

全反式维甲酸对足细胞损伤保护作用的研究进展

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[摘要] 全反式维甲酸(ATRA)是维生素A类衍生物,在调节细胞聚集、分化、凋亡、增殖及炎症反应中起重要作用。近年来ATRA在肾脏疾病的作用,特别是对足细胞保护作用的研究取得了一定进展。该文就足细胞损伤、ATRA作用特点,以及ATRA诱导足细胞分化及再生、抗增殖与纤维沉积、抑制凋亡等保护作用研究进展作综述。
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[关键词] 全反式维甲酸; 损伤; 足细胞

Research advances in the protective effect of all-trans retinoic acid against podocyte injury

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Abstract: All-trans retinoic acid (ATRA) is a vitamin A derivative and plays an important role in the regulation of cell aggregation, differentiation, apoptosis, proliferation, and inflammatory response. In recent years, some progress has been made in the role of ATRA in renal diseases, especially its protective effect on podocytes. This article reviews the research advances in podocyte injury, characteristics of ATRA, podocyte differentiation and regeneration induced by ATRA, and the protective effect of ATRA against proliferation, deposition of fibers, and apoptosis.

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Key words: All-trans retinoic acid; Injury; Podocyte

足细胞、肾小球基底膜及内皮细胞是构成肾小球滤过屏障(glomerular filtration barrier, GFB)的三层滤过膜, GFB功能紊乱导致肾小球滤过作用失调,进而演变成慢性肾脏疾病(chronic kidney disease, CKD)。足细胞损伤被视为肾小球疾病发生发展的早期事件及中心环节。全反式维甲酸(all-trans-retinoic acid, ATRA)是维生素A类衍生物,在调节细胞聚集、分化、凋亡、增殖及炎症反应中起重要作用,近年来ATRA在肾脏疾病中的研究取得一定的进展。本文就足细胞损伤、ATRA作用特点,以及ATRA诱导足细胞分化及再生、抗增殖与纤维沉积、抑制凋亡等作用的研究进展进行综述。

1 足细胞功能特性

肾小球足细胞是有丝分裂后高度分化细胞,具有上皮细胞和间充质细胞的特性,再生能力极弱。足细胞是以肌动蛋白为基础的微管从胞体到足突延伸的趾状突结构,由初级和次级足突相连形成高度网状结构包绕在肾小球基底膜(glomerular basement membrane, GBM)外侧,这一结构能够抵抗来自肾小球的压力,维持毛细血管袢的空间构象。足细胞足突是由肌动蛋白、肌球蛋白、 α 辅肌动蛋白、黏着蛋白等组成的具有收缩能力的结构,受到刺激后足突收缩进而调节肾小球超滤系数、肾小球滤过功能^[1]。相邻足细胞通过各自初级、

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次级足突桥接形成的裂孔隔膜是一种跨膜结构,由多种蛋白组成,如nephrin、podocin、CD2AP、TRPC6、ZO-1、P-cadherin等,这些蛋白高度有序地相互交联形成包涵多种受体和离子通道并具有电荷屏障作用的蛋白质网^[1],对血浆白蛋白或大分子起主要滤过作用。足细胞还具有分泌功能,分泌GBM的组分和降解酶以维持GBM的平衡^[2]。此外,足细胞还具有抗原递呈、吞噬、分泌补体等免疫作用^[3]。因此,GFB的稳定性有赖于足细胞结构及功能的完整。

2 足细胞应对损伤的改变

足细胞损伤是肾小球疾病进展的初始环节。引起足细胞损伤的因素很多,如基因突变、氧化损伤、免疫损伤和血液动力学的机械压力等^[4-5]。应对损伤足细胞可发生足突融合、细胞分离、细胞增殖、细胞肥大等改变。

足细胞损伤后最为经典的改变是足突融合。Kriz等^[6]提出,足细胞损伤、足突改变经历2个阶段。第一阶段,足细胞回缩使得裂孔隔膜结构消失或者位移。这样的改变可能是可逆的,例如甾体类激素能够减轻部分肾病综合征微小病变患者的足细胞损伤;3D重建肾小球技术也显示足细胞在一定程度高灌注压下可通过动态改变胞体及足突来适应压力^[7-8]。第二阶段,足细胞进一步回缩,足细胞骨架蛋白重排,足突融合,裂孔隔膜蛋白丢失,导致CBF功能受损、大量蛋白尿,这些改变是不可逆的。

足突融合导致足细胞分离,并失去与GBM的粘连作用^[9]。足细胞数目减少还与凋亡、自噬、有丝分裂障碍等机制有关^[10]。足细胞的调节通路异常则导致其在鲍曼氏囊增殖,与皮层细胞粘连形成半月体,加之纤维修复,形成局灶节段性硬化^[11]。持续损伤刺激引起足细胞胞体萎缩、肥大,进而从GBM脱落,最后由壁层上皮细胞粘附到裸露的GBM^[12]。因此,早期的足细胞损伤如得不到及时干预则导致肾小球疾病进展。

3 ATRA 生物学功能及作用特点

维甲酸(retinoic acid, RA)是维生素A类的

一种活性代谢衍生物,对调节细胞聚集、分化、凋亡、增殖及炎症反应起重要作用^[13]。维甲酸包括ATRA、9-顺式-RA、13-顺式-RA等多种形式。9-顺式-RA是ATRA的异构体,发挥内源性活性作用的主要是ATRA。ATRA通过调节细胞生长、分化、增殖和凋亡相关的基因发挥生物活性作用,也是首个成功应用于临床的分化诱导剂^[14]。

维甲酸在细胞核内的两组受体维甲酸受体(retinoic acid receptors, RARs)和维甲酸X受体(retinoid X receptors, RXRs)均属于细胞核受体家族,是配体依赖的转录调节子^[15]。这两种核受体普遍存在于各种细胞中,均具有 α 、 β 、 γ 3种不同类型,各类型具有不同的剪接变体,其中RAR α 有7个剪接变体,RAR β 有4个剪接变体,RAR γ 有7个剪接变体;RXR共有6个剪接变体^[16-17]。ATRA主要通过与其高亲和力特异性地结合而发挥生物学效应,9-顺式-RA主要与RXR结合发挥作用^[15,17]。

当缺乏ATRA时,RAR/RXR形成异质二聚体,与ATRA调控的靶基因启动区域维甲酸反应元件(retinoic acid response element, RARE)结合,同时募集阻遏蛋白和组蛋白脱乙酰化酶(histone deacetylases, HDAC)至相应DNA区域,HDAC从组蛋白转移乙酰基,改变染色质结构从而对基因转录起到抑制作用^[18]。当ATRA存在时,ATRA与RAR高亲和力结合,RAR发生构型改变,释放阻遏蛋白,同时募集共激活蛋白如组蛋白乙酰转移酶,激活ATRA调控的靶基因转录及表达,从而发挥对细胞的生物活性调控作用^[19]。

4 ATRA 对足细胞损伤的作用

4.1 诱导足细胞分化

研究发现,在常见肾小球疾病模型中,如嘌呤霉素肾病模型,HIV相关肾病(HIV-associated nephropathy, HIVAN)足细胞损伤模型,阿霉素诱导肾小球硬化(glomerulosclerosis, GS)大鼠模型,阿霉素致足细胞损伤模型等,ATRA干预能够减少蛋白尿和减轻足细胞损伤,并维持足细胞分化^[20-23]。提示ATRA对肾脏保护作用的机制可能与诱导肾祖细胞(renal progenitor cells, RPCs)向足细胞分化有关。RPCs主要定位在肾脏的鲍曼氏囊,具有移

行成足细胞的潜能^[24]。ATRA可使CD133和CD24表达阳性的RPCs分化为足细胞,并促进壁层上皮细胞向足细胞转化^[25-26]。ATRA增加膜性肾病模型大鼠肾小球PAX2和WT1的共定位(PAX2为壁层上皮细胞的标志物,WT1是足细胞的特异标志物),诱导RPCs向足细胞分化,以及诱导壁层上皮细胞向足细胞转化,可一定程度补充足细胞^[26-27]。Peired等^[28]发现,白蛋白可与内源性ATRA结合而影响RARE介导的转录进而阻止RPCs向足细胞分化,加速局灶节段性肾小球硬化;而足量的ATRA治疗可一定程度恢复RARE活性,促进足细胞分化、增加足细胞数目,从而减少蛋白尿和减缓局灶节段性肾小球硬化的进展。此外,联合应用ATRA、激活素A、BMP7能诱导人间充质干细胞向足细胞分化^[29-30]。

RAR对于ATRA的生物活性发挥起重要作用。RAR α 特异激动剂Am580、BD能够减轻蛋白尿和肾小球疾病进展^[31-32]。Khurana等^[33]发现ATRA必须在 α -激动蛋白4存在的前提下,通过增加RAR α 活性并与之结合,才能减少足细胞损伤。Lu等^[34]通过HIVAN小鼠研究发现,ATRA促进足细胞分化,并通过激活cAMP/PKA信号通路,诱发CREB和USF1与MKP1基因启动子结合以减少MAPK的磷酸化,提出RAR α /cAMP/PKA/CREB信号通路在ATRA维持足细胞分化、保护肾脏中起关键作用。本课题组研究^[22]发现,应用ATRA干预阿霉素致足细胞损伤模型,RAR α 和RAR γ 的表达与足细胞分化标志物nephin, podocin表达呈正相关,而RAR β 的表达与其无明显相关性,推测RAR α 和/或RAR γ 可能参与足细胞损伤制。

锌指蛋白转录因子(Krüppel-like factor 15, KLF15)家族是锌指蛋白超家族中的一员,富含于肾脏等组织,通过C端的锌指结构与DNA结合,调控相关基因表达^[35]。ATRA能够诱导KLF15表达。Mallipattu等^[36]证实,ATRA对足细胞的促进分化及对肾脏保护作用与增加KLF15表达有关。研究还发现,ATRA增加KLF15与nephin及podocin启动子的结合可能是ATRA促进分化的机制之一^[37-38]。因此认为KLF15在肾脏发育及足细胞分化中起着重要作用。

4.2 抗增殖与抗纤维沉积

机械性压力作用于足细胞时,血管紧张素II

(angiotensinII, ANG II)的1类受体(AT1R)上调,在不影响收缩压的情况下可引起足突结构消失及蛋白尿^[39]。肾素-血管紧张素(RAS)抑制剂,如ACEI及ARB类能够降低肾小球球囊压及促进肾小球重构,对肾脏起保护作用;还可以通过直接影响AT1R的作用而保护足细胞。研究发现,ATRA与贝那普利对于GS大鼠具有相似疗效,均可降低肌酐、减少蛋白尿、延缓GS进展^[40]。此外,Dechow等^[41]通过系膜增生性肾小球肾炎大鼠模型发现,ATRA能够减少ANG II合成及AT1R表达,减少肾小球细胞增殖。提示ATRA可能通过影响RAS发挥对肾脏及足细胞的保护作用。

ATRA在膜增生性肾病大鼠模型中可减少蛋白尿和肾小球细胞增生,减轻毛细血管闭塞及纤维沉积,血小板源生长因子 β 和TGF- β 1及其受体被显著抑制^[42]。提示ATRA在抗纤维化中起重要作用。此外,ATRA也能减轻GS大鼠模型的肾小球纤维沉积^[21]。进一步体外研究发现,在阿霉素诱导足细胞损伤模型中,RAR α 和RAR γ 表达与基质金属蛋白酶2(matrix metalloproteinase 2, MMP2)和基质金属蛋白酶9(matrix metalloproteinase 9, MMP9)表达呈正相关^[43]。提示ATRA保护足细胞损伤的机制中可能存在RAR/MMP信号通路参与,其确切机制有待进一步探讨。

4.3 抑制炎症与减少足细胞凋亡

ATRA对糖尿病足细胞损伤的保护作用可能与抗炎机制有关。Han等^[44]报道高糖条件能刺激体外培养的足细胞单核细胞趋化蛋白-1(monocyte chemotactic protein 1, MCP-1)转录和蛋白表达,而ATRA干预可抑制MCP1和ED-1(活化的单核巨噬细胞标志物)表达,阻止糖尿病大鼠肾脏受累进展。研究还发现,ATRA能减少抗基底膜肾病模型的蛋白尿、纤维沉积,减少PCNA、ED-1阳性细胞数目以及炎症相关因子如TNF- α 、IL-1 β 、C/EBP δ 的表达^[45]。

足细胞凋亡是足细胞数目减少进而引起蛋白尿和局灶节段性肾小球硬化的重要原因。ATRA对大部分细胞起到促凋亡作用,但在嘌呤霉素致足细胞损伤模型中除了起到抗炎作用,还具有抗足细胞凋亡的作用^[46];在H₂O₂诱导系膜细胞的凋亡中也起到抗凋亡作用^[47]。

5 总结与展望

ATRA在肾病模型中对损伤足细胞具有一定的保护作用,为肾小球疾病的治疗提供了新的思路。但机制尚不完全清楚,加之其毒性作用如肝毒性、神经毒性、皮肤粘膜副作用等可能限制临床应用^[13,48-49],有待进一步临床研究。

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