

音猬因子信号通路在缺血性脑卒中不同病期中的作用

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[关键词] 音猬因子通路; 缺血性脑卒中; 动脉粥样硬化

[摘要] 音猬因子(Shh)信号通路不仅在胚胎发育过程中发挥着举足轻重的作用,而且还参与多种病理生理过程。缺血性脑卒中的病理机制复杂,治疗手段也仅限于急性期的时间窗内。随着对Shh信号通路对缺血性脑卒中的作用深入研究,发现该信号通路的相关基因和蛋白参与缺血性脑卒中的各个阶段并发挥不同作用。本文综述了Shh信号通路在缺血性脑卒中的发病前期、急性期和恢复期三大阶段中所发挥的主要作用。期望对探讨缺血性脑卒中发病机制和药物新靶点有所帮助。

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Differential roles of Sonic hedgehog signaling pathway in different stages of ischemic stroke

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[KEY WORDS] Sonic hedgehog pathway; ischemic stroke; atherosclerosis

[ABSTRACT] Sonic hedgehog (Shh) signaling pathway not only plays an important role in embryo development, but also participates in many physiological and pathophysiological processes. The pathological mechanism of the occurrence and development of ischemic stroke is complex, which has not been fully understood, and the current treatment is limited to an acute time window. As the role of Shh signaling pathway in ischemic stroke being increasingly reported, it was found that genes and proteins related to the pathway may participate in various stages of ischemic stroke and play different roles. This review summarizes main findings of the role played by Shh signaling pathway in the early stage, acute stage and recovery stage of ischemic stroke. It is expected that further study of Shh signaling pathway will help to provide valuable reference to new mechanisms and drug new target for the ischemic stroke.

脑卒中是一种常见突发性疾病,其中缺血性脑卒中大约占85%左右^[1]。缺血性脑卒中主要是由于脑血管内血栓形成后导致脑组织供血、供氧不足引起的,是导致死亡,长期残疾和世界范围内巨大社会经济损失的主要原因^[2-3]。目前,唯一被美国食品和药物管理局批准的治疗急性缺血性脑卒中

药物为组织型纤溶酶原激活物(tissue plasminogen activator, tPA)。然而, tPA的有效治疗窗口狭窄(4~6 h)且具有出血转化的风险^[4],使其在临床中的应用受到了严重限制。据统计,自1996年以来,仅2%~5%的缺血性脑卒中患者从tPA的治疗中获益^[4]。而数百种用于缺血性脑卒中的神经保护剂

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在临床试验中均以失败告终^[5-6],这说明对于脑卒中的防治还远远不足,开发新的缺血性脑卒中防治药物仍是当务之急。

音猬因子(Sonic hedgehog, Shh)是一种分泌蛋白,不仅在胚胎及大脑发育中起着重要调节作用^[7],而且在生理及病理过程中发挥着多种生物学功能^[8]。Shh 可以与其特定的受体碎片蛋白 1 (patched1, Ptch1)结合,解除 Ptch1 对 G 蛋白偶联受体平滑蛋白(smoothed, Smo)的抑制,使下游转录因子胶质瘤相关癌基因同源物(glioma-associated oncogene homolog, Gli-1)激活,从而启动与细胞周期进程相关的靶基因的表达。越来越多的研究结果证明上述经典的 Shh 信号通路的激活在缺血性脑卒中的不同发展阶段均都发挥着重要作用,而 Shh 的基因敲除及其抑制剂环巴胺的使用能够加重脑损伤^[9-11],因此靶向 Shh 信号通路是一个有前景的防治缺血性脑卒中的策略。本文对现有文献进行了详细地梳理与讨论,并重点关注 Shh 信号通路在脑缺血前如何阻止脑血栓和缺血状态的形成、在脑缺血时如何减少缺血再灌注(ischemia/reperfusion, I/R)所造成的损伤以及在脑缺血发生后又如何加快其功能的恢复。通过这“三步曲”详细阐述 Shh 信号通路在缺血性脑卒中发生发展中的作用,为缺血性脑卒中的防治提供新的思路。

1 Shh 信号通路在脑血栓形成前所发挥的预防作用

至今虽无研究结果证明 Shh 信号通路能够直接参与血小板活化、凝血、纤溶等生理过程而发挥抗血栓作用,但越来越多的实验结果证明其能够参与动脉粥样硬化进程。众所周知,动脉粥样硬化是导致缺血性脑卒中的主要原因^[3],并且其中涉及到了诸多“环节”,即血管内皮功能障碍后低密度脂蛋白(low density lipoprotein, LDL)在内皮下基质中积累,导致巨噬细胞摄取氧化型低密度脂蛋白(oxidized low density lipoprotein, ox-LDL)形成泡沫细胞,最终形成斑块,而血管平滑肌细胞的迁移和增殖形成了斑块的“帽子”,如果斑块在脑血管中,在“帽子”被破坏后将形成原位血栓,若斑块在其他动脉中形成并破裂,由于血流动力学原因使脱落的斑块可能移至脑血管并形成血栓^[12-13]。

研究表明,Shh 信号通路能够通过参与上述“环节”对缺血性脑卒中中进行预防。如 Shh 信号通路通过激活细胞外调节蛋白激酶(extracellular regulated

protein kinase, ERK)和磷脂酰肌醇 3 激酶(phosphatidylinositol 3 kinase, PI3K)的合成和磷酸化,诱导内皮细胞释放一氧化氮(nitric oxide, NO),使活性氧的产生减少,以改善内皮损伤,这将有利于预防斑块的形成^[14]。而在斑块形成过程中,Shh 信号通路可以调节血浆脂质水平,并通过抑制巨噬细胞摄取 ox-LDL 来减少泡沫细胞的形成,这将有效抑制斑块的扩大。此外,Shh 信号通路的激活能够提高微管相关蛋白 1 轻链 3II(microtubule-associated protein 1 light chain 3-II, Maplc3-II)的表达水平,促进蛋白激酶 B(protein kinase B, PKB/Akt)的磷酸化,最终导致自噬小体形成来诱导血管平滑肌细胞自噬^[15]。血管平滑肌细胞的自噬可以增加斑块的稳定性,这对预防血栓的形成起到了重要作用。Shh 信号通路所发挥的作用能够显著抑制动脉粥样硬化进程,这可能是有效预防缺血性脑卒中的新靶点(图 1)。

2 Shh 信号通路在脑卒中急性期所发挥的保护作用

2.1 抗氧化应激

在脑卒中急性期,由于脑缺血所造成的机体内氧自由基代谢失衡会导致氧化应激的发生,这是 I/R 损伤的主要机制之一^[16-17]。而 Shh 信号通路则能够通过增加谷胱甘肽过氧化物酶(glutathione peroxidase, GSH-PX)和超氧化物歧化酶(superoxide dismutase, SOD)的活性发挥抗氧化应激的作用^[18-19]。利用大脑中动脉阻塞(middle cerebral artery occlusion, MCAO)大鼠模型发现在 I/R 损伤后的 6 h、12 h、24 h 以及 48 h 均检测到 Shh 蛋白的下游靶点(Ptch1 和 Gli1)以及 SOD1 在其皮质中显著表达,但在 72 h 表达则不明显,这可能是由于 Shh 信号通路主要在脑卒中急性期发挥抗氧化应激作用^[19]。同时,在体外实验中通过利用过氧化氢(H₂O₂)处理皮质神经元细胞来模拟氧化应激损伤也发现 Shh 信号通路能够通过显著降低 ERK 的磷酸化水平以及上调 PI3K 途径的直接下游效应因子 Akt 的磷酸化,从而改善 H₂O₂ 引起的氧化应激反应^[20](图 1)。据报道,脑源性神经营养因子(brain-derived neurotrophic factor, BDNF)被普遍认为是大脑中一种十分重要的神经营养因子,BDNF 能减轻氧化应激对脑组织产生的影响^[21],而用外源性 Shh 蛋白预处理原代神经元细胞后能明显上调其 BDNF 的表达^[18]。

2.2 抗兴奋性神经毒性

谷氨酸诱导的兴奋性神经毒性作为 I/R 损伤又一重要机制,在缺血性脑卒中急性期发挥着重要的作用^[22]。研究表明,在谷氨酸刺激后的早期,神经型一氧化氮合酶(neuronal nitric oxide synthase, nNOS)能够被转运到神经元细胞的细胞核中,通过与性别决定区 Y 框(sex determining region Y-box, Sox)转录因子家族的成员 Sox2 相互作用形成 nNOS-Sox2 复合物。nNOS-Sox2 复合物通过激活 Sox2 的下游靶点 Shh 基因的转录,进而减轻谷氨酸诱导的兴奋性神经毒性^[22-24](图 1)。体内和体外实验均证明“nNOS-Sox2-Shh”轴能够在脑卒中急性期减轻兴奋性神经毒性而保护神经元免受 I/R 损伤。

2.3 调控炎症反应

众所周知,神经炎症对于脑损伤是一把“双刃剑”,在其病理过程中发挥着双重作用^[25]。在体外实验中已经证明,利用白细胞介素 1 β (interleukin-1 β , IL-1 β)、肿瘤坏死因子 α (tumor necrosis factor- α , TNF- α)和干扰素 γ (interferon- γ , IFN- γ)等炎症因子处理星形胶质细胞,3 天后能诱导 Shh mRNA 表达。并有研究证明,由众多促炎信号激活的核因子 κ B(nuclear factor- κ B, NF- κ B)途径,其直接转录靶标之一为 Shh。这些炎症因子的刺激能够激活反应性星形胶质细胞中 Shh 的表达,以促进少突胶质细胞转录因子 2(oligodendrocyte lineage transcription factor 2, Olig2)表达的少突胶质细胞增殖^[26]。这对减轻脑损伤起到一定的积极作用(图 1)。Shh 信号通路还能通过抑制 MCAO 大鼠 IL-6、TNF- α 及 IL-1 β 的过度表达,而直接发挥抗炎作用^[27]。这说明 Shh 信号通路不仅能增强早期炎症反应的有益作用以减轻 I/R 损伤,而且对脑卒中急性期过激的炎症反应起到直接的抑制作用。

2.4 抗细胞凋亡

在缺血性脑卒中急性期, I/R 损伤能够导致脑梗死区的组织坏死和细胞凋亡。虽然组织坏死难以逆转,但细胞凋亡能够通过其上游信号的调节而改变。Shh 信号通路不仅能够显著上调 H₂O₂ 处理的大鼠原代神经元细胞中抗凋亡蛋白 B 淋巴瘤 2(B-cell lymphoma-2, Bcl-2)的表达水平,且能下调促凋亡蛋白细胞凋亡调控因子(Bcl-2-associated X protein, Bax)的表达水平,从而产生抗凋亡作用,这将能够保护神经元细胞免受氧化应激诱导的细胞凋亡^[20](图 1)。此外,体内实验均表明 Shh 信号通路的激活能够减少 TUNEL+细胞的数量^[28-29],其抗凋亡作用可能与激活 PI3K/Akt 通路、抑制 p53

途径以及减弱 Caspase-3 活性有关^[20,30-31]。

2.5 保护血脑屏障

血脑屏障(blood-brain barrier, BBB)的破坏,脑微血管的通透性增加,导致脑水肿是缺血性脑卒中发生后的持续损伤原因之一。体内外实验证明, Shh 信号通路通过降低促炎性介质(如 IL-1 β)的表达以及白细胞的黏附和迁移,进而减轻脑微血管内皮细胞(brain microvascular endothelial cells, BMEC)的免疫应答,以保护 BBB 的完整性^[32-33]。此外还有研究发现,脑室注射重组的 Shh 蛋白能够增强 MCAO 大鼠 BBB 的完整性,减轻脑水肿^[34-36]。体外研究表明,在 I/R 损伤后 Shh 可能主要促进星形胶质细胞中血管生成素 1(angiotensin-1, Ang-1)的表达, Ang-1 可促进 BMEC 中咬合蛋白(occludin)和闭锁小带蛋白(zonula occludens-1, ZO-1)的合成,以修复 BBB 的紧密连接结构并降低脑微血管的通透性^[19,34,37](图 1)。这也是 Shh 信号通路能够减轻急性脑卒中脑水肿的重要原因。

3 Shh 信号通路在脑卒中恢复期所发挥的治疗作用

3.1 血管新生

Shh 信号通路在脑损伤后的功能恢复中发挥着举足轻重的作用^[9]。缺血性脑卒中发生后,机体中代偿机制被激活,如血管新生,以增加脑梗死边缘区域(infarction border zone, IBZ)的氧糖供应^[38]。血管新生不仅有助于 IBZ 内神经元的存活,而且也是脑卒中患者神经功能恢复的重要基础^[39-40]。据报道, CD34⁺的内皮细胞(也称为内皮祖细胞)在血管再生过程中起到了关键的作用,但在未激活前的 CD34⁺内皮细胞却表现出较低的再生能力。Shh 蛋白却能激活粒细胞集落刺激因子(granulocyte-colony stimulating factor, G-CSF)动员的 CD34⁺细胞,增强其迁移、增殖、黏附以及向血管谱系细胞分化的潜能,从而直接促进血管新生^[41]。Shh 介导的血管新生可能与其激活 TGF- β 信号通路有关^[41]。通过体外研究也发现,氧-葡萄糖剥夺(oxygen-glucose deprivation, OGD)处理后的星形胶质细胞能够分泌 Shh 蛋白激活 BMEC 中的 RhoA/ROCK 途径,以促进 BMEC 的增殖、迁移和成管,从而在体外发挥促血管生成作用^[42]。除此之外, Shh 还能通过核受体亚家族 2F 组成员 2(nuclear receptor subfamily 2 group F member 2, NR2F2)促进 OGD 后的星形胶质细胞中血管内皮生长因子(vascular endothelial

growth factor, VEGF)、Ang-1 和 Ang-2 的表达^[43](图 1)。体内研究发现,脑室注射的 Shh 能够显著上调其 VEGF 和 Ang-1 的表达,并促进恢复期 MCAO 大鼠脑组织中的血管生成和微血管密度增加^[44-47]。

3.2 神经发生

在脑卒中恢复期,其脑梗死 IBZ 内可观察到神经发生,但脑室下区域(subventricular zone, SVZ)的神经发生最为明显^[48]。Shh 信号通路对 SVZ 神经干细胞的维持和自我更新至关重要,Shh 信号通路激活会诱导 SVZ 内的神经干细胞分化为特定类型的神经元^[49](图 1)。SVZ 内新产生的神经祖细胞具备一定的干细胞特性,不仅在 I/R 损伤后的神经重塑中起到了关键作用,而且会分化为神经前体细胞,并从 SVZ 内迁移至受 I/R 损伤的皮质和纹状体^[9,50]。迁移至受损区域的神经前体细胞分化为成熟的神经元细胞,并与受损严重的神经元细胞融合,以促进神经功能恢复^[51-52]。近几年研究发现,谷氨酸能神经元(mossy cells, MC)生成的 Shh 蛋白不仅有助于海马区细胞的存活,而且能调节其神经发生^[53]。Shh 信号通路的激活能够通过增强 SVZ 内神经前体细胞的增殖以及向神经元和髓鞘少突细胞的分化,促进缺血后 IBZ 内的神经发生,加速神经功能恢复^[11]。此外,激活的 Shh 信号通路还能减轻 I/R 损伤,使受损较轻的神经元细胞加速神经突生长和突触形成^[29]。而 Shh 的敲除和抑制导致神经发生显著减少,这说明 Shh 信号通路可能在神经发生过程中起着举足轻重的作用^[11,29,54-55]。

3.3 神经可塑性

I/R 损伤后,中枢神经系统所表现出的实质性神经可塑性对其神经功能恢复发挥着重要作用,可以弥补有限的神经再生^[52,56]。BDNF 不仅在神经发生过程中起重要作用,而且在神经可塑性中发挥着主要作用^[57]。BDNF 能够增强神经突触的形成、维持、生长和可塑性,而 Shh 能够与其相互作用进而调节神经可塑性^[57-58]。Shh 的增加也能上调再生轴突中 BDNF 水平^[58]。也有研究表明,Shh 信号通路的激活能够促进脑卒中后 IBZ 内神经元细胞的轴突生长以及突触形成,进而参与神经重塑过程^[10,29,59](图 1)。Shh 信号通路能够介导 tPA 所改善的神经可塑性,显著促进缺血后的功能恢复^[10,60]。此外,由于 Shh 信号通路在神经祖细胞(neural progenitor cells, NPC)向神经元的增殖和分化过程中发挥着重要作用,并可调节其轴突的生长、突触形成和突触可塑性^[61],说明 Shh 信号通路不仅仅在其病理状态下参与神经可塑性,而在整个生命过程中都调节神

经回路的形成及其可适应环境的可塑性。

4 Shh 信号通路的相关药物研究

如图 1 所示,在发病前期,Shh 信号通路通过调节 ERK/PI3K/Akt 通路和抑制泡沫细胞的形成来预防脑血栓形成。在急性期,Shh 信号通路能够通过多种作用来发挥神经保护作用,包括调控 Akt/ERK 通路、增加 GSH-PX/SOD1/BDNF 蛋白表达来发挥抗氧化应激的作用;通过调控凋亡蛋白(Bcl-2、Bax)、激活 PI3K/Akt 通路、抑制 p53 通路以及减弱 Caspase-3 活性来发挥抗细胞凋亡的作用;通过参与“nNOS-Sox2-Shh”轴来发挥抗兴奋性神经毒性的作用;通过炎症因子(IL-1 β 、TNF- α 、IFN- γ)的刺激与其过度表达的抑制来调控炎症反应;通过减轻 BMEC 的免疫应答促进 Ang-1 的蛋白表达来保护血脑屏障。而在恢复期,Shh 信号通路可通过促进血管新生、神经发生和神经可塑性来改善神经功能的恢复,其机制可能与增加 BDNF 蛋白表达、促进神经前体细胞/神经干细胞的增殖与迁移、激活 RhoA/ROCK/TGF- β 途径等有关。

值得关注的是,多种靶向 Shh 信号通路的小分子化合物正在被作为药物开发,有些已经应用于临床治疗,如经 FDA 批准可用于治疗基底细胞癌的 Erivedge(又称 Vismodegib),其通过抑制 Shh 信号通路而起效,并且有望用于治疗 Shh 信号通路呈过度激活状态的髓母细胞瘤^[62-64]。其小分子激动剂嘌呤胺(Purmorphamine)已在临床前研究中发现其具有神经保护作用、增强神经发生以及促进血管新生等作用^[65-66]。此外,Shh 蛋白的直接应用也在临床前研究中被证实其能够减轻 I/R 损伤以及改善神经功能的预后^[9,67]。某些具有活血化瘀功能的中药,如丹参和川芎,能够显著调控 Shh-Ptch1-Gli1 介导的轴突导向通路而发挥抗 I/R 损伤作用,说明中药/天然产物可能是开发防治脑卒中药物的新来源。

5 结语与展望

缺血性脑卒中是主要由动脉粥样硬化引起,发病突然、损伤程度重、不易恢复。在不同阶段,缺血性脑卒中所涉及的主要病理因素也有所不同。越来越多的研究表明,Shh 信号通路对于脑卒中的预防和治疗表现出了积极有效的作用,即在脑卒中前期主要通过抗动脉粥样硬化起到预防作用,在急性期通过发挥神经保护作用(主要包括抗氧化应激、

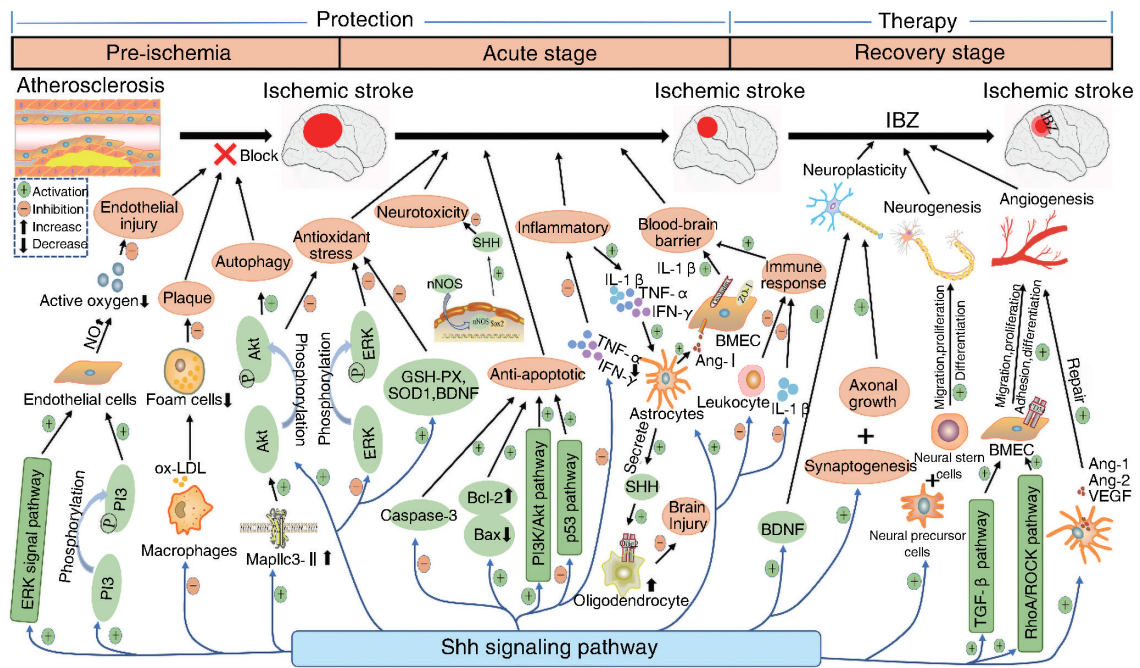


图 1. Shh 信号通路在缺血性脑卒中的发病前期、急性期和恢复期所发挥的主要作用
 Figure 1. The main role of Shh signaling pathway in the pre-ischemic, acute and recovery phases of ischemic stroke

抗兴奋性神经毒性、抗凋亡和炎症反应)减轻 I/R 损伤,在恢复期能够促进血管新生、神经发生和神经可塑性来增强神经功能恢复。

本文通过对 Shh 信号通路在脑卒中不同阶段所发挥的作用进行综述,有助于更加全面地了解 Shh 信号通路对于脑卒中疾病的防治作用。需要指出的是,Shh 信号通路可分为经典信号通路和非经典信号通路。目前 Shh 在缺血性脑卒中的不同发展阶段所发挥的作用均集中于其经典信号通路,而非经典 Shh 信号通路在过程中的作用有待于今后深入研究。

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