动脉粥样硬化中胆固醇逆向转运的研究进展

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摘要 胆固醇逆向转运(reverse cholesterol transport, RCT)是指胆固醇从外周组织细胞流出, 通过高密度脂蛋白转运至肝脏进行代谢转变的过程, 是维持细胞脂质稳态的重要机制。RCT相关基因的功能障碍是动脉粥样硬化脂质沉积、慢性炎症和泡沫细胞形成的主要病因, 已成为抗动脉粥样硬化的主要靶点。该文就近年来关于胆固醇逆向转运的调节和定量分析在动脉粥样硬化中的最新研究进展作一综述。

关键词 胆固醇逆向转运; 巨噬细胞; 淋巴系统; 动脉粥样硬化

Advances on Reverse Cholesterol Transport in Atherosclerosis

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Abstract RCT (reverse cholesterol transport) is a multi-step process, by which peripheral cell cholesterol can be returned to the liver for catabolism by HDL (high density lipoprotein) via the plasma compartment, and it is an essential mechanism to maintain the lipid homeostasis of peripheral tissue cells. The dysfunction of individual genes relavant to RCT may result in lipid deposition, chronic inflammation and foam cell formation in atherosclerosis, and modulation of RCT serves as a valuable therapeutic strategy for atherosclerotic diseases. This article reviews the latest advances in study on regulation and quantification of RCT in atherosclerosis.

Keywords reverse cholesterol transport; macrophages; lymphatic system; atherosclerosis

动脉粥样硬化(atherosclerosis, AS)的特征是动脉壁的脂质沉积和慢性炎症[1]。胆固醇逆向转运(reverse cholesterol transport, RCT)是指胆固醇从外周组织细胞流出, 由高密度脂蛋白(high density lipoprotein, HDL)携带并通过血液循环到达肝脏进行代谢转变的过程。RCT障碍可引起脂质及脂蛋白(包括低密度脂蛋白)沉积在动脉内皮下, 在活性氧自由基的作用下氧化形成氧化型低密度脂蛋白(oxidized low density lipoprotein, oxLDL), oxLDL刺激内皮细胞使其分泌大量炎性介质, 驱动炎性细胞内皮下浸润导致慢性炎症, 最终形成动脉粥样硬化斑块。本文综述了RCT过程的调节靶点和定量分析方法、以及如何通过改善RCT来减缓AS进程。

1 巨噬细胞胆固醇流出的调节

外周组织细胞RCT包括三个连续的步骤。(1)外周组织细胞游离胆固醇(free cholesterol, FC)流出到HDL和(或)载脂蛋白AI(apolipoprotein AI, ApoAI)上; (2)HDL内FC受血浆卵磷脂: 胆固醇脂肪酰基转移酶(lecithin: cholesterol acyl transferase, LCAT)酯化

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形成胆固醇酯(cholesterol ester, CE)并转移至HDL核心; (3)HDL内CE由肝细胞高密度脂蛋白受体、B族I型清道夫受体(scavenger receptor class B type I, SR-BI)摄取并在肝中被代谢[2-3](图1)。

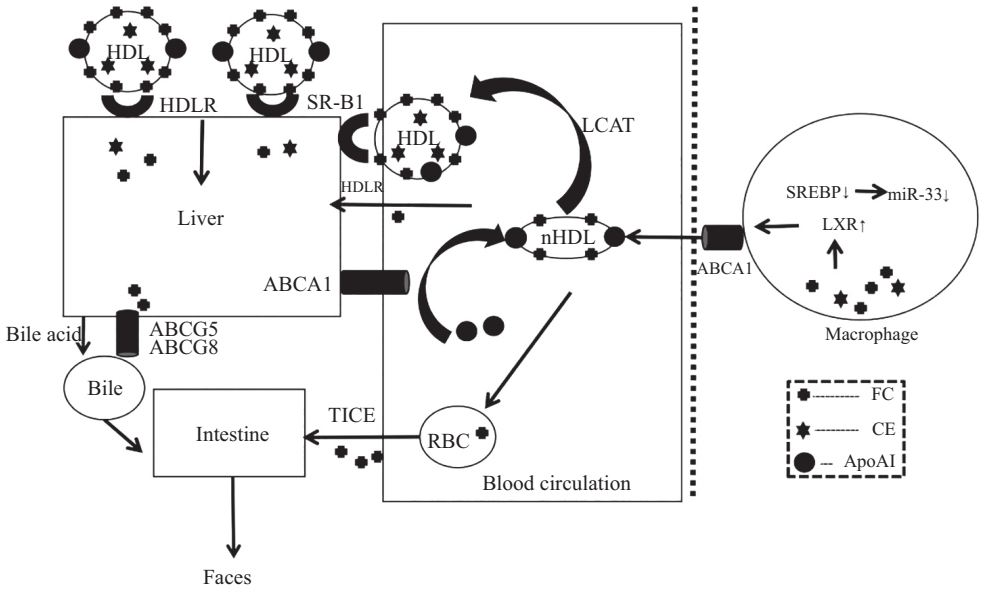
动脉粥样硬化斑块中的泡沫细胞可来源于巨噬细胞和血管平滑肌细胞, 该过程受多种因素的影响。正常巨噬细胞内胆固醇来源于血浆脂蛋白的内化、凋亡细胞的胞吞、以及胞内胆固醇的合成。巨噬细胞可通过SR-BI和低密度脂蛋白受体(low density lipoprotein receptor, LDLR)等摄取脂蛋白(HDL和LDL)中胆固醇[4], 该过程由内体转运系统介导。溶酶体可使结合的LDL与LDLR解离, 水解LDL内CE为FC, 之后FC通过C型尼曼–匹克病蛋白(Niemann-Pick protein C, NPC)运往其他细胞器[5]。LIAO等[6]对此过程的研究表明, 在非经典的核因子-κB(nuclear factor-κB, NF-κB)途径中, NF-κB可直接结合于NPC-2启动子区而促进NPC-2转录。SANDHU等[7]的研究显示, 胆固醇从质膜向内质网(endoplasmic reticulum, ER)转运的过程, 是通过Aster蛋白介导的非囊泡途径进行的。Aster蛋白是调节胆固醇在胞内分布过程的新靶点[7-8]。

巨噬细胞可将胞内多余的胆固醇排出到胞外受体或转化为CE存储在脂滴(lipid droplets, LDs)中, 从而避免胆固醇析出对细胞产生毒性作用。过量的胆固醇可诱导巨噬细胞内肝X受体(liver X receptor, LXR)的表达[9]。LXR可以驱动胆固醇流出途径中诸多基因的表达, 包括ATP结合盒转运蛋白A1/G1(ATP-binding cassette proteins A1 and G1, *ABCA1/G1*)等; 促进低密度脂蛋白受体降解物(inducible degrader of the LDLR, IDOL), 一种E3泛素连接酶的表达, 限制细胞通过LDLR对外源性胆固醇的进一步摄取[10]。LAKE等[11]的最新研究证明, 运输蛋白驱动蛋白结合蛋白2(trafficking protein, kinesin-binding 2, TRAK2)是LXR介导ABCA1表达、胆固醇流出和HDL发生过程的新型调节因子。敲除*TRAK2*基因可增加ABCA1的转录, 进而增加胆固醇向ApoAI和HDL的流出。ZHANG等[12]通过对低水平LDL-胆固醇(LDL cholesterol, LDL-C)的急性心肌梗死(acute myocardial infarction, AMI)患者的研究, 发现启动子低甲基化而上调的羟基类固醇硫酸基转移酶2B1b (hydroxysteroid sulfotransferases 2B1b, SULT2B1b)可通过抑制LXR-β来促进胞内胆固醇累积和炎症反应。因此, 抑制*SULT2B1b*表达可能是一种有效预防冠状动脉疾病(coronary artery disease, CAD)的治疗方法。

对ER中固醇调节元件结合蛋白(sterol regulatory element-binding protein, SREBP)的抑制也可抵御巨噬细胞内胆固醇的累积。抑制SREBP可降低与胆固醇合成(羟甲基戊二酸单酰辅酶A还原酶)和摄取(*LDLR*)相关基因的表达活性, 也可减少SREBP内含子区域编码的miR-33的表达。miR-33可抑制RCT途径中多种关键基因(*ABCA1*、*NPC-1*、*ABC11*和*ATP8B1*)的mRNA翻译[13-15]。FERNANDEZ-HERNANDO小组[16]的最新报告证明, miR-33调节巨噬细胞胆固醇流出和AS的主要机制是抑制ABCA1。

胆固醇必须以游离形式从巨噬细胞内流出。巨噬细胞将LDs内CE水解为FC是胆固醇流出的限速步骤[17-19]。在哺乳动物细胞中, 巨自噬是一种依赖许多自噬蛋白并可大量地或选择性地螯合胞质溶胶的自噬过程。巨噬细胞接受促AS脂蛋白后, 激活巨自噬。自噬蛋白包绕LDs并将其转运至溶酶体, 使LDs在溶酶体中降解释放出FC, 增强巨噬细胞样泡沫细胞的RCT。人类*LIPA*基因编码的溶酶体酸性脂肪酶(lysosomal acid lipase, LAL)可水解与LDs相关的CE[20]。全基因组关联性研究已确定, *LIPA*突变导致的基因功能部分缺失是引起沃尔曼病、胆固醇酯贮积病和CAD的原因[21]。一些研究表明, 抑制miR-33或过表达自噬及溶酶体相关基因的主要转录调节因子——转录因子EB(transcription factor EB, TFEB), 可以恢复斑块中巨噬细胞的自噬, 改善炎症, 最终减缓AS的进展[22-23]。

FC向HDL流出可通过FC的被动扩散、主动转运和ABCA1/G1、SR-BI介导的脂质转运途径进行[24-27]。FC可通过囊泡和非囊泡途径转运至质膜上的ABCA1/G1, 但其确切的机制尚未探明[28-29]。ZHOU等[30]发现, 敲除外周髓鞘蛋白22(peripheral myelin protein 22, *PMP22*)基因会损害ABCA1介导的胆固醇外流能力, 由此提示PMP22在维持细胞胆固醇稳态中的关键作用。胆固醇转运还可由氧化固醇结合蛋白(oxysterol-binding protein, OSBP)-氧化固醇结合相关蛋白(oxysterol-binding related protein, ORP)介导。它们构成了脂质结合/转运蛋白家族, 可以促进胆固醇在脂质双层之间的非囊泡转移, 从而提高亚细胞器膜之间胆固醇转运的效率。OUIMET等[31]研究发



巨噬细胞摄入过量胆固醇上调LXR表达, 抑制SREBP进而下调miR-33表达; FC经肝细胞上ABCA1转运至胞外ApoAI形成新生HDL; 巨噬细胞内胆固醇以FC形式经ABCA1转运至HDL; 新生HDL在血液内LCAT作用下形成成熟HDL; HDL内胆固醇可经HDLR、SR-BI、LDLR及被动扩散转运至肝细胞内代谢; 胆固醇以胆汁酸形式或FC经ABCG5和ABCG8排入胆汁进入肠道, HDL内FC也可经红细胞通过TICE进入肠道, 最终从粪便排出。

Macrophage intakes excessive cholesterol, which up-regulates the expression of LXR and inhibits SREBP, down-regulating the expression of miR-33; free cholesterol in liver cells efflux to ApoAI via ABCA1 (ATP-binding cassette transporter A1) yields nHDL (nascent high-density lipoprotein); macrophage free cholesterol is transported to HDL via ABCA1; nHDL esterification by LCAT (lecithin:cholesterol acyl transferase) gives spherical HDL; liver cells intake HDL cholesterol for catabolism via HDLR, SR-BI, LDLR and passive diffusion; cholesterol transformed to bile acid and free cholesterol via ABCG5 and ABCG8 are excreted to bile, HDL free cholesterol enters the intestine through TICE via red blood cells, and finally be excreted from the faces.

图1 调节RCT的靶点(根据参考文献[47-48]修改)

Fig.1 Targets in regulation of RCT (modified from references [47-48])

现, ORP6可以调节胆固醇外流和HDL内稳态, 因此, 对ORP6和ORP家族其他成员调节该通路机制的研究可能会发现新型RCT途径调节因子。

2 淋巴系统对巨噬细胞RCT的调节

除巨噬细胞等所参与的固有免疫外, 适应性免疫也存在于动脉粥样硬化斑块中。斑块中的适应性免疫细胞主要是T细胞, 在早期的动物研究中, $CD4^+$ T细胞在AS斑块中的作用已得到证实。 最新研究发现, CD137对$CD8^+$ T细胞的共刺激是$CD8^+$ T细胞内皮下浸润的动力, 且这种刺激与AS抗原识别无关[32]。这一发现表明, 血液循环中的效应$CD8^+$ T细胞可能由全身炎症所诱导产生, 并经血液循环浸润至AS斑块内, 持续分泌促炎细胞因子, 募集其他免疫细胞, 并可能与这些细胞共同导致AS斑块的形成和随后发生的血栓事件。

T细胞受体(T cell receptor, TCR)是所有T细胞表面的标志性受体, 是针对AS慢性炎症的潜在靶点。TCR与CD3结合, 形成TCR-CD3复合物。淋巴细胞特异性蛋白质酪氨酸激酶(lymphocyte-specific protein tyrosine kinase, LCK)在TCR下游通路中处于起始核心的位置, 可促进T细胞的增殖与免疫反应, 对维持TCR通路和T细胞活化有着重要作用[33]。LIU等[34]的研究发现, LCK抑制剂会减缓AS的发展并增加斑块稳定性, 还可通过抑制PI3K/AKT/mTOR信号转导来降低Th1细胞与调节性T细胞之比, 可能有抗AS作用。

动脉三级淋巴器官(artery tertiary lymphoid organ, ATLO)是与AS相关的复杂的淋巴细胞聚集物, 具有免疫抑制和免疫促进的双重作用。ATLO通过募集幼稚淋巴细胞、损害脂质流出能力和促进树突状细胞/淋巴细胞群形成来促进免疫反应[35]。但其在调节AS过程中对免疫反应产生何种作用以及如何调节RCT仍需进一步研究。

淋巴系统的作用并不与广义的RCT相违背。位于动脉粥样硬化斑块外膜的淋巴管(lymphatic ves-

sels, LVs), 引流局部的炎细胞和炎性介质, 可抑制局部炎症反应, 延缓AS的发展[36]。在负荷胆固醇的巨噬细胞向血浆输送胆固醇的过程中, 淋巴系统对于巨噬细胞内胆固醇的清除也起了重要作用, 其清除率约占50%[37]。此外, 小鼠的淋巴功能不全会损害正常的脂蛋白代谢(如升高LDL-C和TG水平)和血管稳态, 从而加速AS进展[38]。LVs是由单层淋 巴内皮细胞(lymphatic endothelial cell, LEC)构成的。对LVs的研究可通过LEC上的标记物进行, 如淋巴管内皮透明质酸受体1(lymphatic vessel endothelial hyaluronan receptor 1, LYVE1)和血管内皮生长因子受体3(vascular endothelial growth factor receptor 3, VEGFR3)等。VEGFR3在LEC中高度表达[39]。在胚胎期, VEGFR3作为VEGF-C和VEGF-D的受体, 刺激LVs形成[40]。 抑制VEGFR3介导的信号转导将导致AS病变中T细胞的富集, 但并不影响动脉外膜内淋巴管的数量, 提示淋巴管生长对VEGFR3的非依赖性[41]。但同时MILASAN等[42]的一项研究也表明, VEGF-C152S(激活VEGFR3的重组VEGF-C类似物)对*LDLR*$^{-/-}$小鼠AS斑块的形成有抑制作用。

然而在对ApoE缺陷小鼠AS病变的研究中, 却又得到相反的结论。在斑块进展阶段, 免疫细胞从主动脉壁的流出是减少的[43-44]。且同一研究小组的最新研究报告指出, 在斑块的进展和消退中, 巨噬细胞并不会移出, 从而支持了巨噬细胞可能不会发生穿过动脉中间层进入淋巴管而移出的假设[45]。由此对上述相关标志物的研究可能帮助我们认识LVs及其在AS进程中所发挥的重要作用, 并验证刺激淋巴管生成是否是有意义的抗AS策略。

3 胆固醇的酯化及代谢

FC转移至HDL后, 血浆LCAT将FC酯化为CE并转移至HDL核心, 从而产生成熟的HDL。HDL内CE可通过肝细胞膜SR-BI被选择性摄取。在人体内, HDL核心中的CE也可通过胆固醇酯转运蛋白(cholesterol ester transfer protein, CETP)而转移到富含甘油三酯(triglyceride, TG)的脂蛋白上, 通过LDLR进入肝细胞代谢。 因此, 来源于人外周细胞中的FC经循环转运到肝脏进行代谢的过程涉及2条途径, 直接途径(HDL-SR-BI)和间接途径(HDL-LDL/VLDL-肝LDLR)。最新研究表明, 小鼠大部分新生HDL中的FC是被肝脏所清除而非被血浆LCAT所酯化, 由此SR-BI介导的选择性CE摄取, 即直接途径可能处于一个次要地位[46-47]。同时BASHORE等[48]的研究显示, ABCA1缺失的肝细胞可存在一种新型选择性FC转运途径, 该途径依赖肝细胞表达的LDLR, 并由其介导加速肝对血浆HDL的选择性摄取, 促进胆固醇经肝代谢, 控制肝细胞内FC经HDL再进入血浆, 由此提出肝细胞ABCA1可能在降低血胆固醇浓度方面有重要作用(图1)。

在肝脏转化为胆汁酸是胆固醇代谢的主要途径。SR-BI选择性摄取的CE在肝细胞中被水解, 水解产生的FC或被转化为胆汁酸, 或以原型被ABCG5和ABCG8转运到胆汁中, 随粪便排出。此外, 胆固醇还可通过经肠胆固醇外流途径(transintestinal cholesterol efflux, TICE)直接从血液经肠上皮细胞转运至肠腔[49]。以上两种途径对人体胆固醇清除率的占比分别为65%和35%。法尼醇X受体(farnesoid X receptor, FXR)和LXR是胆固醇排泄过程的重要调节因子, 可以控制众多胆固醇转运蛋白和胆汁合成酶的转录和活性。尽管胆固醇可以原型分泌到胆汁中被排出体外, 但是胆汁酸的合成和排泄仍是哺乳动物胆固醇分解代谢的主要途径[50]。因此, FXR和LXR都为增强TICE和胆汁胆固醇分泌以及促进RCT提供了潜在的治疗靶点[51-52]。

4 RCT的定量指标

高密度脂蛋白胆固醇(HDL-cholesterol, HDL-C)浓度、高密度脂蛋白颗粒(HDL particle, HDL-P)和高密度脂蛋白胆固醇流出量(HDL cholesterol efflux capacity, HDL-CEC)是人体内与HDL相关的RCT标志物, 并可通过这些标志物对RCT进行定量。

HDL-C以前被认为是反映人体RCT能力的可信标志物。但在MADSEN等[53]的研究中, 与较低和较高的HDL-C相比, 中等范围HDL-C反而与人群最低的全因死亡率相关, 且HDL-C与全因死亡率以及心血管死亡率之间的关联是U型的。可见, HDL-C并不是一个合适的可参考的治疗目标。对于HDL-CEC, 达拉斯心脏研究和欧洲癌症诺福克前瞻性研究均显示了其与心血管事件之间独立的负相关性[54-55]。此外, 在多民族AS研究中对CAD和卒中队列HDL-CEC的定量分析表明, HDL介导的胆固醇流出对非卒中的CAD患者有保护作用[56]。HDL-P作为一种新兴的高密度脂蛋白标记物, 可通过核磁共振光谱、离子迁

移率或二维梯度凝胶电泳进行定量[57]。KHERA等[58]通过一项评估瑞舒伐他汀(JUPITER)的干预试验指出, 相较于HDL-CEC、HDL-C和ApoAI浓度, HDL-P是CVD最强的反向预测因子。

此外, 对于RCT的定量, CUCHEL等[59]对传统的方法进行了修改, 使其可以对人体RCT进行定量。在该方法中, 向静脉内输入含有^{3}H-胆固醇的纳米颗粒, 采集血液等样品, 对血浆、非HDL和HDL以及粪便内的示踪剂进行计数, 从而定量人体RCT。将该方法与HDL-CEC定量和先进的成像技术相结合, 例如血管内超声检查、光学相干断层扫描和近红外光谱法, 促进原位斑块成像, 可以更好地评估人体RCT能力并为治疗CAD的新药临床试验提供技术支持[60]。

5 问题与展望

随着生活条件的改善、人口老龄化程度的加剧, 动脉粥样硬化已经成为威胁人类健康的首要病因。关于RCT的研究, 对经典靶点如ABCA1、SR-BI、LXR和LCAT等的作用机制深入的理解, 为干预RCT提供了理论支持。其中调节巨噬细胞胆固醇流出的相关靶点的研究是重中之重, 相关机制的研究也日渐成熟。虽然多种针对上述靶点的药物如CETP抑制剂、LXR激动剂等正处临床试验阶段, 但一些药物的副作用(如LXR致脂肪性肝炎)也使其遭遇新的挑战。在维护血管健康的道路上, 围绕RCT的研究尚有很多亟待解决的问题, 等待我们继续探索并对其进行补充。

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