·综述·

CD_{39}在心肌缺血再灌注中的心脏保护作用的研究进展

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【中图分类号】R542.2　【文献标志码】A　【文章编号】1674-4055(2016)01-0122-02

腺苷循环的活化是缺血再灌注内在重要的病理生理学机制。CD_{39}[1]为膜外二三磷酸核苷水解酶．1（E．NTPDase-1），将细胞外的ATP（三磷酸腺苷）、ADP（二磷酸腺苷）水解产生AMP（一磷酸腺苷），参与腺苷循环的代谢，在缺血再灌注中如肾脏、心脏等起重要的作用。本文通过对CD_{39}的心脏保护作用的研究进展和现状作一综述，特别是在心肌缺血再灌注的发生发展中，以期能全面展示CD_{39}在心肌缺血再灌注损伤中的的应用和前景。

1 CD_{39}的概述

CD_{39}分子[1,2]为膜外二三磷酸核苷水解酶-1（E.NTPDase-1），相对分子质量为70 000~100 000。由510个氨基酸和两个跨膜结构区构成。一个跨膜结构区为NH2末端和COOH末端。另一个为细胞外的分解区域，含有5个三磷酸腺苷双磷酸酶保守区是维持该分子的酶活性、结构完整性和表达于细胞膜上必需的结构。此蛋白主要的翻译后修饰途径主要包括糖基化，棕榈酰化和泛素化[3-5]。

CD_{39}通过阳离子依赖途径分解ATP、ADP产生AMP。研究发现细胞质内的CD_{39}处于失活状态，细胞表面的CD_{39}处于活化状态。其失活与活化的转变主要通过脂质代谢途径，如胞膜窖[6]。

CD_{39}广泛表达于外周血、肺、骨骼肌、肾脏、心脏、脑等组织器官，在外周血白细胞、内皮细胞、角化细胞和神经突触有较高表达[2,7-9]。

2 CD39与心肌缺血再灌注

2.1 心肌缺血再灌注与ATP　心肌缺血主要由于急性冠状动脉（冠脉）血栓引起，进而导致心肌细胞死亡，常见的疾病如急性心肌梗死等，发病率和死亡率在心血管疾病中占很高比例，给国家带来了巨大的健康和经济负担[10]。缺血再灌注会加重组织或细胞的炎症和缺氧。在炎症或缺氧条件下，细胞内[11]的ATP浓度减少，胞外的核苷酸[12]特别是ATP的释放增多。细胞外ATP[13]释放一方面是由于细胞损伤或者破坏，另一方面是由于泛连接蛋白半通道的凋亡细胞和联结蛋白半通道的炎症细胞。释放的ATP[14]通过自分泌或者旁分泌作用于特定的细胞表面P2受体，其包括G蛋白偶联的P2X7受体和ATP门控的P2X非选择性阳离子通道。P2[15]受体介导的信号分子主要是通过核细胞膜外苷酸酶清除ATP来终止缺血再灌注。CD_{39}是主要的细胞膜外苷酸酶，其有起到清除ATP的能力。

2.2 心肌缺血再灌注与血栓　心肌缺血再灌注是由于动脉粥样硬化斑块（血栓）的急性损害导致。血栓形成在其中起到重要作用。血栓形成是血管受到损失导致血小板的活化和聚集。血栓调控机制[2]包括类花生酸的释放，一氧化氮（NO）的产生，硫酸乙酰肝素的表达，腺苷循环中ATP，ADP，AMP等的分解代谢。由此可知在血栓形成过程中，核苷酸酸及其代谢产物腺苷也起到重要作用。

2.3 CD_{39}与腺苷　腺苷为一种内源性的自分泌抗炎因子，通常状态下，在细胞外浓度含量较低。在缺血缺氧条件[16]下转录水平增高，暗示腺苷信号能使组织适应缺氧。当细胞缺氧情况下，细胞外的腺苷水平急剧增高，主要是由于CD_{39}分解细胞外的核苷酸导致。研究发现[17]CD_{39}的转录和蛋白水平增加与腺苷密切相关，高于正常基线水平的9倍。因此缺血再灌注中腺苷循环的活化为其内在的病理生理机制，促使器官、组织耐受进一步的缺血障碍。CD_{39}在缺血再灌注中起到重要作用。

在大鼠的离体心脏的心肌缺血再灌注模型[18]，发现其冠脉血管床缺乏CD_{39}，可见CD_{39}在心肌缺血再灌注中起重要作用。核苷酸在心肌的缺血再灌注中起至关重要的作用。细胞外的核苷酸浓度的改变能够活化腺苷循环，导致血小板的聚集或解聚，改善心肌的缺血再灌注损伤。

2.4 CD_{39}与心肌保护作用　CD_{39}对于调节心肌缺血再灌注起到重要作用。在细胞水平上[19]，细胞内的稳态和纤维反应是由促纤维化的ATP和抗纤维化的腺苷组成的集成信号，CD_{39}在集成信号中起了重要的作用，可能在心肌纤维化形成过程中起一定作用。在小鼠心肌缺血再灌注模型[20]中，CD_{39}的缺乏造成ATP的聚集和腺苷的缺失，通过A2BR机制来使心肌细胞对损害的敏感性增加。相反，在小鼠心肌缺血再灌注模型中[21]，CD_{39}的过度表达组梗死面积较正常组显著减少，研究提示CD_{39}可能具有心脏保护作用。随后有研究将人的CD_{39}基因锲入猪、小鼠的模型中[22,23]，发现其梗死面积相应减少。在人体的研究水平上，不稳定型心绞痛和心肌梗塞患者[24]相对稳定型心绞痛其CD_{39}的免疫活性降低。对于外周的动脉粥样硬化的患者研究发现[25]其相对正常组有较低水平的CD_{39}，并与疾病进程有关。

3 细胞表面的CD_{39}分子

研究证实CD_{39}使胞膜外的ATP和ADP水解为单磷酸腺苷，腺苷可作为免疫抑制因子作用于A2A腺苷受体，抑制促炎性因子γ-干扰素（IFN-γ）、白介素-17（IL17）分泌，促进调节性T细胞表达叉头蛋白P3（forkhead box P3，FoxP3）、白介素-10（IL-10）和血清转化生长因子（TGF-B）分泌，抑制效应性T细胞增殖和功能。

CD_{39}是表达于白细胞和内皮细胞的细胞表面酶。在白细胞亚群中，CD_{39}主要表达于B淋巴细胞、中性粒细胞（PMN）和单核细胞，高达90%，而表达CD_{39}的T淋巴细胞和自然杀伤细胞（NK细胞）为6%。尽管表达CD_{39}的B淋巴细胞数量远高于T淋巴细胞，但T淋巴细胞中的CD_{39}活性可能比B淋巴细胞中的活性高。在正常和缺血再灌注情况下，

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doi：10.3969/j.issn.1674-4055.2016.01.37

CD_{39}表达于心脏循环的白细胞和内皮细胞[26]。在前降支血流堵塞的心肌缺血50 min内，免疫细胞数目增加，特别是粒细胞CD_{39}。研究证实在心肌缺血再灌注过程中，在Treg细胞上的CD_{39}在其中其了重要的作用[27]。在动物水平上，研究称在心肌缺血再灌注的小鼠中Treg细胞快速积累。在调节性T细胞缺乏的小鼠模型中选择性的Treg的缺乏导致心肌的缺血再灌注。对其机制的研究中发现活化的Treg细胞的保护不能被TGF-B1和IL-10抑制剂所阻止以及能被CD_{39}的缺乏所消弱，在人体水平上，通过对经皮冠脉介入术的急性冠脉综合症的患者的外周血分析发现其Treg细胞的减少，伴随着相关CD_{39}细胞的增加。因此推论在心肌的缺血再灌注中活化的treg细胞可通过CD_{39}来起到心肌保护作用。

4 CD^{39}的临床应用前景

血栓形成过程中动脉粥样硬化斑块的急性破裂是导致死亡和致残的主要原因之一，抗血小板聚集对于其发生发展起到重要的作用。现有的抗血小板聚集的药物包括不可逆的环化酶抑制剂（阿司匹林）、P2Y12受体失活（氯吡格雷）和其他（如他汀类药物等）。在体内使用羟甲基戊二酸单酰辅酶A还原酶抑制剂（他汀类药物）药物[28]能引起CD_{39}表达上调，导致凝血酶诱导下的血小板聚集降低。氯吡格雷等[29]可通过肝脏转化作用抑制血管核苷酸分解酶的活性作用。

重组可溶性CD_{39}[30]，是一种新型的抗血小板聚集抑制剂。具有抗血栓作用。研究发现在急性大脑中动脉闭塞的大鼠模型中[31]，重组可溶性CD_{39}具有神经源性保护作用。在急性冠脉球囊损伤的猪模型中[32]，结合肝素的重组可溶性CD_{39}，其血小板和纤维蛋白沉积比率降低趋势减弱。当加入含丰富人类血小板的血浆中，能100%的组织ADP引起血小板聚集。因此结合现代急性血栓事件的标注化治疗方案，重组CD_{39}能进一步抑制血小板聚集。重组CD_{39}能抑制内皮新生[33]，减少血小板聚集。在心血管治疗领域，可提供一种新的治疗策略和拓宽心血管治疗窗口的安全性。研究发现[34,35]8-BUS-ATP衍生物和多金属氧酸酶，为胞膜外的选择性的核苷酸酶抑制剂，对选择性阻滞剂的研究很有必要。

在临床上，磷酸二脂酶抑制剂如西洛他唑[5]具有抗栓作用，其获得美国食品药品监督局批准，作用机制为通过胞内的cAMP的浓度的减少增加CD_{39}的蛋白表达和ATP/ADP分解酶的活性，而不影响CD_{39}转录。其提示CAMP可调控CD_{39}的表达。

5 结论

CD_{39}广泛表达于心脏血管和组织。CD_{39}水解ATP、ADP，最终产生腺苷。腺苷在抑制血小板活化和聚集方面起重要作用。CD_{39}在心肌缺血再灌注的发生发展中起保护作用。

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