Claudin-5介导的BBB功能障碍在脑缺血再灌注中的作用

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摘要 Claudin-5为一种跨膜蛋白, 是脑内皮细胞间紧密连接的重要黏附分子, 参与血脑屏障的组成并调节其通透性和紧密性, 参与介导了脑缺血再灌注损伤的发生发展。该文综述了Claudin-5的结构与功能及其在脑缺血再灌注中的作用, 为脑缺血再灌注损伤的治疗提供新的理论依据。

关键词 Claudin-5; BBB; 脑缺血再灌注

The Role of Claudin-5 Mediated BBB Dysfunction in Cerebral Ischemia Reperfusion

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Abstract As a transmembrane protein and an important adhesion molecule of tight junctions between brain endothelial cells, Claudin-5 participates in the composition of the blood-brain barrier and regulates its permeability and tightness. Also it mediates the occurrence and development of cerebral ischemia reperfusion injury. This article reviews the structure and function of Claudin-5, and its role in cerebral ischemia reperfusion, providing a new theoretical basis for the treatment of cerebral ischemia reperfusion injury.

Keywords Claudin-5; BBB; cerebral ischemia reperfusion

近年来, 脑血管疾病发病率逐年攀升, 其中缺血性脑血管病的发生尤为显著。而缺血性脑血管病的治疗以恢复血流再灌注为主, 但往往会导致更加严重的继发性脑损伤, 即脑缺血再灌注损伤(cerebral ischemia reperfusion injury, CIRI)。在导致CIRI的多种病理生理机制中, 血脑屏障(blood-brain barrier, BBB)功能障碍也参与其中。闭合蛋白-5(Claudin-5)为脑内皮细胞(endothelial cells, ECs)间紧密连接(tight junctions, TJs)的重要组成成分, 介导了BBB细胞旁途径功能障碍以及缺血后出血性转化、致死性脑水肿等, 严重影响疾病的预后。因此, 本文综述了Claudin-5的结构与功能, 脑缺血再灌注(ischemia reperfusion, I/R)后它的表达与调控, 以及其作为可能的脑缺血治疗靶点的相关研究进展。

1 Claudin-5的结构与功能

闭合蛋白(Claudins)是一个大小在207至305个氨基酸范围的跨膜蛋白家族, 它们具有相同的二级

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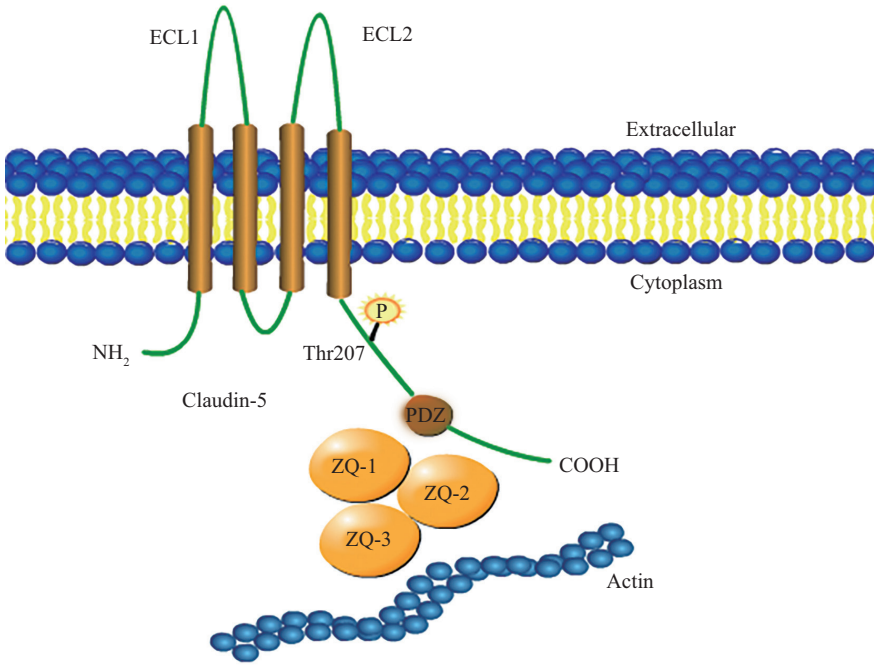


ZO-1: 闭锁小带-1; ZO-2: 闭锁小带-2; ZO-3: 闭锁小带-3。
ZO-1: zonules occludens-1; ZO-2: zonules occludens-2; ZO-3: zonules occludens-3.

图1 Claudin-5的结构图(根据参考文献[1]修改)

Fig.1 The structure of Claudin-5 (modified from reference [1])

结构, 即四个跨膜结构域, 包括朝向胞质溶胶的N末端和C末端结构域、两个细胞外环(extracellular loops, ECLs)和一个短的细胞内环结构域, 通过C末端结构域中含有的PDZ结合基序, 使Claudins与细胞质支架蛋白相互作用进而间接与肌动蛋白细胞骨架连接, 从而稳定Claudins功能并最终维持BBB的通透性[1](图1)。在哺乳动物中, Claudins在肾脏、胃肠道及呼吸道等上皮中及内皮细胞中广泛表达, 由于组织结构的差异, Claudins表达亚型也不同[2]。在ECs中, 除了Claudin-1、3、12等以外, 主要为Claudin-5, 为其中TJs的主要细胞黏附分子, 并通过肌动蛋白的收缩在建立内皮细胞紧张性中发挥核心作用, 导致BBB中的屏障密封比其他组织内的密封性更强[3-4]。

BBB为血液与脑实质的物理屏障, 由血管内皮细胞、基底膜、周细胞、星形胶质细胞足突及其外周的细胞外基质(extracellular matrix, ECM)组成, 它负责选择性运输维持大脑内环境稳态的重要离子和介质, 并向其转运营养素, 去除代谢废物, 抵挡外来有害物质对脑实质的损伤[5]。作为生物体内最严密的屏障, 它可以通过跨细胞途径及细胞旁途径来调控屏障的通透性。而细胞旁途径则涉及ECs的胞旁间隙, 它主要由紧密连接蛋白(tight junction proteins, TJPs)密封, 通过Claudins和咬合蛋白(occludin)及其与细胞骨架支架蛋白相互作用, 将TJPs“连接”到肌动蛋白细胞骨架来实现的[6]。Claudin-5作为主要的成分, 参与了TJs介导的BBB的调节。

2 脑I/R后Claudin-5的表达及调控

2.1 脑I/R后Claudin-5的表达

Claudin-5作为BBB的TJs重要组成成分, 它的表达变化介导了BBB功能障碍, 因此也参与了CIRI。在脑微血管内皮细胞氧糖剥夺复氧(oxygen-glucose deprivation reoxygenation, OGD/R)实验中, 有学者发现在复氧后3 h Claudin-5的表达开始显著下调[7]。另有研究表明, 在缺血半影带中, 紧密连接蛋白Claudin-5和闭锁小带蛋白1(zonula occluden-1, ZO-1)的水平在血管重塑的早期阶段降低, 后期增加, 并且这种动态变化与BBB通透性的动态变化紧密相关[8]。由此可见, 脑I/R后Claudin-5表达的变化参与介导BBB通透性的改变, 而后者的增加恶化了脑缺血的不良后果, 严重影响了疾病的预后。

2.2 脑I/R后Claudin-5的调控

在参与CIRI的众多病理机制中, 血管内皮生长因子(vascular endothelial growth factor, VEGF)等营

养因子、蛋白激酶(protein kinase, PK)及磷脂酰肌醇3激酶(phosphatidylinositol 3 kinase, PI3K)/蛋白激酶B(protein kinase B, Akt)信号转导通路、激素等参与了对Claudin-5的调控, 它们可以通过抗氧化或(和)抗炎等机制从转录前或者翻译后水平上调节Claudin-5的表达、减少其降解或影响其在ECs膜上的再分布, 介导缺血后BBB通透性的改变。

2.2.1 VEGF等营养因子 VEGF是一种具有神经保护作用的血管生成刺激因子, 同时也是一种有效的血管通透性调节因子。它可以通过转录因子蜗牛家族锌指2(snail family zinc finger 2, SNAI2)以及N-myc原癌基因蛋白(N-myc proto-oncogene protein, MYCN)特异性地调节*Claudin-5*的基因表达, 而对它们的沉默则可以抑制VEGF介导的Claudin-5下调[9]。脑缺血后应用VEGF在改善血管和神经生成的同时增加了微血管的渗漏, 导致缺血缺氧性脑血管渗漏成为临床应用VEGF的主要障碍。在对VEGF亚型的研究中, 有学者发现, VEGF-A增加了脑微血管破坏的风险, 而VEGF-B通过与周细胞膜上VEGF受体1特异性结合, 促进了内皮细胞和周细胞之间的相互作用以及损伤区域内稳定的脑微血管的形成, 是一种有吸引力的血管生成因子替代亚型[10]。

表皮生长因子(epidermal growth factor, EGF)在脑I/R后可以通过上调TJPs的表达和降低内皮通透性来改善缺血性BBB损伤[11], 转化生长因子-β(transforming growth factor, TGF-β)以及其他神经营养因子如胶质细胞源性神经营养因子(glial cell line-derived neurotrophic factor, GDNF)与脑源性神经营养因子(brain-derived neurotrophic factor, BDNF)等, 虽然影响Claudin-5表达和BBB跨膜电阻, 但在脑I/R后的研究尚少。

2.2.2 PK PK在细胞信号转导、细胞周期调控的过程中发挥重要作用, 因此也参与了BBB的TJPs中跨膜蛋白Claudin-5的调控。在缺氧及缺氧后复氧过程中, 有研究表明, 蛋白激酶C(protein kinase C, PKC)同工酶nPKC-θ和aPKC-ζ的表达明显增多, 并被磷酸化激活而转移至胞膜, 与TJ结构区域内的TJPs底物结合, 改变TJPs的丝氨酸/苏氨酸磷酸化状态, 介导TJPs的破坏及随后BBB通透性的增加[12]。而H_2S作为一种具有信号转导作用的分子, 可以通过抑制PKC-α、βI、βII和δ的活化, 促进PKC-ε的活化, 增加Claudin-5等的表达改善大鼠脑I/R后BBB的完整性, 减轻脑水肿, 改善神经功能[13]。同样, 相关的信号转导通路也介导了Claudin-5的调控, 影响BBB的功能状态。IL-9增加了OGD/R后TJPs的缺失, 这种作用是通过激活IL-9受体而介导的, 该受体增加了内皮型一氧化氮合酶(endothelial nitric oxide synthase, eNOS)的表达, 并通过上调信号转导子和转录激活子1/3(Signal transducer and activator of transcription 1/3, STAT1/3)以及下调PI3K/Akt信号通路介导BBB的破坏作用[14]。而还原型谷胱甘肽(glutathione, GSH)则通过促进PI3K/Akt途径, 抑制叉头盒蛋白O3(forkhead box protein O3, FOXO3)向细胞核的转运, 促进B淋巴细胞瘤-2(B-cell lymphoma-2, Bcl-2)的表达, 通过增加脑ECs的存活等多种方式维持BBB完整性及功能状态[15]。

Rho激酶为丝氨酸/苏氨酸蛋白激酶, 作为Rho的下游分子, 它的激活可以使Claudin-5的T207位点磷酸化, 从而干扰与TJ锚定分子(如ZOs)的结合, 导致其在质膜上不稳定[16]。而在I/R后, Rho激酶抑制剂法舒地尔, 抑制了促氧化剂NADPH氧化酶的活性、超氧阴离子的释放等, 使紧密连接蛋白Claudin-5表达显著增加[17]。

2.2.3 激素 激素具有抗炎作用, 而由于炎症参与了CIRI, 因此, 激素的应用也可能通过调控TJPs的表达改善I/R后BBB的通透性, 进而发挥神经保护作用。众所周知, 雌激素的神经保护作用是通过经典的雌激素受体途径发挥的, 也有研究表明通过G蛋白偶联雌激素受体-1(G protein-coupled estrogen receptor 1, GPER-1)这一非经典的雌激素受体途径可以增加海马CA1中TJPs以及减少VEGF-A, 进而达到神经保护的效果[18]。除了雌激素外, 孕激素减轻缺血后凝血酶增加诱导的BBB的破坏也是与阻断bEnd.3细胞中Claudin-5和内皮蛋白C受体(endothelial protein C receptor, EPCR)等的降解有关[19]。糖皮质激素如地塞米松通过糖皮质激素受体a(glucocorticoid receptor a, GRa)和核受体共同阻遏物(nuclear receptor co-repressor, N-CoR)与组蛋白H3K27去甲基化酶*JMJD3*基因上游的负性糖皮质激素反应元件(negative glucocorticoid response element, nGRE)结合, 抑制JMJD3的表达, 致使基质金属蛋白酶(matrix metalloproteinase, *MMP*)-*2*、*MMP-3*和*MMP-9*的激活被抑制, 同时, 它还能激活*Claudin5*和*occludin*的表达, 因此减轻肿瘤坏死因子α(tumor necrosis factor-α,

TNF-α)处理后脑微血管内皮细胞TJ的破坏[20]。

神经甾体激素维生素D, 它的缺乏常共存于其他疾病, 有研究表明, 脑I/R后予以足量维生素D, 可以提高BBB的稳定性, 其机制是通过与维生素D受体结合抑制活性氧自由基(reactive oxygen species, ROS)的产生和核因子κB(nuclear factor-kappa B, NF-κB)的激活以及MMP-9的表达等, 增加TJPs水平来保护脑内皮功能[21]。与之相反的是, 储存在突触小泡中起着信号传递作用的锌, 脑缺血后从神经元末梢释放并在微血管中积聚, 通过激活自由基-MMPs途径降解Claudin-5, 在体内外对BBB通透性的增加起着至关重要的作用[22]。

2.2.4 *其他* 除上述的分子、激素及其相应的信号转导通路之外, BBB各组成成分以及各成分之间的相互作用, 也可以调控Claudin-5的表达, 进而影响BBB通透性。

内皮细胞黏附连接(adhesion junctions, AJs)中两种黏附连接蛋白β-连环蛋白(β-catenin)及血管内皮钙黏蛋白(vascular endothelial cadherin, VEC)也参与缺血后Claudin-5表达的调控。在全脑I/R模型中, 激活经典的Wnt/β-catenin信号通路也可以增加ZO-1、Claudin-5、β-catenin等的表达, 降低MMP-9、糖原合酶激酶-3β(glycogen synthase kinase 3β, GSK-3β)的表达对BBB具有保护作用[23]。VEC在血管成熟和稳定过程中起着重要作用, 是内皮细胞AJs的主要成分, 该分子由ECs特异性表达, 介导细胞–细胞接触处的亲和力黏附, 可以通过上调*Claudin-5*等在血管稳定中起关键作用的内皮特异性基因来维持血管稳定性, 但在脑I/R后的研究尚少[24]。

基质金属蛋白酶(matrix metalloproteinases, MMPs)在脑缺血后发挥着双重作用, 早期介导ECM的降解及随后BBB的损伤, 而在恢复期促进了血管的生成。脑I/R后, 缺血侧Claudin-5染色强度降低但MMP-2免疫活性增强, 合并图像显示MMP-2与Claudin-5共定位, 并且通过免疫印迹证明Claudin-5表达减少伴随其碎片化的表达明显增多, 予以MMPs抑制剂后Claudin-5表达的降低被显著抑制, 支持MMPs对Claudin5的降解作用。然而, I/R后不同时期, 可由不同的MMPs发挥作用, 介导BBB通透性的改变[25]。

小窝蛋白(caveolin-1)为细胞膜内陷形成质膜的主要成分, 广泛存在于内皮细胞、平滑肌等细胞中, 利于质膜内陷形成小窝、内吞胞吐以及细胞信号的转导[26]。有研究表明, 脑缺血后*caveolin-1*基因敲除小鼠水通道蛋白4(aquaporin-4, AQP4)表达减少并在血管周围的覆盖受损, 导致脑组织肿胀增加, 可能与星形胶质细胞形态的改变以及eNOS的抑制等有关[27], 表明其对缺血后脑肿胀具有保护作用。而另一项研究发现, 常氧状态下, Claudin-5分布在脑微血管内皮细胞膜上, 而低氧诱导其从胞膜上脱位并聚集在在胞质溶胶中, 该过程是由依赖caveolin-1的内吞作用介导的, 但这种异常集聚的Claudin-5与自噬标记物LC3B共定位, 通过自噬的激活降解caveolin-1和Claudin-5, 影响Claudin-5的再分布, 维持BBB功能状态[28]。因此, caveolin-1在脑缺血中的作用仍有争议, 可能与其对BBB通透性不同途径的调节有关。

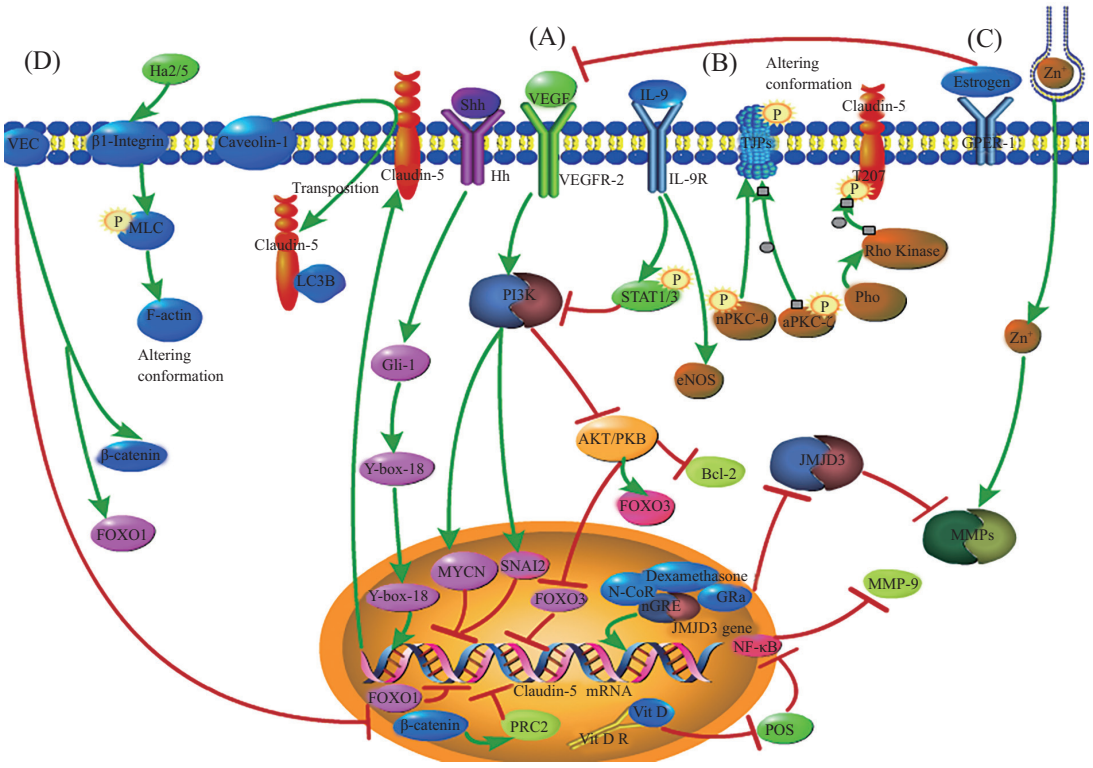
BBB组成成分相互作用同样也可以调控缺血后Claudin-5的表达。微血管内皮细胞β1整合素与下层基底膜的黏附是维持BBB完整的必要条件, 通过β1整合素依赖的肌球蛋白轻链(myosin light chain, MLC)信号和F-肌动蛋白构象, 对TJP进行正确定位。单克隆抗β1整合素IgM-Ha2/5对内皮细胞β1整合素–基质黏附的干扰增加了MLC磷酸化改变了F-肌动蛋白构象等, 但在予以MLC激酶(MLC kinase, MLCK)和Rho相关蛋白激酶(Rho-associated protein kinase, ROCK)抑制剂后则消除了Ha2/5依赖性增加的内皮通透性[29]。刺猬(hedgehog, hh)通路是血管周围星形胶质细胞与BBB-ECs进行通讯的途径, 在BBB水平上具有双重保护作用, 通过促进屏障形成和作为内源性抗炎系统发挥作用。星形胶质细胞分泌声波刺猬(sonic hedgehog, Shh)诱导BBB-ECs中hh受体的表达, hh激活后致使Gli-1转录因子及随后性别决定区域Y-box-18(sex-determining region Y -box-18, SOX-18)向BBB-ECs核内上调和移位, 诱导靶基因*Claudin-5*的表达, 促进屏障形成[30], 该通路对Claudin-5调控的机制在脑I/R后需进一步探究(图2)。

3 脑I/R后基于Claudin-5的治疗

基于脑I/R后Claudin-5的调控, 可以靶向相关分子及信号转导通路、激素等提高缺血后Claudin-5的表达以稳定BBB, 改善缺血不良后果。

3.1 调控VEGF、激素等相关信号通路

IL-9增加缺血后星形胶质细胞中VEGF-A的水平, 因此, 予以抗IL-9中和抗体则可以下调VEGF-A、



绿色箭头代表上升或者激活, 红色线条代表下降或者抑制。Ha2/5为抗β-1整合素IgM, PRC2为聚梳抑制复合物2。

The green arrow represents rising or activation, and the red line represents falling or inhibition. Ha2/5 is anti-β-1 integrin IgM, and PRC2 is polycomb repressive complex 2.

图2　脑缺血后Claudin-5的调控

Fig.2　Regulation of Claudin-5 after cerebral ischemia

修复受损的TJPs来减轻BBB的破坏[31]。Src蛋白酪氨酸激酶抑制剂可以减少Src的磷酸化, 降低VEGF-A增加Claudin-5的表达来保护I/R后损伤的BBB[32]。临床上通常予以高渗盐水减轻水肿, 其机制可能是通过抑制VEGF-VEGFR2介导的ZO-1、Claudin-5的下调以及小胶质细胞中NLRP3炎性小体的活化进而减少细胞中VEGF的表达来实现的[33-34]。除了下调VEGF或其受体外, 予以相应的对抗因子如色素上皮衍生因子(pigment epithelium-derived factor, PEDF), 逆转VEGF介导的Claudin-5的降低, 也可以减轻BBB的损伤[35]。而在激素治疗方面, 同样可以通过对激素或(和)相应受体或者下游信号转导通路的激活或抑制, 调控Claudin-5的表达, 进而发挥BBB的保护作用。

3.2　调控PK通路

调控相应的PK通路, 也可以增加BBB Claudin-5的表达, 改善脑I/R后BBB通透性及预后。DL-3-正丁基苯酞通过降低氧化应激和激活Akt/GSK-3β/β-catenin信号通路, 增加磷酸化的Akt与GSK-3β, 使细胞核内β-catenin增加, 减少MMP-9对Claudin-5及ZO-1的降解, 改善慢性低灌注大鼠BBB的损伤[36]。小GTP结合蛋白Rho家族, 又称Rho GTP酶(Rho GTPases), 是肌动蛋白骨架的关键调控因子, 影响了I/R后BBB的功能状态, 而来源于腹蛇的血小板膜糖蛋白Ib受体拮抗剂安菲博肽(Anfibatide), 可以抑制脑I/R后Toll样受体4(toll-like receptors 4, TLR4)介导的RhoA/ROCK信号通路减少Claudin-5等TJPs的降解来维持BBB的完整性[37]。

3.3　干预BBB结构成分

细胞旁间隙各成分或者其相互作用可以影响Claudin-5, 因此, 干预前者可以增加Claudin-5的表达或者影响其在脑ECs上的分布, 最终达到治疗效果。

ECs中miR-15a/16-1基因缺失减轻了脑卒中小鼠BBB渗漏, 降低了脑含水量和脑梗死体积, 抑制了梗死周围区M1型小胶质细胞/巨噬细胞极化, 减少了外周巨噬细胞和中性粒细胞的浸润, 这些作用可能是通过上调*Claudin-5*来实现的[38]。通心络胶囊则通过激活Shh通路上调TJPs来实现对永久性脑缺血

小鼠BBB的保护作用[39]。

3.4 非药物治疗

除了药物治疗外, 非药物治疗在脑I/R后BBB保护方面的治疗也取得了相当不错的进展。发生缺血性脑卒中时, TJPs可被降解, 移植的少突胶质细胞前体细胞释放Wnt7a, 作用于内皮细胞膜上相应的受体Fzd, 然后激活Wnt/β-catenin途径并上调Claudin-5[40]。而予以短暂的双侧颈总动脉闭塞缺血预处理后, TJPs Claudin-5及occludin的表达降低, 炎症反应增加, 激活内源性血管保护和抗炎机制以响应缺血预处理诱导的BBB破坏和炎症反应, 从而减少随后严重的缺血性脑损伤, 而这种保护作用被细胞外信号调节激酶1/2(extracellular signal-regulated kinase1/2, ERK1/2)抑制剂逆转[41]。同样, 肢体远端缺血后处理也可以减少BBB的损伤, 对大脑发挥保护作用, 该机制是通过抑制MMP-9对Claudin-5的降解来实现的[42]。物理治疗在脑I/R后的BBB保护中也表现出明显的优势。亚低温可以减少TJs和AJs VEC的破坏、抑制MMP-9和VEGF的表达以及上调血管生成素-1(angiogenin-1, Ang-1), 减轻猪心肺复苏模型脑水肿和BBB破坏[43]。而在传统的针灸治疗中, 电针预处理及后处理均可以上调Claudin-5等TJPs的表达而减轻脑损伤和相关的行为缺陷[44-45]。强制性运动疗法也可以促进脑I/R后血管生成和神经发生, 增加Claudin-5的表达部分是通过克服轴突生长抑制剂(Neurite outgrowth inhibitor-A, Nogo-A)/RhoA/ROCK信号通路实现的[46]。

4 小结与展望

Claudin-5作为一种重要的脑内皮细胞黏附分子, 同时也是一种重要的跨膜蛋白, 介导脑缺血后BBB通透性改变的同时也与脑I/R后其他病理机制相互促进, 进一步加重缺血后的不良后果。未来应进一步深入研究其在CIRI汇总的病理生理学意义, 以期为脑缺血的治疗提供可能的新的靶点。

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