·综述·

脑缺血后血管内皮细胞对小胶质细胞的调节作用及机制

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摘要 缺血性卒中目前仍然是人类死亡和长期残疾的主要病因之一。血脑屏障作为大脑与外周之间的重要防线，在维持环境稳态中发挥着重要作用，而血脑屏障的主要成分内皮细胞与神经血管单元其他细胞的相互作用更为关键。缺血性卒中后，血脑屏障渗漏和内皮细胞功能障碍导致血浆成分外渗和循环炎症细胞浸润，进一步激活小胶质细胞等，加剧病灶损伤。本文结合最新的研究进展，对缺血性卒中后内皮细胞对小胶质细胞的作用途径及机制予以综述。

关键词 内皮细胞；血脑屏障；小胶质细胞；缺血性脑卒中

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Pathway and Mechanism of Vascular Endothelial Cells on Microglia after Ischemic Stroke

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Abstract Ischemic stroke remains one of the leading causes of death and long-term disability in humans. As an important barrier between the brain and the periphery, the blood-brain barrier (BBB) plays an important role in maintaining environmental homeostasis. The interaction between endothelial cells, a major component of the BBB, and other cells in the neurovascular unit is particularly critical. After ischemic stroke, BBB leakage and endothelial cell dysfunction lead to extravasation of plasma components and infiltration of circulating inflammatory cells, further activating microglia and aggravating lesion injury. This article will review the pathways and mechanisms of endothelial cells on microglia after ischemic stroke based on the latest research progress.

Keywords endothelial cells; blood-brain barrier; microglia; ischemic stroke

脑卒中已经成为全球第二大导致死亡的原因，具有发病率高、致残率高的特点，其中缺血性卒中是所有脑卒中类型中占比最高的一种。血脑屏障主要是指脑毛细血管壁与神经胶质细胞形成的血浆与脑细胞之间的屏障，能够阻止某些有害物质由血液进入脑组织，是中枢神经系统维持正常功能和稳态的重要结构基础[1]。脑血管内皮细胞（endothelial cells, ECs）是血脑屏障的主要组成部分之一，与周围其他细胞密切相互作用，以维持血脑屏障的特性。而小胶质细胞，特别是血脑屏障周围的小胶质细胞，作为中枢神经系统的固有免疫细胞，与内皮细胞保持着不断的双向交流。许多研究已经证明了血脑屏障破坏和脑驻留小胶质细胞激活之间存在非常紧密的时空相关性[2-4]。本文将对缺血性脑卒中后内皮细胞对小胶质细胞产生的作用途径进行综述。

1 缺血性卒中后内皮细胞的变化及对局部炎症微环境的影响

缺血性卒中会导致血脑屏障受损，而内皮细胞作为血脑屏障的主要成分，在脑卒中后不同阶段产生不同的病理改变，导致血脑屏障通透性的改变[5,6]。内皮功能障碍是缺血再灌注后炎症损伤和血管损伤的最早事件。缺血损伤后，内皮细胞和星形胶质细胞产生大量趋化因子和细胞因子刺激内皮细胞上粘附分子的表达，导致内皮紧密连接蛋白（tight junction protein，TJP）和细胞外基质的降解[7]；同时招募和激活炎性细胞（中性粒细胞、T细胞和单核/巨噬细胞等），使这些细胞通过细胞旁和细胞间途径迁移穿过血脑屏障并浸润中枢神经实质[1]，释放炎症介质和活性氧触发脑内局部炎症并激活小胶质细胞，导致血脑屏障进一步受损和内皮细胞退化[8]，进一步破坏血管完整性，加剧卒中不良结局[9]。

2 缺血性卒中后内皮细胞对小胶质细胞的作用途径

2.1 趋化因子（chemokine）

趋化因子是一种刺激白细胞趋化的促炎细胞因子[10]。在脑缺血损伤状态下，内皮细胞可产生大量的趋化因子，通过促进内皮细胞与白细胞的直接相互作用促进白细胞的募集，触发外周急性期反应[11]；同时也可通过趋化和激活小胶质细胞，加剧中枢神经系统的固有免疫反应[12,13]。

CXC趋化因子配体5（chemokine ligand 5，CXCL5）是CXC趋化因子家族的一个小细胞因子，

也被称为上皮中性粒细胞激活肽-78。CXCL5在许多不同的细胞中表达，包括内皮细胞、单核细胞和肺泡上皮II型细胞[12,14,15]。有研究表明，脑脊液中趋化因子CXCL5的水平在缺血性卒中24 h内即升高，并与早期脑损伤的严重程度呈正相关[16]；在炎症性疾病中，内皮细胞的激肽B1受体激活，自身分泌更高水平的CXCL5，导致白细胞募集[11]；在早产儿中，缺血缺氧导致脑白质损伤的24 h内，小胶质细胞和血管内皮细胞的CXCL5表达都上调，增加中性粒细胞的浸润。而CXCR2是一种趋化因子受体，表达在内皮细胞、少突胶质细胞和各种免疫细胞上[17]，与中性粒细胞浸润与白细胞募集有关[18]；通过抑制CXCR2减弱CXCL5的功能，可以显著减弱小胶质细胞的激活，减轻血脑屏障损伤，对白质损伤有保护作用[12]。

CX3CL1，也被称为Fractalkine，是一种跨膜趋化因子，在炎症期间由分离整合素和金属蛋白酶（A disintegrin and metalloproteinase，ADAM）-10从质膜释放，通过其G蛋白偶联受体CX3CR1作用于小胶质细胞和免疫细胞[4,19]。研究发现内皮细胞CX3CL1似乎对脑缺血后脑内白细胞运输的控制很重要，在脑卒中小胶质细胞与内皮细胞通讯中起重要作用，从而控制白细胞浸润；事实上，在缺血48 h和7 d后，CX3CL1也在梗死区内皮细胞中被诱导合成，而CX3CR1的表达可在活化的小胶质细胞中诱导血管募集白细胞到梗死区域[20]。另一方面，也有报道称CX3CL1在啮齿类动物永久性局灶性脑缺血后具有神经保护作用[21]。CX3CL1信号缺乏会抑制小胶质细胞的增殖，并促进实验性脑卒中后M2样小胶质细胞的激活[22]。

MIP-1β是一种从缺血脑微血管内皮细胞（brain microvascular endothelial cells，BMECs）释放的趋化因子，已被证明能刺激小胶质细胞的增殖[23]。氧糖剥夺（oxygen and glucose deprivation，OGD）损伤的BMECs表现为MIP-1β的大量分泌和表达，CCR5的上调。同时，经过OGD处理的BMEC的培养基（BMECs-CM）可以显著增加小胶质细胞的增殖活性[24]。一项研究调查表明BMECs-CM可抑制MIP-1β诱导的小胶质细胞的增殖和迁移。此外，BMECs-CM显著抑制MIP-1β受体CCR5的表达以及p38和JNK的磷酸化，提示BMECs-CM可抑制MIP-1β诱导的小胶质细胞活化[23]。

2.2 血管内皮生长因子（vascular endothelial growth factor，VEGF）

VEGF家族包括VEGF-A, -B, -C, -D, -E和胎盘生长因子，是一种众所周知的内皮细胞有丝分裂原、血管生长和通透性因子[25-28]，其中VEGF-A的2种亚型VEGF165、VEGF121最常见。VEGF主要由血管周围的细胞产生，并通过旁分泌机制作用于内皮细胞，并在促进血管形成、抑制内皮细胞的凋亡及提高血管通透性等方面发挥重要作用。在脑缺血中也存在内皮源性VEGF诱导的小胶质细胞激活[29]。在缺氧和缺血的情况下，VEGF及其受体在数小时内迅速诱导神经元和神经胶质细胞[30]。另一方面，内皮VEGF165以VEGFR1依赖的方式降低清道夫受体a的表达，抑制脑缺血后小胶质细胞的激活，降低IL-1β、TNF-α和iNOS的分泌，有效抑制缺血诱导的神经炎症和随后的脑损伤，发挥神经保护作用[28]。此外，VEGF通过vegfr1介导的信号传导，驱动巨噬细胞和小胶质细胞迁移到受损的中枢神经系统[31-33]，使小胶质细胞向抗炎表型转化[26]。

2.3 内皮源性细胞外囊泡

细胞间的通信是由分泌的生物活性分子，如短肽、蛋白质、脂类和核酸介导的。这些小分子通常由细胞释放，并与靶细胞上的特定受体结合，诱导细胞内信号转导，改变靶细胞的病理生理状态[34]。而细胞外囊泡（extracellular vesicles，EVs），包括微颗粒（microvesicents）、微囊泡（microvesicents）和外泌体（exosomes），也从不同的细胞类型中释放出来[35,36]，并且有新的证据表明，EVs是这些生物活性分子的载体[37,38]。

血管内皮细胞是向血流中释放EVs的主要来源之一，并且血液循环中内皮源性EVs的数量与疾病的严重程度相关[39]。有研究发现，被系统性炎症激活的大脑内皮细胞会通过分泌的外泌体进一步激活神经血管单元中的邻近细胞，并且内皮源性EVs在不同病理条件下的循环血液中均上调[34]。脑卒中后，受损的内皮细胞释放促炎因子和EVs，通过渗漏的血脑屏障，激活星形胶质细胞和小胶质细胞释放促炎因子（TNFα、IL1β）[40]，加重病灶的损伤[41]。

微小核糖核酸（microRNAs，miRNAs）是一种内源性的小的非编码RNA分子，通过与一个或多个mRNA的3个非翻译区域结合，以一种序列特异性的方式抑制基因表达[42]。脑血管内皮细胞富含多种调节复杂内皮生理功能的miRNAs[43]，内皮源性EVs通过向靶细胞输出miRNAs等介导细胞之间的通信；研究发现，炎症刺激诱导的内皮源性EVs中携带大量特异性的miRNAs，可以通过调节周细胞的基因表达谱来介导内皮细胞的炎症反应[34]。与此同时，在脑缺血模型中，内皮细胞miR-15a/16-1可能通过直接抑制血管紧密连接蛋白Claudin-5在微血管内皮细胞中的表达，加重血脑屏障破坏，增强中性粒细胞和巨噬细胞的浸润，使小胶质细胞从M2型向M1型极化，加重缺血后的炎症损伤[42]。

2.4 其他影响途径

基质金属蛋白酶（matrix metalloproteinases，MMPs）是一类在组织重塑和修复中起重要作用的细胞外蛋白酶。MMPs通过调节细胞外基质来调节炎症[44]，在组织重塑中发挥积极作用[45]。有研究表明，脊髓损伤后血管内皮细胞产生和分泌的MMP-3诱导小胶质细胞激活，随后p38MAPK激活和pro-NGF产生，从而介导少突胶质细胞的细胞死亡[46]。在中枢神经系统中，CD200主要表达在神经元和内皮细胞表面，通过与表达在小胶质细胞上的CD200受体（CD200R）相互作用，触发小胶质细胞的免疫抑制[47]。此外，与OGD的星形细胞激活的小胶质细胞相比，OGD的内皮细胞激活的小胶质细胞更活化，具有神经毒性，释放更多的促炎因子TNFα、IL-1β、IL-10，降低胰岛素样生长因子（insulin-like growth factor，IGF）-1的表达，迁移和吞噬能力受到抑制[48]。

3 小结与展望

脑缺血后血脑屏障受损可能是其关键的始动病理环节。内皮细胞缺血损伤后，通过释放各种趋化因子、炎症因子和细胞外囊泡，以及与血管内皮生长因子结合等方式，对小胶质细胞、星形胶质细胞、白细胞等产生一系列的影响，发挥加重或者缓解病灶损伤的双重作用，影响疾病进程。综上所述，内皮细胞及血脑屏障在脑卒中的发生发展中起着重要作用，还需要进一步探索其中的机制。

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