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基于自噬溶酶体形成机制调控缺血性脑卒中后神经元自噬流*

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摘要 脑卒中是由脑血管阻塞或出血引发的急性脑血管病，约84%的临床脑卒中患者由脑缺血引起。研究表明，自噬广泛参与并显著影响脑卒中病理生理进程。自噬是一个将陈旧蛋白质、损伤细胞器及多余胞质组分等呈递给溶酶体进行降解的代谢过程，其包括自噬的激活、自噬体的形成和成熟、自噬体与溶酶体融合、自噬产物在自噬溶酶体内消化和降解等过程。自噬流通常被定义为自噬/溶酶体信号机制。最近发现，自噬流障碍是导致缺血性脑卒中后神经元损伤的重要原因，而在自噬过程中任一步骤发生障碍均可导致自噬流损伤。本文重点对自噬体-溶酶体融合的机制，以及该机制在缺血性脑卒中后发生障碍的致病机理进行详细阐述，以期基于自噬体-溶酶体融合机制对神经元自噬流进行调节，进而诱导缺血性脑卒中后的神经保护。本文可为脑卒中病理机制研究指明方向，为脑卒中治疗探寻新的线索。

关键词 缺血性脑卒中，神经元，自噬流障碍，自噬体-溶酶体融合，神经保护
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缺血性脑卒中是导致人类死亡和永久性残疾的主要原因，多由心源性血栓、脑部小血管闭塞和影响脑部血液循环的动脉粥样硬化引起，导致脑组织供血减少，进而导致脑实质氧气和营养素严重不足，引发一系列级联性病理损伤[1]，包括血脑屏障功能障碍[2]、氧化应激、神经炎症、Ca^{2+}超载、内质网应激等[3]。在这些致病因素作用下，自噬可被不同程度地激活，通过控制细胞内成分的清除和再利用来维持细胞稳态，利于细胞存活[4]。

自噬是一种高度保守的溶酶体依赖性细胞代谢途径，可降解和回收错误折叠的蛋白质、陈旧胞质组分和损伤细胞器[5]。自噬流常被人们用来衡量自噬体随机或选择性地募集细胞质成分，并通过细胞内膜转运系统将自噬底物运送至溶酶体进行降解这个连续动态过程的活性[6-7]。而上述过程中的任意一个步骤发生紊乱都会引发自噬流障碍[8]。在众多细胞中，神经元又高度依赖自噬途径以清除代谢产物并及时更新损伤细胞器维持细胞稳态[9]。因此，缺血性脑卒中后保持自噬流通畅对提高神经元存活显得尤为重要。

自噬进程中，溶酶体通过与成熟自噬体的外膜融合，释放酸性水解酶来降解自噬体内容物，从而达到循环利用细胞营养物质和代谢中间体的目的[10]。而当细胞持续性遭受病理因素刺激时，溶酶体水解产物的释放又能通过多种途径抑制自噬，导致自噬流障碍尤其是自噬体-溶酶体融合障碍[11]。故本文拟阐述缺血性脑卒中后由自噬流障碍引发的神经元损伤机制、自噬体-溶酶体融合障碍的致病机理，以及基于该融合障碍机理挽救自噬神经元可采取的可能措施，由此寻求减轻神经损伤的途径和有效的治疗线索。

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1 缺血性脑卒中后自噬流障碍导致神经元损伤

作为一种细胞在压力刺激下的应激保护机制，自噬涉及双膜囊泡吞噬细胞质成分、细胞自噬体形成、自噬体-溶酶体融合、自噬溶酶体降解4个步骤。上述4个步骤发生任何问题都会导致自噬流发生障碍[12]。新近研究表明，脑缺血后哺乳动物雷帕霉素靶蛋白（mammalian targets of rapamycin，mTOR）/Unc-51样自噬激活激酶1（Unc-51-like autophagy-activating kinase 1，ULK1）通路被激活，导致自噬激活受阻，引发自噬流障碍[13-14]。脑缺血后自噬体的形成也会被抑制，导致底物积累，引起神经元受损[15-16]。当自噬体-溶酶体融合发生障碍后，自噬体的积累将加剧脑缺血诱导的神经元损伤[17]。另有研究发现，神经元中组织蛋白酶D蛋白水平和活性还会随脑缺血后的病理进程逐渐下降，转录因子EB的核转位也会被抑制，引发溶酶体功能缺陷，导致底物堆积，严重时引起细胞凋亡[13，18]。近期研究还指出，脑缺血后引发的溶酶体功能失衡会导致自噬溶酶体堆积，进而加重缺血性损伤[19]。由此可见，自噬进程中的任意一步发生紊乱都会引发严重的自噬流障碍，进而导致神经元损伤，加速缺血性脑卒中的病理进程。

2 自噬体–溶酶体融合障碍是引发神经元损伤的重要原因

自噬体-溶酶体融合是自噬进程中的限速步骤，也是保证自噬体高效代谢的关键。所以，当融合发生障碍时将引发严重的神经元损伤。研究表明，脑缺血后出现严重的自噬体-溶酶体融合障碍，导致自噬体、溶酶体异常堆积[20]，破坏神经元内环境稳态，加重缺血性损伤[11]。有研究发现，融合发生障碍后导致受损细胞器清除不足，进而加剧神经元氧化应激[21]，还会造成炎症小体过度激活，加重炎症反应[22]。此外，融合障碍加剧Ca^{2+}超载现象，导致一系列Ca^{2+}依赖性酶被过度激活，使细胞内的DNA和蛋白质过分水解，最终导致神经元因兴奋性毒性死亡[23]。另有研究表明，脑缺血后融合障碍诱导内质网中未折叠蛋白积累从而诱发内质网应激，而应激发生后又会导致内质网释放大量Ca^{2+}，Ca^{2+}在线粒体中积累进而破坏线粒体膜，导致产生过多的活性氧，最终触发内质网应激及氧化应激依赖性细胞凋亡[24-25]。由于融合障碍引发上述严重后果，故下文继续探究缺血性脑卒中后自噬体-溶酶体融合具体机制以及针对机制进行调控的研究进展，以期找到缓解神经损伤的新思路。

3 自噬体–溶酶体的融合机制

研究发现，参与介导融合过程的关键蛋白质属于以下3个家族：可溶性NSF附着蛋白受体（soluble NSF attachment protein receptor，SNARE）、衔接蛋白和小GTP酶Rab7[26]（图1）。

3.1 SNARE介导自噬体–溶酶体融合的机制

SNARE是介导自噬体-溶酶体融合的关键因子[17]。在哺乳动物中主要通过突触融合蛋白17（syntaxin17，STX17）-膜突触相关蛋白29（synaptosome associated protein 29，SNAP29）-囊泡相关膜蛋白8（vesicle-associated membrane protein 8，VAMP8）SNARE复合物来介导融合[27]。

在神经元自噬过程中，STX17主要定位于自噬体，VAMP8主要定位于溶酶体，它们从胞质中招募SNAP29，组装成STX17-SNAP29-VAMP8反式SNARE复合物，来调控自噬体-溶酶体融合[25，28-29]。其中，STX17的招募与微管相关蛋白轻链3（microtubule-associated protein light chain 3，LC3）有关[30]，目前，在脑缺血/再灌后的研究中观察到STX17和LC3之间共定位减少现象，也恰好证明了两种因子的互作[31]。并且，抑制脑缺血/再灌后相关mRNA（LC3、STX17、VAMP8、SNAP29）的转录水平，也会造成神经元自噬流障碍[32]，由于STX17等SNARE蛋白是典型的参与自噬体-溶酶体融合的关键因子，因此可以合理推测，脑缺血后神经元的自噬流障碍很大一部分原因是由于自噬体-溶酶体融合障碍，并且STX17等SNARE蛋白在其中发挥了重要作用。而新近研究也发现，STX17在脑缺血后自噬体-溶酶体融合过程中发挥重要作用[33]。还有研究通过诱导海马神经元中STX17的敲低，使自噬体-溶酶体融合发生严重障碍[9]。而一项针对帕金森病的研究发现，敲除神经元中的SNAP29，会引发严重的自噬体-溶酶体融合障碍[34]。此外，在再灌后12 h，由于SNAP29持续减少，皮层神经元会发生严重的自噬流障碍[35]，其具体机制可能也与自噬体-溶酶体融合障碍有关。

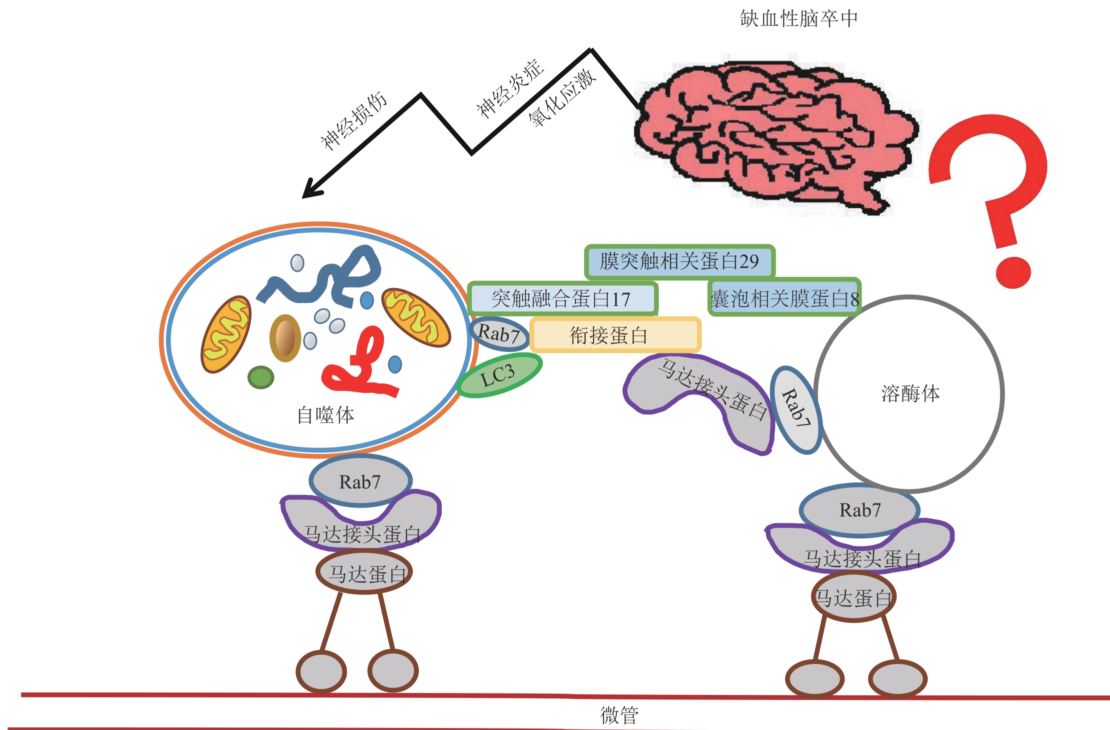

Fig. 1　The possible fusion mechanism of neuronal autophagy with lysosome after ischemic stroke

图1　缺血性卒中后神经元自噬体-溶酶体融合的可能机制

缺血性脑卒中后，自噬在各种刺激下被激活，自噬体-溶酶体融合是在SNARE复合物、小GTP酶、衔接蛋白、各种马达蛋白及其接头蛋白和其他未知因素的帮助下进行的。根据目前的研究进展，绘制出缺血性脑卒中后自噬体-溶酶体融合的可能机制图。LC3：微管相关蛋白轻链3；Rab7：小GTP酶。

3.2　衔接蛋白介导自噬体-溶酶体融合的机制

衔接蛋白是介导自噬体-溶酶体融合的另一种关键因子，以往研究发现衔接蛋白中的同型融合和蛋白质分类复合体（homotypic fusionand protein transport，HOPS）对于大部分融合事件都是必不可少的[10]，因此，HOPS也被视为自噬体-溶酶体融合的关键纽带。在哺乳动物中，还发现了另外两种衔接蛋白Atg14、EPG-5[36-37]。其中，Atg14通过与STX17结合参与自噬体-溶酶体融合，并稳定SNARE复合体[38]。有研究在再灌后的神经元中观察到Atg14和LC3之间共定位减少现象，这其中的具体机制可能与Atg14衔接功能受损以及Atg14与SNARE复合物互作减弱，使自噬体-溶酶体融合发生障碍有关[31]。而EPG-5则通过与Rab7和LC3结合被招募到SNARE复合物中，从而促进SNARE复合物组装和自噬体-溶酶体融合[37]。新近研究发现，大鼠脑缺血/再灌后EPG5的转录和翻译水平显著降低[39]，其具体作用机制可能也与自噬体-溶酶体融合障碍及向溶酶体的货物输送受损有关。在神经元中，HOPS可能通过STX17被招募到自噬体，进而促进自噬体-溶酶体融合[40]，并且HOPS的亚基Vps33A还会促进SNARE复合物的组装，防止其解体，进一步促进神经元内自噬体-溶酶体融合的发生[28]。还有研究在敲除HOPS复合体亚基Vps33A、Vps16或Vps39后发现自噬流发生障碍，自噬体积累现象严重[41]。此外，紫外线辐射抗性相关基因蛋白（UV radiation resistance-associated gene，UVRAG）也可以通过与自噬体相互作用来招募HOPS，以促进融合发生[25, 42]。而新近研究也发现，在短暂性全脑缺血模型（transient global cerebral ischemia，tGCI）中，UVRAG的缺乏会破坏Vps16和Rab7之间的相互作用，阻止自噬体-溶酶体融合并最终导致神经受损[17]，但是这其中是否涉及HOPS募集的减弱还需进一步探究。

3.3　Rab7介导自噬体-溶酶体融合的机制

哺乳动物细胞中还有多种小GTP酶参与介导自噬体-溶酶体融合，其中Rab7被视为介导自噬体-溶酶体融合的关键参与者[10]。在神经元自噬过

程中Rab7主要定位在自噬体和溶酶体上[43]，自噬体以Rab7鸟嘌呤核苷酸交换因子复合物依赖的方式募集并激活Rab7，参与自噬体-溶酶体融合[44]。而Rab7可以通过与多种马达接头蛋白（RILP（Rab7-interacting lysosomal protein）、PLEKHM1（pleckstrin homology and RUN domain containing M1））结合来调控自噬体、溶酶体沿微管运动，促进融合发生[45]。在溶酶体上，Rab7通过招募其效应子PLEKHM1和RILP分别与HOPS亚基Vps39和Vps41结合，来促进自噬体-溶酶体融合[46-47]。而目前研究表明，脑缺血后Rab7主要行使促进自噬体成熟的功能[17]，但也有研究发现，在神经元应激期间，通过增加Rab7与RILP的相互作用，可以恢复自噬流，减轻神经元损伤[48]，其主要机制可能与自噬体-溶酶体共定位后融合增强有关。脑缺血后Rab7与自噬体-溶酶体融合之间的准确关系还需进一步研究。

综上，采取多种方法调控融合事件，减轻自噬流障碍，发挥神经元保护作用就显得尤为重要。

4 神经元自噬流障碍调控及靶标研究

近年来，越来越多人关注自噬流障碍在缺血性脑卒中发生后的作用机制，尤其是自噬体-溶酶体融合障碍。故许多研究以其为治疗靶点，寻求减轻神经损伤的途径和有效的治疗线索，以期缓解乃至治愈相关疾病。

4.1 调控SNARE减轻自噬流障碍

通过调控SNARE可以减轻自噬流障碍，发挥神经保护功能。研究表明，海藻糖可以减轻自噬流障碍并减少神经元中的蛋白质聚集，从而在多种神经退行性疾病中产生保护作用[49]，有研究在缺血/再灌后利用海藻糖来调控自噬流障碍，发现其能在一定程度上挽救由STX17敲除引起的自噬体-溶酶体融合障碍和内质网应激，减轻神经元损伤[33]。还有研究通过引入外源STX17改善受损的自噬流，显著逆转自噬体-溶酶体融合缺陷，增加溶酶体与马达蛋白的结合，减轻海马神经元损伤[9, 50]。

而一项针对阿尔茨海默病（Alzheimer's disease，AD）的研究表明，早老蛋白1磷酸化后，通过膜联蛋白A2与VAMP8相互作用，并且促进VAMP8与自噬体SNARE结合，从而调节自噬体-溶酶体融合[51]，这也为通过调控VAMP8来辅助治疗缺血性脑卒中提供了新思路。

还有研究通过阻断超极化激活的环核苷酸门控通道来干扰SNAP29的募集进而显著逆转氯喹对自噬体-溶酶体融合的抑制，促进自噬-溶酶体降解，诱导脑卒中后的神经保护作用[52-53]。另有研究发现，SNAP29的O-连接β-N-乙酰氨基葡萄糖（O-linked β-N-acetylglucosamine，O-GlcNAc）修饰对自噬体-溶酶体之间的SNARE依赖性融合具有负调节作用[54]。因此，通过基因编辑等手段，敲除O-GlcNAc转移酶或突变SNAP29的O-GlcNAc位点可能起到促进自噬体-溶酶体融合的作用。

此外，通过调控mTOR也可以间接减轻自噬体-溶酶体融合障碍。研究发现，mTOR连续再激活造成再灌后神经元中的自噬流障碍，抑制自噬体形成、STX17锚定和自噬溶酶体降解[31]，还会抑制再灌后与融合相关mRNA转录水平，造成神经元自噬流障碍[32]，因此，未来可以此为靶点，通过抑制mTOR磷酸化减轻自噬流障碍情况，这可能为减少脑缺血/再灌注损伤提供内源性干预策略。

4.2 调控衔接蛋白减轻自噬流障碍

提高衔接蛋白的招募与表达可以恢复自噬流，减轻神经元损伤。有研究通过诱导UVRAG过表达减轻细胞和小鼠模型中的自噬流障碍，抑制神经元坏死[55]，其机制可能与HOPS的招募障碍有关。另一项针对大鼠tGCI的研究表明，缺氧预处理通过介导UVRAG-Vps16相互作用，可以显著逆转氯喹对自噬溶酶体形成的抑制，进而促进自噬体-溶酶体融合，引起对全脑缺血的神经保护作用[17]。新近研究则通过薯蓣皂苷元治疗，提高大鼠再灌后Rab7效应因子EPG5的表达，逆转由自噬体-溶酶体融合障碍引起的自噬损伤，并促进自噬溶酶体形成[39]。还在小鼠脑缺血/再灌的体内模型中，蛋白激酶Cγ的表达异常升高使得LC3和Atg14之间共定位减少，发生自噬流障碍[31]，这可能与Atg14的衔接功能受损有关，这也为间接调控Atg14促进自噬体-溶酶体融合提供了可能。新近研究则通过增强动脉粥样硬化后Atg14的表达，促进自噬体-溶酶体融合，减缓病变、解除炎症[56]。该研究也为调控Atg14促进脑缺血后自噬体-溶酶体融合、减轻神经损伤提供了方向。

4.3 调控Rab7蛋白减轻自噬流障碍

关于通过调控Rab7来减轻自噬流障碍的研究较少，有研究通过间歇性禁食，提高再灌后Rab7的表达，减轻自噬流障碍，其具体机制可能与调控自噬体-溶酶体融合有关[57]。此外，姜黄素可以增加与Rab7相互作用的RILP表达，解决自噬体积累

问题，发挥神经保护作用[58]。还有研究通过增强Rab7和马达接头蛋白之间的相互作用，从而促进自噬溶酶体形成恢复自噬流[59]。这也为未来调控缺血性脑卒中后自噬流障碍提供了线索。

4.4 其他方法减轻自噬流障碍

另外还有几项减轻自噬流障碍的方法。例如，神经突蛋白是一种营养因子，因其促进突触生长、轴突再生和神经元存活的能力而得名。有研究发现，神经突蛋白可以促进再灌后的自噬激活，增加自噬体-溶酶体融合，但具体机制未明[6]。假人参皂甙F11通过减轻大鼠脑缺血后的自噬体-溶酶体融合障碍，显著减少梗塞体积、脑水肿、神经功能缺损和皮质神经元死亡，但这其中是否涉及上述介导融合的几个关键因子还需进一步探究[60]。此外，丰富环境处理会显著增强再灌后溶酶体相关膜蛋白活性，减轻神经功能缺损和神经元死亡，促进自噬体-溶酶体融合[61-62]。未来还需进一步研究其促进融合的潜在机制。

5 前景与展望

本文详细阐述缺血性脑卒中发生后引起的自噬流障碍及其严重后果，特别聚焦于自噬体-溶酶体融合障碍机制以及其作为调控靶点的研究进展。但因其与胞内多种因子、多种信号通路相互调节、交互作用，未来还需要对缺血性脑卒中后的自噬流障碍进行更全面、更准确的探究，尤其是自噬体-溶酶体融合障碍的具体机制、发生的具体时间、引发的具体后果，以期发现更广泛的选择性试剂和治疗靶点来调控自噬流，减轻缺血性脑卒中所致的损伤。当然，缺血性脑卒中发生后，损伤不仅仅局限于单个神经元，其所处的神经血管单元亦会受到较大影响，而其中的具体机理与自噬流障碍是否相关以及如何相关，仍需进一步探究。在研究脑缺血病理机制时，应更加关注神经元与微血管之间结构和功能的密切联系，以及它们对脑缺血的协同损伤效应，由此探寻维持神经-血管单元结构和功能完整性的方法。

参 考 文 献

[1] 吴致远,邓仪昊,张永杰,等.雷帕霉素促进缺血性脑卒中后神经血管单位修复.中国病理生理杂志, 2020, **36**(9): 1709-1714
Wu Z Y, Deng Y H, Zhang Y J, *et al*. Chin J Pathophys, 2020, **36**(9): 1709-1714

[2] Jiang X, Andjelkovic A V, Zhu L, *et al*. Blood-brain barrier dysfunction and recovery after ischemic stroke. Prog Neurobiol, 2018, **163-164**(4-5): 144-171

[3] Shi Q, Cheng Q, Chen C. The role of autophagy in the pathogenesis of ischemic stroke. Curr Neuropharmacol, 2021, **19**(5): 629-640

[4] Ajoolabady A, Wang S, Kroemer G, *et al*. Targeting autophagy in ischemic stroke: from molecular mechanisms to clinical therapeutics. Pharmacol Ther, 2021, **225**(9): 107848

[5] Kalra P, Khan H, Kaur A, *et al*. Mechanistic insight on autophagy modulated molecular pathways in cerebral ischemic injury: from preclinical to clinical perspective. Neurochem Res, 2022, **47**(4): 825-843

[6] Zhang L, Wang Y, Pan R L, *et al*. Neuritin attenuates oxygen-glucose deprivation/reoxygenation (OGD/R) -induced neuronal injury by promoting autophagic flux. Exp Cell Res, 2021, **407**(2): 112832

[7] Klionsky D J, Abdalla F C, Abeliovich H, *et al*. Guidelines for the use and interpretation of assays for monitoring autophagy. Autophagy, 2012, **8**(4): 445-544

[8] Sha S, Tan J, Miao Y, *et al*. The role of autophagy in hypoxia-induced neuroinflammation. DNA Cell Biol, 2021, **40**(6): 733-739

[9] Xu H, Zhu Y, Chen X, *et al*. Mystery of methamphetamine-induced autophagosome accumulation in hippocampal neurons: loss of syntaxin17 in defects of dynein-dynactin driving and autophagosome-late endosome/lysosome fusion. Arch Toxicol, 2021, **95**(10): 3263-3284

[10] Lorincz P, Juhasz G. Autophagosome-lysosome fusion. J Mol Biol, 2020, **432**(8): 2462-2482

[11] Liu Y, Xue X, Zhang H, *et al*. Neuronal-targeted TFEB rescues dysfunction of the autophagy-lysosomal pathway and alleviates ischemic injury in permanent cerebral ischemia. Autophagy, 2019, **15**(3): 493-509

[12] Cheng A, Tse K H, Chow H M, *et al*. ATM loss disrupts the autophagy-lysosomal pathway. Autophagy, 2021, **17**(8): 1998-2010

[13] Fu X, Liu Y, Zhang H, *et al*. Pseudoginsenoside F11 ameliorates the dysfunction of the autophagy-lysosomal pathway by activating calcineurin-mediated TFEB nuclear translocation in neuron during permanent cerebral ischemia. Exp Neurol, 2021, **338**(4): 113598

[14] Lai Z, Gu L, Yu L, *et al*. Delta opioid peptide [d-Ala2, d-Leu5] enkephalin confers neuroprotection by activating delta opioid receptor-AMPK-autophagy axis against global ischemia. Cell Biosci, 2020, **10**(6): 79

[15] Liu Y, Che X, Zhang H, *et al*. CAPN1 (Calpain1) -mediated impairment of autophagic flux contributes to cerebral ischemia-induced neuronal damage. Stroke, 2021, **52**(5): 1809-1821

[16] Yihao D, Tao G, Zhiyuan W, *et al*. Ginkgo biloba leaf extract (EGb-761) elicits neuroprotection against cerebral ischemia/reperfusion injury by enhancement of autophagy flux in neurons in the penumbra. Iran J Basic Med Sci, 2021, **24**(8): 1138-1145

[17] Zhan L, Chen S, Li K, *et al*. Autophagosome maturation mediated by Rab7 contributes to neuroprotection of hypoxic preconditioning against global cerebral ischemia in rats. Cell

Death Dis, 2017, **8**(7): e2949

[18] Hossain M I, Marcus J M, Lee J H, *et al*. Restoration of CTSD (cathepsin D) and lysosomal function in stroke is neuroprotective. Autophagy, 2021, **17**(6): 1330-1348

[19] Zhang X, Wei M, Fan J, *et al*. Ischemia-induced upregulation of autophagy preludes dysfunctional lysosomal storage and associated synaptic impairments in neurons. Autophagy, 2021, **17**(6): 1519-1542

[20] Hou K, Xu D, Li F, *et al*. The progress of neuronal autophagy in cerebral ischemia stroke: mechanisms, roles and research methods. J Neurol Sci, 2019, **400**(5): 72-82

[21] Hu M, Wang R, Chen X, *et al*. Resveratrol prevents haloperidol-induced mitochondria dysfunction through the induction of autophagy in SH-SY5Y cells. Neurotoxicology, 2021, **87**(6): 231-242

[22] Wang C, Liu C, Gao K, *et al*. Metformin preconditioning provide neuroprotection through enhancement of autophagy and suppression of inflammation and apoptosis after spinal cord injury. Biochem Biophys Res Commun, 2016, **477**(4): 534-540

[23] Yagami T, Yamamoto Y, Koma H. Pathophysiological roles of intracellular proteases in neuronal development and neurological diseases. Mol Neurobiol, 2019, **56**(5): 3090-3112

[24] Lin M, Ling J, Geng X, *et al*. RTN1-C is involved in high glucose-aggravated neuronal cell subjected to oxygen-glucose deprivation and reoxygenation injury *via* endoplasmic reticulum stress. Brain Res Bull, 2019, **149**(14): 129-136

[25] Shen Q, Shi Y, Liu J, *et al*. Acetylation of STX17 (syntaxin17) controls autophagosome maturation. Autophagy, 2021, **17**(5): 1157-1169

[26] Tian X, Teng J, Chen J. New insights regarding SNARE proteins in autophagosome-lysosome fusion. Autophagy, 2021, **17**(10): 2680-2688

[27] Takats S, Glatz G, Szenci G, *et al*. Non-canonical role of the SNARE protein Ykt6 in autophagosome-lysosome fusion. PLoS Genet, 2018, **14**(4): e1007359

[28] Saleeb R S, Kavanagh D M, Dun A R, *et al*. A VPS33A-binding motif on syntaxin17 controls autophagy completion in mammalian cells. J Biol Chem, 2019, **294**(11): 4188-4201

[29] Tang B L. Vesicle transport through interaction with t-SNAREs 1a (Vti1a)'s roles in neurons. Heliyon, 2020, **6**(8): e04600

[30] Kumar S, Jain A, Farzam F, *et al*. Mechanism of STX17 recruitment to autophagosomes *via* IRGM and mammalian Atg8 proteins. J Cell Biol, 2018, **217**(3): 997-1013

[31] Hua R, Han S, Zhang N, *et al*. cPKCgamma-modulated sequential reactivation of mTOR inhibited autophagic flux in neurons exposed to oxygen glucose deprivation/reperfusion. Int J Mol Sci, 2018, **19**(5): 1380

[32] Hua R, Wei H, Liu C, *et al*. Phosphorylated mTORC1 represses autophagic-related mRNA translation in neurons exposed to ischemia-reperfusion injury. J Cell Biochem, 2019, **120**(9): 15915-15923

[33] Chen L, Xia Y F, Shen S F, *et al*. Syntaxin17 inhibits ischemic neuronal injury by resuming autophagy flux and ameliorating endoplasmic reticulum stress. Free Radic Biol Med, 2020, **160**(22): 319-333

[34] Tang Q, Gao P, Arzberger T, *et al*. Alpha-Synuclein defects autophagy by impairing SNAP29-mediated autophagosome-lysosome fusion. Cell Death Dis, 2021, **12**(10): 854

[35] Yan W, Fan J, Zhang X, *et al*. Decreased neuronal synaptosome associated protein 29 contributes to poststroke cognitive impairment by disrupting presynaptic maintenance. Theranostics, 2021, **11**(10): 4616-4636

[36] Diao J, Liu R, Rong Y, *et al*. ATG14 promotes membrane tethering and fusion of autophagosomes to endolysosomes. Nature, 2015, **520**(7548): 563-566

[37] Wang Z, Miao G, Xue X, *et al*. The vici syndrome protein EPG5 is a Rab7 effector that determines the fusion specificity of autophagosomes with late endosomes/lysosomes. Mol Cell, 2016, **63**(5): 781-795

[38] Zhang Y, Han X, Tang Y, *et al*. Weakened interaction of ATG14 and the SNARE complex blocks autophagosome-lysosome fusion contributes to fluoride-induced developmental neurotoxicity. Ecotoxicol Environ Saf, 2021, **230**(12): 113108

[39] Zhang X, Wang X, Khurm M, *et al*. Alterations of brain quantitative proteomics profiling revealed the molecular mechanisms of diosgenin against cerebral ischemia reperfusion effects. J Proteome Res, 2020, **19**(3): 1154-1168

[40] Jia R, Guardia C M, Pu J, *et al*. BORC coordinates encounter and fusion of lysosomes with autophagosomes. Autophagy, 2017, **13**(10): 1648-1663

[41] Jiang P, Nishimura T, Sakamaki Y, *et al*. The HOPS complex mediates autophagosome-lysosome fusion through interaction with syntaxin17. Mol Biol Cell, 2014, **25**(8): 1327-1337

[42] Liang C, Lee J S, Inn K S, *et al*. Beclin1-binding UVRAG targets the class C Vps complex to coordinate autophagosome maturation and endocytic trafficking. Nat Cell Biol, 2008, **10**(7): 776-787

[43] Xing R, Zhou H, Jian Y, *et al*. The Rab7 effector WDR91 promotes autophagy-lysosome degradation in neurons by regulating lysosome fusion. J Cell Biol, 2021, **220**(8): e202007061

[44] Yasa S, Modica G, Sauvageau E, *et al*. CLN3 regulates endosomal function by modulating Rab7A-effector interactions. J Cell Sci, 2020, **133**(6): jcs234047

[45] Amaya C, Militello R D, Calligaris S D, *et al*. Rab24 interacts with the Rab7/Rab interacting lysosomal protein complex to regulate endosomal degradation. Traffic, 2016, **17**(11): 1181-1196

[46] Gillingham A K, Bertram J, Begum F, *et al*. *In vivo* identification of GTPase interactors by mitochondrial relocalization and proximity biotinylation. Elife, 2019, **8**(7): e45916

[47] Wijdeven R H, Janssen H, Nahidiazar L, *et al*. Cholesterol and ORP1L-mediated ER contact sites control autophagosome transport and fusion with the endocytic pathway. Nat Commun, 2016, **7**(3): 11808

[48] Bains M, Zaegel V, Mize-Berge J, *et al*. IGF-I stimulates Rab7-RILP interaction during neuronal autophagy. Neurosci Lett, 2011,

488(2): 112-117

[49] Wang X Y, Yang H, Wang M G, *et al*. Trehalose protects against cadmium-induced cytotoxicity in primary rat proximal tubular cells *via* inhibiting apoptosis and restoring autophagic flux. Cell Death Dis, 2017, **8**(10): e3099

[50] Sun L, Lian Y, Ding J, *et al*. The role of chaperone-mediated autophagy in neurotoxicity induced by alpha-synuclein after methamphetamine exposure. Brain Behav, 2019, **9**(8): e01352

[51] Bustos V, Pulina M V, Bispo A, *et al*. Phosphorylated presenilin 1 decreases beta-amyloid by facilitating autophagosome-lysosome fusion. Proc Natl Acad Sci USA, 2017, **114**(27): 7148-7153

[52] Mauthe M, Orhon I, Rocchi C, *et al*. Chloroquine inhibits autophagic flux by decreasing autophagosome-lysosome fusion. Autophagy, 2018, **14**(8): 1435-1455

[53] Chen C, Liu L, Shu Y Q, *et al*. Blockade of HCN2 channels provides neuroprotection against ischemic injury *via* accelerating autophagic degradation in hippocampal neurons. Neurosci Bull, 2020, **36**(8): 875-894

[54] Guo B, Liang Q, Li L, *et al*. O-GlcNAc-modification of SNAP-29 regulates autophagosome maturation. Nat Cell Biol, 2014, **16**(12): 1215-1226

[55] Xu C, Wu J, Wu Y, *et al*. TNF-alpha-dependent neuronal necroptosis regulated in Alzheimer’s disease by coordination of RIPK1-p62 complex with autophagic UVRAG. Theranostics, 2021, **11**(19): 9452-9469

[56] Zhang H, Ge S, Ni B, *et al*. Augmenting ATG14 alleviates atherosclerosis and inhibits inflammation *via* promotion of autophagosome-lysosome fusion in macrophages. Autophagy, 2021, **17**(12): 4218-4230

[57] Jeong J H, Yu K S, Bak D H, *et al*. Intermittent fasting is neuroprotective in focal cerebral ischemia by minimizing autophagic flux disturbance and inhibiting apoptosis. Exp Ther Med, 2016, **12**(5): 3021-3028

[58] Liang J, Zhou F, Xiong X, *et al*. Enhancing the retrograde axonal transport by curcumin promotes autophagic flux in N2a/APP695swe cells. Aging (Albany NY), 2019, **11**(17): 7036-7050

[59] Xiao W, Yeerken D, Li J, *et al*. Nlp promotes autophagy through facilitating the interaction of Rab7 and FYCO1. Signal Transduct Target Ther, 2021, **6**(1): 152

[60] Liu Y Y, Zhang T Y, Xue X, *et al*. Pseudoginsenoside-F11 attenuates cerebral ischemic injury by alleviating autophagic/lysosomal defects. CNS Neurosci Ther, 2017, **23**(7): 567-579

[61] Deng Y H, Dong L L, Zhang Y J, *et al*. Enriched environment boosts the post-stroke recovery of neurological function by promoting autophagy. Neural Regen Res, 2021, **16**(5): 813-819

[62] Mering S, Jolkkonen J. Proper housing conditions in experimental stroke studies-special emphasis on environmental enrichment. Front Neurosci, 2015, **9**(3): 106

Regulation of Autophagic Flux in Neurons Based on The Mechanism of Autolysosome Formation After Ischemic Stroke*

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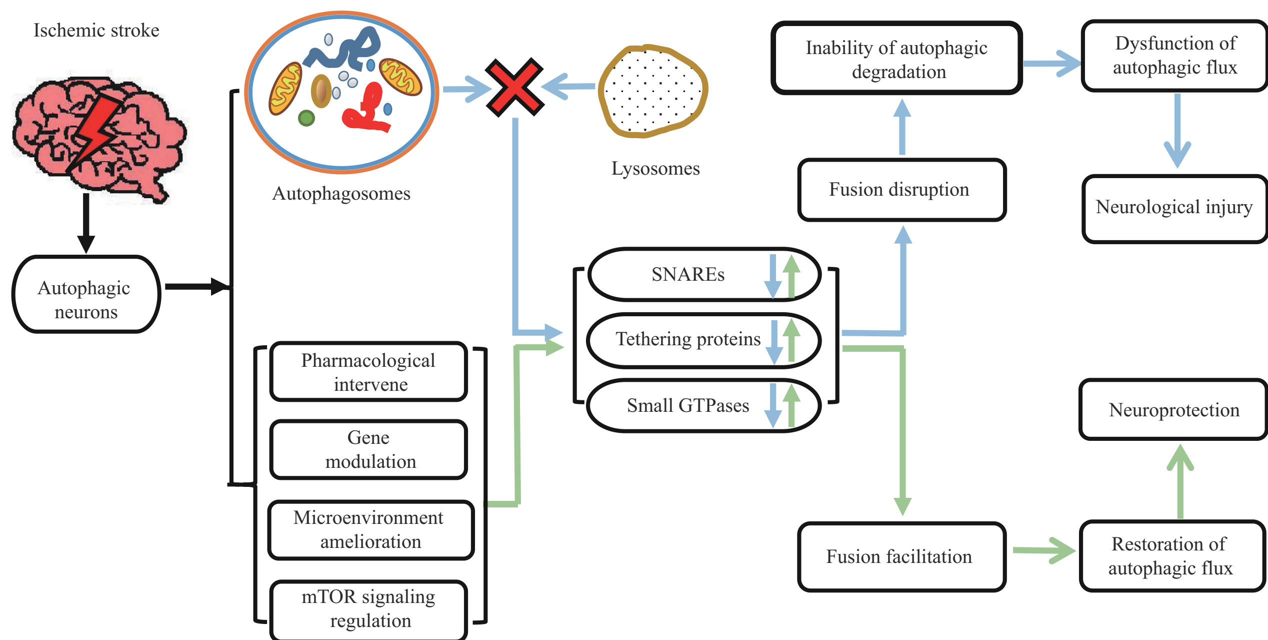
Graphical abstract



Abstract Stroke is an acute cerebrovascular disease caused by cerebrovascular occlusion or hemorrhage, and approximately 84% of clinical stroke patients is suffered from cerebral ischemia (Ischemic stroke). Studies indicated that autophagy is extensively involved and prominently affects the pathophysiological development of stroke. Autophagy is a metabolic process by which delivers old proteins, damaged organelles and superfluous cytoplasmic components to lysosomes for degradation. It comprises a series of processes including activation of autophagy, formation and maturation of autophagosomes, fusion of autophagosomes with lysosomes, and

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digestion and degradation of autophagic substrates in autolysosomes. Autophagic flux is usually defined as autophagic/lysosomal signaling machinery. Recent studies reveal that dysfunction of autophagic flux is a critical pathogenesis of neuronal injury after ischemic stroke. However, disruption in any step in the autophagic/lysosomal pathway can lead to impairment of autophagic flux. This article is to be reviewed from the following four items. Firstly, excessive activation of autophagy, deficiency of autophagosome formation, fusion blockage of autophagosomes with lysosomes, as well as lysosomal inefficiency can drive dysfunction of autophagic flux and thereby aggravating neuronal injury. Secondly, fusion disruption between autophagosomes and lysosomes is an important cause of autophagic/lysosomal dysfunction in neurons. Consequently, a massive of autophagic substrates is accumulated within cells to worsen post-stroke damage. Thirdly, the fusion of autophagosomes with lysosomes is mainly mediated by the membrane-to-membrane fusion machinery *via* the three core elements: NSF (N-ethyl-maleimide sensitive factor ATPase), SNAP (soluble NSF attachment protein), and SNAREs (soluble NSF attachment protein receptors). SNAP is an adaptor attaching NSF to SNAREs, which are the proteins directly mediate the membrane fusion. After membrane-membrane fusion, SNAREs must be reactivated by NSF for the next round of fusion. It is vital that NSF is the sole ATPase to regenerate active SNAREs. SNF inactivation represses the reactivation of SNAREs and thereby disrupting the fusion between autophagosomes and lysosomes after ischemic stroke. Subsequently, the autophagic/lysosomal dysfunction in neurons is created to aggravate the neurological injury. Additionally, the insufficiency of the tethering proteins and inefficiency of the GTPases are also the pathologies to interrupt the fusion between autophagosomes and lysosomes. Accordingly, the impaired autophagic flux in neurons may be restored by facilitating fusion of autophagosomes with lysosomes, *via* pharmacological intervene, gene modulation, microenvironment amelioration, or mTOR signaling regulation. Finally, based on the mechanism of autolysosome formation, more therapeutic clues may be sought to alleviate neurological injury after ischemic stroke.

Key words ischemic stroke, neurons, autophagy flux disorder, fusion of autophagosome with lysosome, neuroprotection

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