自噬流障碍在缺血性脑卒中后的神经损伤机制

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摘要 自噬是一种细胞分解代谢途径, 通过降解长寿或错误折叠的蛋白质以及受损的细胞器, 以维持细胞内稳态和正常细胞功能。相反, 自噬流发生障碍会影响细胞内蛋白质和细胞器的降解, 破坏细胞稳态, 最终导致神经元死亡。研究表明, 缺血性脑卒中所致脑损伤的主要原因是能量消耗、氧化应激和炎症, 它们与应激后神经元自噬流的改变显著相关。该文回顾了自噬流障碍在缺血性脑卒中后的神经损伤机制及其相关的治疗药物与手段。

关键词 自噬流障碍; 调控; 缺血性脑卒中; 神经保护

Mechanism of Autophagy Flux Disorder in Nerve Injury after Ischemic Stroke

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Abstract Autophagy is a catabolic pathway, which degrades long-lived or misfolded proteins and damaged organelles in order to maintain homeostasis and normal cell function. Contrarily, if autophagy flux is disturbed, it may affect the degradation of intracellular proteins and organelles, disrupt cell homeostasis, and ultimately lead to the death of neurons. Studies have shown that ischemic stroke may induce brain injury primarily by energy consumption, oxidative stress and inflammation, which are significantly correlated with changes in autophagy flux of neurons after stress. This article reviews the mechanism of autophagy flux disorder after ischemic stroke and its related therapeutic drugs and methods.

Keywords autophagy flux disorder; regulate; ischemic stroke; neuroprotection

脑卒中是一种急性脑血管疾病, 它是全球三大致死疾病之一[1]。在我国卒中是成人致死、致残的首位病因, 其具有高发病率、高致残率、高死亡率、高复发率、高经济负担五大特点[2]。最新研究显示, 我国总体脑卒中终生发病风险为39.9%, 位居全球首位, 近几年脑卒中发病率有爆发式增长的态势, 并呈现出低收入群体快速增长、性别和地域差异明显以及年轻化等趋势[3]。脑卒中分为出血性脑卒中和缺血性脑卒中, 其中缺血性脑卒中约占所有脑卒中患者的87%[4]。卒中发生后侧支循环不足、脑供血中断, 引起神经系统损伤, 进而导致神经元缺血性死亡。神经元死亡的原因复杂, 其病理机制也复杂多样, 包

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括能量衰竭、兴奋性毒性、神经炎症、线粒体损伤和氧化应激等[5]。自噬流(autophagy flux)充分参与了脑卒中的病理生理过程, 自噬流产生障碍会导致严重的神经元自噬性损伤, 通过适当调控自噬–溶酶体通路可以达到诱导脑卒中后神经保护的效果[6]。随着脑卒中与自噬流关系研究的不断深入, 现已发现许多药物及手段能够调控自噬–溶酶体通路进而减轻脑卒中后的损伤。

1 自噬流广泛参与脑卒中后的病理生理过程

自噬是一种细胞自我保护的分解代谢途径, 通过降解更新长寿或错误折叠的蛋白质以及受损的细胞器来维持组织稳态[7-8]。胞质内膜结构包裹自噬底物形成自噬小体, 自噬小体与溶酶体相结合形成自噬溶酶体, 溶酶体进一步将自噬底物降解的这一动态过程被称为自噬流[9]。自噬流不是细胞被诱导后发生自噬的一种特定状态, 而是细胞从被诱导到最终完成自噬所经历的一个动态过程[10]。最新的研究表明, 缺血性脑卒中病理进程通常伴随着自噬流的改变[11]。

1.1 缺血性脑卒中发生后的自噬流现象

大量研究表明, 在缺血性脑卒中发生后, 自噬相关信号通路被显著激活[12], 例如在脑卒中发生3 h后, 自噬流信号靶点蛋白激酶PI3K/Akt的表达水平显著降低; 12 h后, 高水平的神经生长因子激活蛋白激酶PI3K/Akt信号通路, 从而减少对缺血性脑组织的损伤[13]。此外mTOR、Beclin1、5’-单磷酸腺苷活化蛋白激酶(5’-adenosine monophosphate-activated protein kinase, AMPK)、转录因子EB(transcription factor EB, TFEB)等一系列自噬相关蛋白及信号通路也被证实参与了缺血性脑卒中后的神经元调节[14]。研究表明, 急性和严重缺血可能导致“过度自噬”, 从而引发神经元的死亡和损伤; 慢性和轻度缺氧状态则会引起“适度自噬”, 从而清除受损组织和蛋白质以保护神经元[15]。与此同时, 自噬的诱导率和激活时间的长短也可以决定自噬是有益还是有害的[16]。目前有研究发现, 在营养匮乏的情况下, 自噬细胞的数量在4 h后达到峰值, 但在12 h后逐渐减少到几乎为零[17]。基于此类研究, 实验为平衡自噬的“双刃剑”作用, 通常设置梯度浓度来探究药物在激活自噬后对脑卒中的影响[18]。

1.2 自噬流与神经元兴奋性毒性

缺血性脑卒中后大脑动脉闭塞导致神经细胞缺氧缺糖、脂质不足, 进而产生神经损伤[19]。神经元缺血性损伤后, 谷氨酸释放, *N*-甲基-*D*-天冬氨酸受体(*N*-methyl-*D*-aspartic acid receptor, NMDA)被过度激活, 大量Ca^{2+}进入细胞, 一系列Ca^{2+}依赖性酶被过度激活, 细胞内的DNA和蛋白质过分水解, 导致神经元因兴奋性毒性而死亡[20]。其中钙调磷酸酶被激活释放, 通过结合TFEB并使其去磷酸化, 从而促进TFEB核转位, 进而引起细胞自噬[21]。自噬溶酶体通过降解这些受损蛋白质来维持细胞稳态, 避免神经元因兴奋性毒性死亡[20]。

1.3 自噬流与线粒体功能障碍

线粒体参与细胞代谢, 是维持细胞稳态方面必不可少的细胞器[22]。有研究表明, 线粒体是氧化磷酸化和呼吸作用的位点, 可产生活性氧(reactive oxygen species, ROS), 因此线粒体在应激状态下更容易发生氧化损伤, 而线粒体功能障碍是缺血性脑卒中发生后神经元凋亡和坏死的早期阶段[23-24]。在脑卒中病理进程中, 当线粒体功能受损时, 线粒体外膜上的关键分子直接与LC3相互作用, 诱导LC3II表达, 从而促进线粒体自噬, 进而降解功能失调的线粒体以维持细胞稳态[25-26]。

1.4 自噬流与氧化应激

细胞内过量的氧化剂或ROS会引起神经元氧化应激, 在缺血性脑卒中发生后营养匮乏的情况下, ROS大量而快速地增加, 进而诱导了早期自噬的激活[27]。ROS由超氧化物(O^{2-})、羟基自由基(OH^{-})和过氧化氢(H_2O_2)等组成[28]。过量的ROS导致细胞内大分子及细胞器发生不可逆的损伤[29]。H_2O_2能够抑制mTOR并诱导Beclin1表达, O^{2-}诱导了AMPK激活, 进一步促进了自噬小体的形成[30]。自噬的增强能够修复并降解氧化应激造成的细胞器及大分子的损伤, 降低氧化应激存在下的细胞毒性[31]。

1.5 自噬流与神经炎症

神经炎症是缺血性脑卒中后病理进程的重要因素之一, 因此如何诱导抗炎机制引起了广泛关注[32-33]。近年来的研究在调控自噬流抑制脑卒中神经炎症上取得了较好的结果[34]。Toll样受体(Toll-like receptor, TLR)是先天免疫系统的关键组成部分, 脑缺血损伤激活TLR信号, 通过调节细胞因子和趋化因子诱导炎症反应。炎症小体能诱导自噬的激活, 而自噬流

则通过将炎症小体泛素化、捕获和降解炎症小体来抑制炎症的发生。此外自噬流还可以通过调节细胞因子信号如: 丝氨酸/苏氨酸蛋白激酶TOR激酶(mTOR)、AMPK和NF-κB等抑制炎症反应[35]。

综上所述, 缺血性脑卒中后自噬流被激活, 而自噬流减轻了神经元兴奋性毒性、线粒体功能障碍、氧化应激、神经炎症等, 维持了细胞的稳态, 故自噬流广泛参与了脑卒中后的病理进程, 并在其中起着关键作用[36]。

2 自噬流障碍是脑卒中后神经元自噬性损伤的重要原因

自噬小体形成受损、自噬–溶酶体障碍均会导致自噬流障碍, 进而引起神经元的自噬性损伤[37]。

2.1 自噬小体形成受损致神经元自噬性损伤

自噬小体的生成是自噬发生的标志, 在自噬小体形成过程中, 细胞质中出现一个小的扁平的膜结构, 被称为隔离膜或吞噬体, 它膨胀、弯曲、变成球形, 随后将孔闭合, 最终形成了双膜囊状自噬小体[38]。当自噬小体形成受损时, 错误折叠的蛋白质、受损的细胞器堆积不能进入溶酶体中进行降解更新, 导致神经元稳态被破坏。此外, 溶酶体异常堆积, 自噬活性下降, 最终导致神经元自噬性损伤[39]。

2.2 自噬–溶酶体通路障碍致神经元自噬性损伤

目前, 脑缺血后的自噬–溶酶体通路(autophagy/lysosomal pathway, ALP)障碍受到广泛关注[40]。ALP是细胞中一个重要的生理过程, 在维持细胞、组织和机体内环境稳定中起着重要作用[41]。有研究发现, 神经元中自噬小体主要在轴突终末进行生物合成, ALP障碍会使自噬小体异常堆积, 轴突积累, 导致神经元受到严重的自噬性损伤[42]。发生脑卒中后, ALP障碍会使溶酶体异常积累, 进而使溶酶体组织蛋白酶B(cathepsin B, CTSB)、组织蛋白酶D(cathepsin D, CTSD)活性下降[40,43]。CTSD减少导致了更加严重的溶酶体障碍, 进而使细胞中错误折叠的蛋白质聚集和有毒废物积累[44]。因此, ALP功能障碍会使细胞内环境稳态遭到破坏, 最终导致神经元自噬性损伤[45]。

3 调控自噬–溶酶体信号通路可诱导脑卒中后的神经保护

在缺血性脑卒中发生后, 多个信号通路参与了细胞自噬的调控[39], 现已证明Beclin1/Bcl-2、AMPK、TFEB、unc -51样激酶1(unc-51-like kinase 1, ULK1)、mTOR等靶点在其中具有关键作用[35]。在上文有提到, 自噬活性不足或过度自噬均被认为是有害的, 自噬活性不足会导致自噬底物堆积而造成神经元自噬性损伤, 而过度自噬则会引发神经元自噬性死亡, 甚至加速脑卒中病理进程, 因此通过调控自噬流中的信号通路以达到适度的状态对治疗缺血性脑卒中而言是尤为重要的[46]。当自噬活性不足时可通过上调AMPK/Nrf2/HO-1信号通路的表达激活自噬, 提高自噬活性, 以达到神经保护作用。相反, 当自噬过度时则激活mTOR/ULK1通路, 抑制自噬, 减轻自噬性损伤。

诱导TFEB核转位可以激活自噬进而产生神经保护作用。TFEB是正向调控溶酶体以及自噬的主要转录因子[47]。有研究发现, TFEB不仅受到mTOR介导的磷酸化负调控, 还受到由Ca^{2+}依赖的钙调神经磷酸酶介导的TFEB去磷酸化正调控, 去磷酸化的TFEB可以被自由运输到细胞核中, 从而诱导溶酶体和自噬基因表达[48]。在缺血性脑卒中后, TFEB功能逐渐下降, TFEB核积聚减少, 致使ALP功能的逐渐下降。研究表明, 诱导TFEB核转位既能促进自噬小体与溶酶体的结合, 也能增强溶酶体活性, 减轻自噬–溶酶体障碍, 进而减弱了神经元兴奋性毒性, 产生神经保护作用[40,48]。

减弱Beclin1/Bcl-2相互作用激活自噬能够产生神经保护作用。Beclin1是Bcl-2同源(BH)-3结构域蛋白, 分布于质膜、细胞质和细胞核中[49]。Beclin1可结合核心脂质激酶Vps34, 产生不同的PI3K复合物。Atg14L介导Beclin1与Vps34形成复合物I促进了自噬小体形成, 而由UVRAG介导Beclin1与Vps34形成复合物II则与空泡蛋白的分选途径有关[50]。Bcl-2家族蛋白能与Beclin1结合并能够破坏Beclin1和Vps34之间的相互作用, 从而负调控Beclin1-Vps34复合物激酶活性, 抑制自噬小体形成[49]。有研究表明, 通过减弱Beclin1/Bcl-2相互作用, 可以释放Beclin1, 促进自噬小体合成, 进而激活自噬, 减弱卒中后神经元氧化应激、神经炎症等达到神经保护作用[51-52]。

上调AMPK/Nrf2/HO-1信号通路可激活自噬产生神经保护作用。AMPK是丝氨酸/苏氨酸(Ser/Thr)激酶中的一员, 广泛分布于各种细胞和器官中[53]。它是一种重要的内源性防御分子, 可对脑缺血、脑出血和

神经退行性疾病等有害刺激作出反应[54]。在能量消耗的条件下, AMPK被高AMP:ATP值的上游激酶例如丝氨酸/苏氨酸蛋白激酶STK11、钙/钙调素依赖性蛋白激酶2(Calcium/calmodulin-dependent protein kinase 2, CAMKK2)和丝裂原活化蛋白激酶激酶7等激活[55]。在缺氧缺糖情况下, O^{2-}诱导AMPK磷酸化, AMPK通过磷酸化Ser317和Ser777直接激活ULK1促进自噬[56], 并激活抗氧化调节因子相关因子2(nuclear factor erythroid 2-related factor, Nrf2)[57]。Nrf2是一种Cap'n'Collar(CNC)碱性区亮氨酸拉链(basic-region leucine zipper, bZIP)转录因子, 通过控制抗氧化反应元件(antioxidant response element, ARE)驱动的基因, 在内源性抗氧化应激系统中, 诱导抗氧化应激基因的表达, 而内源性抗氧化蛋白可以消除细胞内氧化剂和ROS, 从而减轻神经元氧化应激以及抑制线粒体自噬, 产生神经保护作用[27,58]。

激活mTOR/ULK1通路抑制自噬可诱导脑卒中后的神经保护作用。ULK是自噬激酶启动的复合体, 作为一个支架来招募下游因子, 并通过磷酸化丝氨酸或苏氨酸残基调节其功能。哺乳动物的ULK复合体由ATG13、FIP200、ATG101和ULK1或ULK2组成[38]。雷帕霉素靶蛋白(mammalian target of rapamycin, mTOR)是一种非典型丝氨酸/苏氨酸激酶, 包含mTOR复合物1(mTOR complex 1, mTORC1)和mTOR复合物2(mTOR complex 2, mTORC2)[59], 它能调节细胞生长和代谢[60]。mTOR可促进核糖体的形成以及蛋白质、核苷酸、脂肪酸和脂质等的合成[61]。mTORC1受如葡萄糖、氨基酸和ATP等多种代谢信号的调节, 其会在神经元应激的情况下失活并提升自噬活性[60]。但当神经元在长期处于缺糖缺氧状态下时, 溶酶体水解产物的释放能够重新激活mTOR进而抑制自噬[62]。此外, mTORC1通过磷酸化ULK1 Ser757并破坏ULK1和AMPK之间的相互作用来阻止ULK1激活, 与此同时mTORC1磷酸化还能抑制TFEB核转位, 进而抑制自噬小体的合成, 从而抑制急性缺血性脑卒中发生后的神经元过度自噬, 促进神经元存活[62-63]。

综上, 在缺血性脑卒中后适度调控自噬, 可增强细胞活性, 减小梗死面积从而达到神经保护的目的[64]。

4 调节自噬–溶酶体通路的方法及途径

一些药物通过调控自噬–溶酶体通路增强自噬小体的合成, 清除细胞内受损细胞器和降解错误蛋白, 以达到神经保护作用。黄芪甲苷(Astragaloside IV)处理大脑中动脉闭塞(middle cerebral artery occlusion, MCAO)组小鼠后可抑制Bcl-2的表达水平, 释放Beclin1, 促进自噬小体的形成与成熟, 提高细胞活力, 从而发挥神经保护作用[65]。五味子醇甲(schisandrin A, Sch A)治疗MCAO大鼠后可调控AMPK/Nrf2通路, 其通过促进AMPK磷酸化抑制了NF-κB表达进而抑制炎症的发生[34]。其还能增强Beclin1和LC3II的表达, 下调缺血半影区内CTSB、P62及泛素(ubiquitin, UB)的表达水平, 这表明Sch A促进了自噬小体的合成, 减少了自噬产物堆积而产生抗脑缺血再灌注损伤的作用[34,66]。此外还有一些药物之外的方法激活自噬, 例如富集环境(enriched environment, EE)治疗卒中发作的大鼠, 其能明显抑制Bcl-2的表达, 释放Beclin1并提升LC3II表达水平, 减少自噬底物积累, 增强自噬和溶酶体活性, 改善脑缺血诱导的溶酶体功能障碍[67](表1)。

而通过调控自噬–溶酶体通路可促进自噬小体与溶酶体相融合、改善溶酶体功能, 减轻自噬–溶酶体障碍也能起到卒中后的神经保护作用。海藻糖(Trehalose)直接作用于溶酶体, 可促使mTORC1

表1 增强自噬小体合成的方法及途径

Table 1 Methods and approaches to increase autophagosome synthesis

目的 Purpose	药物 Drugs	途径 Pathway	方法 Method	参考文献 References
Increase autophagosome synthesis	Astragaloside IV	Bcl-2/Beclin1	Inhibition	[65]
	Enriched environment	Bcl-2/Beclin1	Inhibition	[67]
	methylcobalamin	Bcl-2/Beclin1	Inhibition	[68]
	Sch A	AMPK	Activation	[66]
	Berberine	AMPK	Activation	[69]
	AICAR	AMPK	Activation	[70]

表2 调节自噬–溶酶体融合并改善溶酶体功能的方法与途径

Table 2 Methods and approaches to regulate autophagy-lysosomal fusion and improve lysosome function

目的 Purpose	药物 Drugs	途径 Pathway	方法 Method	参考文献 References
Autophagy-lysosomal fusion and improve lysosome function	Formononetin	TFEB	Activation	[72]
	Melibiose	TFEB	Activation	[76]
	GSK-3β inhibition	TFEB	Activation	[77]
	Vitexin	mTOR/ULK1	Inhibition	[73]
	Everolimus (RAD001)	mTOR/ULK1	Inhibition	[74]
	Carbamazepine	mTOR/ULK1	Inhibition	[78]

失活, 激活TFEB, 促进自噬–溶酶体融合, 减缓溶酶体功能障碍, 从而促进自噬[71]。刺芒柄花素(Formononetin)能够磷酸化AMPK并促进TFEB的核易位, 促进溶酶体的合成, 进而降解自噬底物[72]。此外, 牡荆素(Vitexin)可以抑制mTOR/ULK1通路, 促进自噬–溶酶体融合, 改善溶酶体功能, 减轻MCAO诱导的缺血性脑卒中对神经元的损伤[73]。依维莫司[Everolimus(RAD001)]是mTOR抑制剂, 可通过抑制mTOR激酶, 激活ULK1与AMPK之间的相互作用, 促进自噬小体与溶酶体的融合[74]。近年来, 有研究利用各种药物及手段调节自噬–溶酶体通路, 进而辅助治疗多种疾病[75](表2)。

5 展望

自噬流广泛参与脑卒中的病理进程, 卒中后适度自噬可以产生神经保护作用, 自噬流障碍则会导致自噬底物大量堆积, 造成更严重的损伤。许多药物和手段通过对自噬流进行调控, 起到了脑卒中后的神经保护效果。但因自噬流涉及的信号通路错综复杂, 目前其对缺血性脑卒中后的神经损伤机制尚未被完全说明, 故未来需要进一步了解各通路之间的联系, 寻找更优的治疗方法以期达到辅助治疗缺血性脑卒中的目的。

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