·专题综述·

常染色体显性遗传性脑动脉病伴皮质下梗死和白质脑病病理学及分子机制研究进展

孙瑗璟 范玉华

【摘要】 常染色体显性遗传性脑动脉病伴皮质下梗死和白质脑病(CADASIL)是*Notch3*基因突变导致的遗传性脑小血管病。目前*Notch3*基因突变致CADASIL的分子机制尚不明确。本文综述CADASIL的病理改变、基因突变以及可能的相关分子机制,包括Notch3蛋白及其下游靶标的异常信号转导,细胞内质网应激和RhoA/Rho激酶活化,血管平滑肌细胞收缩异常及其自噬机制紊乱、增殖分化异常。

【关键词】 CADASIL; 受体,Notch3; 病理学; 病理学,分子; 综述

Pathological changes and molecular - genetic mechanisms of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy

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【Abstract】 Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a inherited cerebral small vessel disease caused by *Notch3* gene mutation. The molecular-genetic mechanisms of CADASIL have been still unclear. This review includes the physiopathology and gene mutation of CADASIL. We will focus on the underlying pathogenesis based on worldwide researches and literatures in recent years, including abnormal signaling of Notch3 protein and downstream targets, endoplasmic reticulum stress and activation of RhoA/Rho kinases, abnormal contraction, proliferation and differentiation of vascular smooth muscle cell, and autophagy defect of vascular smooth muscle cell.

【Key words】 CADASIL; Receptor, Notch3; Pathology; Pathology, molecular; Review

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常染色体显性遗传性脑动脉病伴皮质下梗死和白质脑病(CADASIL)是*Notch3*基因突变导致的非淀粉样变性、非动脉粥样硬化性、常染色体显性遗传性脑小血管病(CSVD)。通常于中青年发病,临床主要表现为反复的短暂性脑缺血发作(TIA)和缺血性卒中、认知功能障碍、情感障碍、偏头痛等。亚洲人群以缺血性卒中为首要或主要表现,偏头痛少见,欧美人群则常以偏头痛为首发症状。影像学表现包括脑白质高信号(WMH)、皮质下梗死和脑微出血(CBMs),T_2WI和FLAIR成像高信号好发于侧脑室周围和前颞极、外囊、额顶叶白质,目前认为双侧前颞叶长T_2信号即"O'Sullivan征"在CADASIL的诊断中具有较高的敏感性和特异性,可资与其他脑小血管病相鉴别。病变主要累及微小动脉,光学显微镜下可见血管平滑肌细胞(VSMC)缺失变性,血管中膜纤维化,管壁增厚,病变广泛,多位于侧脑室周围、基底节和脑干;超微结构可见颗粒性电子致密

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嗜锇物质(GOM)沉积于血管平滑肌细胞基膜凹陷处,是CADASIL的诊断"金标准"之一。基因检测检出*Notch3*突变是确诊依据,目前文献报道*Notch3*基因有200余种突变与CADASIL发病相关。本文拟对CADASIL病理改变、基因突变及可能的相关分子机制进行综述。

一、CADASIL血管病理改变

CADASIL主要累及直径50~100 μm的微小动脉。光学显微镜下可见皮下组织小动脉纤维化,管壁增厚,血管中膜平滑肌细胞缺失变性等退行性变,且随病程进展,管腔进行性狭窄,出现血管功能障碍。电子显微镜观察,皮肤组织活检标本在血管平滑肌细胞基膜处可见特征性GOM沉积,小动脉和毛细血管周围均有沉积,多围绕血管平滑肌细胞,而血管内皮细胞和周细胞周围较少见。此外,亦累及血管平滑肌细胞和毛细血管内皮细胞,胞质嗜锇染色深,吞饮小泡、空泡和致密颗粒形成突向管腔,胞核形状不规则,充满异染色质。针对GOM化学性质、来源和功能的研究是目前重点。Joutel等[1]在CADASIL患者脑组织中发现相对分子质量为210×10^3的Notch3裂解产物沉积,但并未发现Notch3蛋白细胞内结构域(Notch3ICD),表明*Notch3*基因突变可破坏细胞表面Notch3蛋白细胞外结构域(Notch3ECD)的清除,表明Notch3ECD是GOM的主要成分。晚近一项研究采用斑点免疫结合试验(DIBA)在CADASIL患者皮肤组织匀浆中观察到Notch3ECD沉积物[2]。晚近一项CADASIL转基因小鼠模型显示,GOM沉积并非静止过程,其大小、形态和数目不断变化,位于管壁细胞管腔侧的GOM随疾病进展自最初的小圆形,逐渐演变至无定形的块状沉积物,导致基膜突起和管壁细胞凹陷[3]。还可在老年小鼠脑部微血管观察到各时期形态的GOM共存,进一步证实GOM在CADASIL病程中是不断演变的。Ruchoux等[4]制备CADASIL转基因小鼠模型,并在电子显微镜下无GOM沉积的血管观察到血管内皮细胞和血管平滑肌细胞退行性变,表明CADASIL病程中*Notch3*基因产物积聚或GOM沉积对血管平滑肌细胞损伤均非必要条件,提示血管内皮损伤可能发生于GOM沉积前。Pescini等[5]发现,CADASIL严重损伤血管平滑肌细胞,周细胞周围的*Notch3*基因突变造成周细胞数目显著减少,周细胞足突覆盖毛细血管的范围缩小,使与毛细血管接触的星形胶质细胞末端脱离微血管,神经元-神经胶质细胞-血管单元整体受损,影响神经血管耦合过程,表明CADASIL主要累及神经血管单元(NVU)[6-7]。周细胞的退行性变破坏脑白质微循环,导致纤维蛋白沉积和脑灌注减少,造成脑白质功能障碍[8],与血小板源性生长因子受体-β(PDGFR-β)信号转导异常相关,但周细胞覆盖率的减少以及血-脑屏障破坏无法解释脑白质病变的相关机制[9]。此外,*Notch3*基因突变还上调微血管环境中纤维母细胞平滑肌蛋白的表达[10],亦参与GOM形成和管壁增厚的病理生理学过程。Zellner等[11]采用免疫荧光染色在CADASIL患者脑血管中观察到高温需求因子A1(HTRA1,丝氨酸蛋白酶家族成员)与Notch3ECD沉积物的共定位,前者对维持血管完整性具有重要作用,缺乏HTRA1介导的细胞外基质蛋白加工亦是血管病变的影响因素之一。

二、*Notch3*基因突变

CADASIL的致病基因为定位于19p13.2~13.1的*Notch3*,包含33个外显子,编码跨膜蛋白Notch3。目前报道的CADASIL相关*Notch3*基因突变多达200余种,多位于外显子3和4,尤其常见于外显子4。

1. CADASIL相关基因突变 目前发现的CADASIL相关基因突变类型主要是错义突变(95%),而框内缺失、移码缺失或剪接位点突变较少见。大多数突变可导致半胱氨酸残基丢失或增加,产生奇数个半胱氨酸,未配对的半胱氨酸破坏正常的二硫键形成,导致受体错误折叠,形成Notch3聚集体。除半胱氨酸数目改变外,其他氨基酸变异也可能导致受体错误折叠。Arnardottir等[12]报告不涉及半胱氨酸的基因突变类型,导致其他氨基酸替换,并不改变表皮生长因子样重复序列(EGFr)中半胱氨酸数目,但其致病作用尚不明确。Mizuno等[13]在两个R75P突变家系中发现,Notch3蛋白结构变化可能与脯氨酸替代有关,阻碍受体β-折叠,导致蛋白质构象变化,亦有观点认为上述突变可能是与CADASIL无关的多态性。研究显示,累及半胱氨酸的*Notch3*突变体在普通人群中较预期的更普遍[14],但其临床意义目前尚不清楚。

2. 基因突变类型与临床表型的相关性 Rutten等[15]发现,基因型与临床表型的相关性取决于表皮生长因子受体(EGFR)结构域的突变位点:与*EGFR7~34*变异患者相比较,*EGFR1~6*变异患者脑卒中发病更早,MRI病变更加严重,生存率更低。Baron-Menguy等[16]在*TgNotch3* R169C小鼠模型中

观察到，与配体结合域中的突变相比，R169C及类似突变所对应的临床表型更严重，与既往认为的存在热点突变患者较配体结构域突变具有更严重的认知功能下降相吻合。晚近一项回顾性研究显示，*Notch3*基因突变的致病性与突变位点有关[17]。Liu等[18]的横断面研究显示，国人在Notch3表皮生长因子样重复序列位点的基因突变在遗传学上易患年龄相关的脑小血管病，且较之表皮生长因子样重复序列保留的突变类型，对应更明显的脑白质高信号和更高的疾病负担。目前认为，遗传和环境因素均可影响疾病进程，同一家族中甚至同卵双生子之间也存在明确差异，表明除基因突变类型外，环境、生活习惯等因素对临床表型的影响更甚，二者之间存在复杂的相互作用[19]。*SQSTM1*基因编码一种多功能泛素结合蛋白SQSTM1/P62，参与细胞信号转导、氧化应激和自噬调控。近年研究发现，*Notch3*和*SQSTM1*致病性变异（p.Ser275Phefs*17）共存可能加重疾病进程[20]。但*SQSTM1*基因对CADASIL临床异质性的作用尚不明确，需扩展到与CADASIL表型谱相关的其他基因行基因组学分析以进一步确定。

三、CADASIL分子机制

Notch3蛋白是Notch受体家族成员之一，由包含34个表皮生长因子样重复序列的Notch3ECD和包含跨膜区域的Notch3ICD通过非共价连接而成。Notch蛋白与Delta/Jagged配体家族相结合，被解整合素-金属蛋白酶（ADAM）和γ-分泌酶复合物裂解，后者释放Notch3ICD，该结构域位于胞核，与CSL DNA结合蛋白和MAML蛋白（核转录激活蛋白家族成员）形成三聚体形式激活蛋白，将CSL蛋白从转录阻遏物转化为激活物，激活Notch信号转导通路下游靶基因。Notch3受体激活导致依赖RBP-J的*Notch*靶基因（*HES1*、*HELL*、*HRT1*、*HRT2*等）表达上调，可能促进管壁细胞生长并调节其凋亡。

1. Notch3信号转导异常　Peters等[21]发现，*Notch3*基因突变位于配体结合位点时，信号转导活性明显降低，抑制RBJ-Jκ等转录因子的激活；而配体结构域以外位点突变时，与配体结构域位点突变相比，Notch3ECD沉积无明显变化。部分CADASIL突变体不影响信号转导，Karlström等[22]在R142C突变小鼠模型中发现，细胞内完成S1裂解的Notch3蛋白减少，Notch3蛋白易在细胞内聚集可能是由于分泌增加或传导减慢，但Delta/Jagged配体的信号转导无明显异常。因此推测，CADASIL的发病机制并非仅是Notch3信号转导异常。但CADASIL相关基因突变如何影响Notch3蛋白活性，目前尚无定论[23]，不同突变位点对Notch3信号转导的影响不同。

2. 内质网应激和血管平滑肌细胞收缩　内质网应激是内质网中未折叠或错误折叠的蛋白质聚集诱发的细胞反应，与心血管病血管功能障碍相关。除细胞内蛋白质合成和加工外，内质网还调控钙离子释放，促进线粒体代谢和细胞凋亡[24]。Ihalainen等[25]在CADASIL患者中观察11种差异表达的蛋白质，均参与蛋白降解和折叠、血管平滑肌细胞收缩和应激。亦有研究显示，Notch3蛋白错误折叠可以引起内质网应激和未折叠蛋白反应（UPR），使活性氧（ROS）含量增加，抑制细胞增殖，并上调锰超氧化物歧化酶（MnSOD）保护性代偿，从而导致谷胱甘肽耗竭。由于泛素羧基末端水解酶L1（UCH-L1）无法识别突变的Notch3ECD，后者在血管平滑肌细胞表面进一步聚集，阻碍受体内化和再循环。*TgNotch3* R169C小鼠模型显示，CADASIL外周血管功能障碍与钙离子稳态失衡、氧化应激和内皮型一氧化氮合酶（eNOS）/可溶性鸟苷酸环化酶（sGC）/环磷酸鸟苷（cGMP）信号转导异常有关，其过程涉及Rho激酶（ROCK）和内质网应激[26]。Neves等[27]发现，CADASIL患者血管平滑肌细胞活性氧含量增加，与其他脑小血管病研究结果相似[28]，认为Notch3蛋白激活诱导Nox5衍生的活性氧生成，诱发RhoA/Rho激酶与内质网应激相互作用，导致小血管内皮功能障碍、内皮依赖性血管舒张受损，使脑血管反应性（CVR）降低，血管平滑肌细胞生长和细胞骨架重组等结构改变亦与之相关。血管平滑肌细胞和血管损伤动物模型业已证实，内质网应激可以促进Rho激酶激活[29]，小Rho家族GTP酶与多种细胞反应有关，如细胞生长和凋亡。因此，将Notch3-Nox5/内质网应激/Rho激酶信号转导通路定义为调节CADASIL患者的血管信号转导的假定上游系统。此外，收缩蛋白水平升高提示血管平滑肌细胞收缩的信号转导改变，Notch3ECD在细胞表面聚集可上调血管紧张素Ⅱ调节反馈通路，增强细胞对血管紧张素Ⅱ刺激的反应，后者通过激活转化生长因子-β（TGF-β）信号转导通路促进Ⅰ型胶原表达，这一过程可以解释CADASIL脑小血管纤维化。

3. 血管平滑肌细胞增殖分化与自噬异常　近年研究发现，CADASIL发生发展过程中血管平滑肌细胞增殖速度明显降低。Dziewulska等[30]通过尸检发

现，CADASIL患者血管平滑肌细胞有丝分裂呈不稳定状态，推测*Notch3*基因突变可能与其有丝分裂时相紊乱、细胞周期停滞、衰老及退化相关。Panahi等[31]的研究显示，CADASIL患者血管平滑肌细胞TGF-β水平升高与血管平滑肌细胞增殖减少有关，并影响周围其他血管成分增殖。此外，自噬异常也可以影响血管平滑肌细胞增殖、迁移和其他重要功能。Viitanen等[32]认为，CADASIL患者血管平滑肌细胞线粒体功能减退、数目增加，增多的线粒体碎片使溶酶体功能超负荷，导致血管平滑肌细胞增殖减慢和细胞活性损害。Gatti等[33]发现，CADASIL患者脑血管内膜含表达成熟平滑肌标志物的细胞，究其原因，可能是由于动脉中膜平滑肌细胞（SMC）在血管损伤后成为内膜细胞来源之一，平滑肌细胞中膜向内膜细胞实现再分布，病理性增厚的内膜主要由去分化的平滑肌细胞组成，平滑肌细胞表型从纺锤形向上皮样形态转变，蛋白合成增加，自中膜向内膜迁移[34]；另一可能的解释是，此类内膜细胞起源于多能血管干细胞，血管损伤使后者渗透至血管内皮，再进入内膜，在生长因子[血小板源性生长因子（PDGF）和细胞外基质等]作用下，诱导分化合成平滑肌细胞[35]。相应地，在分化的平滑肌细胞还可以观察到PDGFR-β水平升高和丝状肌动蛋白网络分布异常[36]。Hanemaaijer等[37]推测，错误折叠的Notch3蛋白可能导致自噬机制失调：$VSMC^{R133C}$可见Notch3蛋白表达和积累增加，而在自噬体-溶酶体融合中突变的*Notch3*基因与自噬标志物微管相关蛋白1轻链3（LC3）的共定位信号减弱，表明$VSMC^{R133C}$自噬过程受限。Takahashi等[38]也得出相似结论，与野生型相比，突变型Notch3蛋白更易形成聚集体，该聚集体可部分抗降解，进而影响血管平滑肌细胞增殖。此外，$VSMC^{R133C}$还可能通过改变细胞骨架结构抑制噬溶酶体功能，导致自噬体-溶酶体融合缺陷和延迟[39]。外泌体分泌和自噬体-溶酶体途径是协同参与异常蛋白质清除的细胞反应。Gao等[40]的研究显示，CADASIL患者血浆外泌体数目较正常对照者显著降低，且其降低幅度与脑白质病变（WML）严重程度相关。

4.血流动力学改变　Ling等[41]的研究显示，广泛的脑低灌注可能导致CADASIL中枢神经系统损害。Dong等[42]在CADASIL患者脑膜小动脉中观察到内膜增厚，但不影响管径，平滑肌细胞样细胞参与该过程，表明管壁增厚可能与管腔狭窄并无直接关系，因此，CADASIL脑低灌注可能归因于其他血流动力学改变，如脑血管反应性损害[43]、血-脑屏障破坏等。de Boer等[44]的研究显示，CADASIL患者内皮依赖性血管平滑肌细胞舒张功能受损，基线脑血流量（CBF）减少、血管反应性下降，影响乙酰唑胺的血管舒张反应，提示其血流动力学储备受损。Dabertrand等[45]在*TgNotch3* R169C小鼠模型脑血管中观察到血管平滑肌细胞膜电位去极化和肌源性张力降低，推测与电压门控性钾离子通道（VGKC）数目增加有关，但是在肠系膜动脉中未见这一改变，提示CADASIL主要表现为脑血管病的可能基础。外源性表皮生长因子受体激动剂肝素结合性表皮生长因子（HB-EGF）通过降低电压门控性钾离子通道数目以恢复肌源性张力，纠正脑血管功能障碍[46]。Capone等[47]认为，脑血管平滑肌细胞电压门控性钾离子通道数目增加是金属蛋白酶组织抑制因子3（TIMP3）在血管外基质中聚集所致，是导致脑血管功能障碍的关键因素，在*TgNotch3* R169C小鼠模型中加入外源性解整合素-金属蛋白酶17（exogenous ADAM17）可以恢复脑动脉张力和血管反应性。

综上所述，Notch3蛋白及其下游靶标的异常信号转导可能是CADASIL脑血管损伤和功能障碍的主要分子机制。突变型Notch3蛋白可以促进细胞内质网应激和氧化应激以及RhoA/Rho激酶活化，抑制血管平滑肌细胞增殖，影响血管平滑肌细胞收缩、迁移等重要功能，导致CADASIL脑血管功能障碍。此外，血管平滑肌细胞自噬机制紊乱、增殖分化异常等表现，进一步解释CADASIL的血管病理与皮肤活检结果。截至目前，*Notch3*基因突变致CADASIL的分子机制仍是研究重点，不同基因突变类型的分子机制是否各异尚待进一步研究。

利益冲突　无

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