进一步调节自噬水平^[46]。Beclin1(酵母 Atg6 同源物)信号通路在自噬调节中发挥重要作用,Beclin1 蛋白中 BH3(Bcl-2-homology3)、中央螺旋区(CCD)和进化保守区(ECD)3 个结构域可与其他因子如 Bcl-2/XL、DAPK、Mst1、JNK1 和 Class III PI3K 等相互作用,影响细胞自噬^[47]。而 *P53* 作为一个主要的抑癌基因,可通过调控 AMPK 和 Akt/mTOR 通路及 *ATG12* 的表达来调节自噬^[48]。此外,细胞应激通路也参与细胞自噬的调控^[49]。其中,细胞应激通路主要包括线粒体氧化应激和内质网应激 2 条途径。目前,认为 MCs 对细胞自噬效应的诱导主要通过诱发细胞应激而实现,即与线粒体氧化应激和内质网应激途径有关。

2.2.1 线粒体氧化应激途径

MCs 能造成细胞线粒体形态损伤、脂质过氧化以及线粒体膜电位变化,并能抑制烟酰胺腺嘌呤二核苷酸(nicotinamide adenine dinucleotide, NADH)脱

氢酶活性和增强琥珀酸脱氢酶(succinate dehydrogenase, SDH)活性,进而影响线粒体电子传递链的正常功能,致使细胞产生过多的 ROS 如超氧阴离子($O_2^{\cdot-}$)、羟自由基($\cdot OH$)和过氧化氢(H_2O_2)等^[50]。目前,大量文献表明,ROS 可通过活化自噬的不同阶段的多条信号通路来调控自噬^[51-52]。例如,研究显示,ATG4 可使 ATG8-PE 水解从而分解自噬体,而 ROS 可抑制 ATG4 半胱氨酸蛋白酶活性从而促进 ATG8 脂化进而促进细胞自噬^[53]。ROS 通过 ATM 依赖机制活化 AMPK 而抑制 mTORC1 从而激活 ULK1,进一步诱导细胞自噬^[54]。ROS 还能通过激活 BNIP3 和 HMGB1 的表达而促进这 2 种蛋白与 Bcl-2 竞争性结合,或活化 JNK 而介导 Bcl-2 磷酸化,从而活化 Beclin-1 诱导细胞自噬^[55]。此外,ROS 氧化低密度脂蛋白可上调 *Beclin1* 基因表达而活化细胞自噬^[56]。目前,已有的研究报道证实,MC-LR 诱导的细胞自噬与氧化应激密切相关。Liu 等^[41]利用 MC-LR 处理小鼠卵巢颗粒(KK-1)细胞和小鼠卵巢组织后检测到 ROS 含量显著上升,而在 ROS 抑制剂 N-乙酰半胱氨酸(N-acetyl-L-cysteine, NAC)联合 MC-LR 共同作用后,其自噬相关蛋白 LC3 II/LC3 I、ATG12、ATG5-ATG12、ATG16 和 Beclin1 的表达下调,即氧化应激介导了 MC-LR 诱导细胞自噬。而对 PC12 细胞进行 MC-LR 染毒后发现,细胞内 ROS 水平显著升高,线粒体自噬相关基因 *PINK1* 和 *Parkin* 的表达也上升了,推测 MC-LR 是通过氧化应激激活 *PINK1/Parkin* 线粒体的自噬^[44]。综上所述,MC-LR 通过增加细胞内 ROS 的含量而造成氧化应激,从而进一步诱导细胞自噬的发生。

2.2.2 内质网应激途径

内质网(endoplasmic reticulum, ER)参与细胞内如蛋白质合成与修饰、多肽链折叠、钙代谢和脂代谢等多种生物学功能^[57-58]。当机体受到刺激(有毒物质等)时,因内质网应激(endoplasmic reticulum stress, ERs)产生过多的未折叠或错误折叠的蛋白致使机体启动未折叠蛋白反应(unfold protein reaction, UPR)^[59]。UPR 主要由存在于内质网膜上的 3 个跨膜分子 PERK(pancreatic ER kinase-PKR-like kinase)、ATF6(activating transcription factor 6)、IRE1(inositol-requiring enzyme 1)参与调控。目前,越来越多的研究表明 PERK、ATF6 和 IRE1 与自噬的发生密不可分^[60]。证据表明,PERK-eIF2 α -ATF4 途径及 PERK-AMPK-mTORC 途径参与 ERs 介导的细胞自

噬^[61-62]。其中,PERK-eIF2 α -ATF4 途径可激活一系列自噬相关基因(autophagy related genes, ATGs)包括 *LC3B*、*ATG3*、*ATG7*、*ATG10*、*ATG12*、*ATG16L1* 和 *Beclin1* 等的表达而促进自噬^[63]。此外,IRE1-JNK/XBP1-Beclin1 途径诱导的自噬也得到了验证^[64]。而 ERs 可导致机体内 Ca^{2+} 平衡紊乱,诱导 Ca^{2+} 从内质网释放到细胞质中,从而激活 mTOR 和 AMPK 等自噬上游信号通路^[65]。为探讨 ERs 与自噬之间的关系,Zhang 等^[40]分别应用 MC-LR 联合 ERs 抑制剂 4-PBA 或自噬抑制剂 3-MA 共同处理 CHO 细胞 24 h,结果显示,抑制 ERs 后,自噬标志性蛋白 Beclin1 和 LC3 II 表达降低,LC3 I 表达升高,而细胞凋亡率明显下降;抑制自噬后,ERs 标志蛋白如 GRP78、ATF6、PERK、IRE1 和 CHOP 表达升高而细胞凋亡率显著增加。由此可知,ERs 是 MC-LR 诱导自噬的重要因素之一,ERs 促进细胞凋亡,而自噬作为保护机制能抑制细胞凋亡的发生。Liu 等^[41]的研究结果表明,MC-LR 能够通过上调 ERs 相关蛋白 eIF2 α 、CHOP、XBP-1、GRP78 及 PERK 和自噬相关蛋白 ATG16、ATG12、ATG5、LC3 II/LC3 I 及 Beclin1 的表达,诱导 KK-1 细胞 ERs 和自噬,推测 MC-LR 诱发的 ERs 可通过 PERK/eIF2 α /ATG12 和 XBP-1/Beclin1 信号通路诱导细胞自噬,而细胞经 NAC 预处理后,MC-LR 通过下调上述蛋白的表达抑制 ERs 和自噬,这表明氧化应激介导了 MC-LR 诱导的 ERs,随后通过 ERs 相关途径调控自噬。然而 ERs 在 MCs 诱导细胞自噬的具体信号调控机制有待今后进一步研究。

3 结论及展望(Conclusions and prospects)

目前,水质恶化日趋严重,广泛存在于水体中的 MCs 备受人们关注,因此,深入研究 MCs 的毒性机理及自噬在机体受 MCs 胁迫时发挥的具体作用具有重要意义。研究表明,MCs 能够通过线粒体氧化应激和内质网应激 2 种途径诱导细胞自噬,细胞自噬可能是机体应对 MCs 毒性的早期反应,通过自噬清除受损细胞器以提高细胞的生存,而随着暴露时间延长及暴露浓度的上升,细胞凋亡及坏死被诱导。

目前针对 MCs 诱导细胞自噬的研究已逐渐开展,但还存在很多不足,今后可从以下几个方面进行深入研究。(1)现阶段的研究主要集中于高等哺乳动物,而要真正全面地揭示调控 MCs 诱导细胞自噬的机制,还需对长期暴露于 MCs 中的低等水生生物如鱼类等进行更深入细致的研究。(2)现有研究表明,MC-LR 的转运和代谢途径基因的多态性可以影响

毒素的转运和代谢,导致 MC-LR 暴露机体敏感性存在差异,从而致使 MC-LR 进入细胞内发挥的毒性作用存在差异^[5,66],这可能是目前 MC-LR 诱导细胞自噬的研究主要集中在肝细胞、肾细胞、生殖细胞和神经细胞等上,而还未在其他组织细胞深入开展的原因之一。因此,后续可以从 MC-LR 是否诱导其他不同组织细胞自噬以及诱导机制是否存在差异等方面进行探索,以期全面剖析 MC-LR 的毒性机理。(3)MCs 种类繁多,已鉴定的 100 多种异构体之间存在差异,而目前的研究主要针对 MC-LR 对动物机体的影响,而其他种类 MCs 的毒性作用机理的异同尚未可知。(4)自噬的调控机制错综复杂,而现有 MCs 诱导细胞自噬机制的研究主要针对自噬体形成过程,其上游调控信号通路及自噬溶酶体的降解途径未见详细报道,对自噬流的研究尚不系统,且自噬在机体受 MCs 胁迫时具体发挥的作用以及途径还需进一步研究。

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