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结缔组织生长因子在糖尿病视网膜病变纤维化中的作用

丛春霞 综述 韩梅 审校

(天津市眼科医院眼底病科，天津 300020)

[摘 要] 糖尿病视网膜病变(diabetic retinopathy，DR)是糖尿病中最常见的微血管严重并发症之一，是具有特异性改变的眼底病变。当DR发展到增殖性糖尿病视网膜病变(proliferative diabetic retinopathy，PDR)阶段，由纤维血管膜的收缩牵拉引起的玻璃体积血及视网膜脱离是导致PDR患者视力下降甚至失明的主要因素。纤维血管膜的形成与视网膜的纤维化作用密切相关，而结缔组织生长因子(connective tissue growth factor，CTGF)是与DR纤维化密切相关的一种细胞因子，可刺激成纤维细胞和血管内皮细胞的生长、黏附，促进细胞外基质的沉积，造成毛细血管基底层增厚，是视网膜纤维化程度的预测指标。CTGF还可通过外源性调节和血管生长因子(vascular endothelial growth factor，VEGF)水平之间的平衡，有效延缓视网膜纤维化进程，但其具体机制尚不明确。

[关键词] 增殖性糖尿病视网膜病变；纤维化；结缔组织生长因子；血管内皮生长因子

Role of connective tissue growth factor in diabetic retinopathy fibrosis

CONG Chunxia, HAN Mei

(Department of Fundus Diseases, Tianjin Eye Hospital, Tianjin 300020, China)

Abstract Diabetic retinopathy (DR) is one of the most serious complications of diabetic microangiopathy, which has many special fundus lesions. When it is the phase of proliferative diabetic retinopathy (PDR), vitreous hemorrhage and the retinal detachment caused by the contraction of fibrovascular membrane become the main factors of visual decreasing and even blindness. Fibrovascular membrane formation was closely related with retinal fibrosis, and connective tissue growth factor (CTGF) is a cytokine closely related to the DR fibrosis. It can stimulate the growth and adhesion of fibroblast and vascular endothelial cell, the accumulation of extracellular matrix (ECM), the proliferation, adhesion and migration of cell, then effectively relieve retinal fibrosis process, but its mechanism has not been yet completely clear.

Keywords proliferative diabetic retinopathy; fibrosis; connective tissue growth factor; vascular endothelial growth factor

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通信作者 (Corresponding author)：韩梅，Email: hanmay69@sina.com

随着社会生活水平的提高，糖尿病的发病率逐年上升，糖尿病视网膜病变(diabetic retinopathy，DR)也逐步发展为当今世界四大主要致盲眼病之一[1-4]。DR发展到增殖性糖尿病视网膜病变(proliferative diabetic retinopathy，PDR)阶段，主要表现为新生血管的出现，导致出血、渗漏以及多种炎性及免疫因子的释放，使纤维组织在新生血管附近增生，最终形成纤维血管膜。而纤维血管膜的收缩牵拉引起的玻璃体积血及视网膜脱离是PDR导致患者视力下降甚至失明的主要原因[4-5]。结缔组织生长因子(connective tissue growth factor，CTGF)是富含半胱氨酸的分泌性基质蛋白，作为关键的促纤维化细胞因子，在创伤修复或纤维化疾病中发挥重要作用，并在肺、肾、心肌纤维化、动脉粥样硬化及肿瘤等病变中表达水平升高[6-9]。研究[9-12]显示：在PDR患者玻璃体中，CTGF的蛋白和mRNA表达均升高，且CTGF的浓度与视网膜纤维化的程度呈正相关，证实CTGF可能参与并促进纤维血管膜的生成。CTGF参与PDR纤维化的机制包括刺激成纤维细胞和血管内皮细胞的生长、黏附及促进细胞外基质的沉积[10,13-15]。提示CTGF在DR纤维化过程中发挥至关重要的作用。

1 CTGF 的结构与功能

CTGF又称为CCN2，是一种分泌性多肽，属于即刻早期基因CCN家族。CNN家族目前包括CCN1(CyT61)，CCN2(CTGF)，CCN3(Nov)，CCN4，CCN5和CCN6。CTGF富含半胱氨酸，含349个氨基酸，分子质量36~38 ku，其基因位于染色体6q23.1，包含5个外显子和4个内含子；其外显子编码1个信号肽和4个结构域，分别是胰岛素样生长因子结合区、Von Wille因子C型重复区、血小板反应蛋白I型重复区和生长因子半胱氨酸群。CTGF通过上述4种模块，与细胞外基质成分、细胞膜表面受体等结合，发挥其生物学作用[16]。CTGF功能广泛，参与调节许多生长因子的活动和细胞外基质的形成、重塑及创伤的修复和纤维化，与许多组织细胞的生理性或病理性纤维化相关[17]。

2 CTGF 的生物学作用

CTGF的生物学作用广泛，其表达主要和人体的病理状态相关。在正常组织中，CTGF的表达水平较低，但在结构修复时表达上调，如伤口愈合、纤维化障碍、血管生成或机体患有某种癌症时[18]。CTGF是第1个被发现的可以影响成纤维细胞的内皮生长因子[19]。CTGF参与多种信号通路，被多种生长因子和环境因素激活，并结合众多蛋白或因子，起到广泛生物学调节作用，如基底膜增厚、细胞迁移、凋亡、血管生成、细胞转分化、肌成纤维细胞活化和纤维化等[20-23]。

2.1 TGF-β/Smad/CTGF 信号通路

Zavadil等[24]研究发现：转化生长因子-β1(TGF-β1)是上皮间充质转变的关键因素，可通过Smad2/3信号通路的磷酸化诱导上皮间充质转变(epithelial-mesenchymal transition，EMT)和细胞外基质(extracellular matrix，ECM)合成。CTGF是TGF-β介导的早期即刻基因，其作为TGF-β下游因子发挥促纤维化作用，在PDR过程中扮演重要角色[10]。

Kucich等[15]对lamin A/C基因突变导致的心脏病进行研究，结果证实：TGF-β/Smad信号通路参与活化细胞外调节蛋白激酶(extracellular regulated protein kinases，ERK1/2)，导致CTGF/CCN2活化作用的改变，从而调节纤维化。CTGF作为TGF-β通路的下游调控因子，在刺激细胞外基质成分生成方面发挥作用[25]。

Jun等[17]研究发现：敲除成纤维细胞中CCN2基因，并不影响皮肤组织的修复、胶原纤维的增加，且CCN2基因的表达在细胞增殖和成熟期迅速下降；而直接去除皮肤损伤中的CCN2蛋白却能导致细胞的衰老和相关的分泌表型(senescence-associated secretory phenotype，SASP)，包括MMPs的上调、胶原纤维和TGF-β的下调，最终导致胶原纤维的减少。

2.2 TGF-β1/PI3K/CTGF 信号通路

Shi等[26]研究表明：TGF-β1/PI3K/CTGF信号通路在肺纤维化过程中至关重要；CTGF作为

TGF-β1/PI3K通路的下游影响因素，可促进细胞外基质的产生；PI3K抑制剂通过反转EMT和下调CTGF与胶原纤维的表达，阻止肺纤维化。

2.3 HIF-1/CTGF 信号通路

CTGF可由缺氧诱导。研究[27]表明：在小鼠的肾小管上皮细胞中，缺氧诱导因子(hypoxia-inducible factor 1，HIF-1)可通过TGF信号通路，诱导CTGF表达，从而调节血管新生、组织纤维化等病理过程。

CTGF还可通过调节其它生长因子活性来调节信号传导，例如联合CTGF和VEGF可抑制后者介导的血管生成；用基质金属蛋白酶切割CTGF，可恢复VEGF促血管生成的活性[28]。CTGF不仅是一种基础的生长因子，还是其它生长因子的活性调节因子[26]。

3 CTGF 参与调控视网膜膜纤维化

3.1 CTGF 与 MMPs，Müller 细胞

Müller细胞是参与糖尿病视网膜前膜形成的细胞之一。Bringmann等[29]认为：前膜形成过程与反应性胶质细胞增生、纤维化和细胞迁移有关。Barcelona等[30]研究发现：在增殖性糖尿病患者中，金属基质蛋白酶的表达，包括MMP2和MMP9，可促进Müller细胞的转移。促红细胞生成素(erythropoietin，Epo)可抑制肾小管上皮细胞向间叶细胞转化，从而减缓肾纤维化。Hu等[31]证明：EP0可延缓Müller细胞反应性胶质细胞增生过程。Luo等[32]的研究显示：Epo可减少Müller细胞中CTGF的生成，从而减轻纤维化的严重程度。

3.2 CTGF 与基底层增厚

毛细血管基底层增厚、周细胞丢失和无细胞毛细血管，是DR的早期视网膜血管变化。视网膜毛细血管基底层的主要成分是IV型胶原蛋白、层黏连蛋白和纤连蛋白。CCN家族尤其是CTGF是基底层成分表达的强有力的诱导物，其表达在糖尿病视网膜早期病变中上调[10]。Kuiper等[33]通过建立链霉素诱导的糖尿病小鼠模型，发现CTGF等位基因缺失通过减少CTGF的蛋白表达可阻止视网膜毛细血管基底膜的增厚。Van Geest等[34]研究发现：在CTGF等位基因缺失的小鼠中，在长期(6~8个月)糖尿病条件下，周细胞及无细胞毛细血管生成减少、视网膜毛细血管基底膜增厚可得到良好控制。上述研究提示CTGF在视网膜血管结构性改变方面发挥极大作用，将CTGF作为靶目标能够安全、有效地延缓DR的结构改变。

3.3 CTGF 与 VEGF 的相互作用

在PDR发展过程中，VEGF是新生血管生长到纤维增殖形成过程中的关键因素，同时还是PDR血管通透性增强和血管生成的重要媒介。VEGF可诱导视网膜内皮细胞和周细胞上的CTGF、纤维黏连蛋白和TIMP-1的mRNA和蛋白水平增长，表明VEGF可通过诱导CTGF的表达造成基底膜增厚[35]。有研究[36]表明：PDR患者玻璃体腔的CTGF的N终端结构水平明显上升与纤维化程度密切相关。提示CTGF是PDR纤维化和瘢痕化的关键因素。CTGF在血管生成方面的作用隐蔽，主要依靠调节特定条件下血管生成因子如VEGF的活性发挥作用，进而抑制VEGF介导的新生血管生长。因此CTGF和VEGF之间的平衡最终决定PDR纤维血管化的进程，其比率是纤维化的最强预测数值[36]。

国内一项研究[37]发现：与直接行手术治疗的对照组相比，术前10 d注射抗VEGF因子的研究组的增殖膜上VEGF水平明显降低，但CTGF水平升高，与新生血管向纤维化转换的临床现象一致。Kuiper等[38]认为：在PDR患者中，CTGF与VEGF间存在着动态平衡，当CTG水平达到阈值时，CTGF活性升高，VEGF活性降低时，CTGF/VEGF比值突破某临界值，平衡被打破，PDR病变由新生血管向纤维化增殖方向发展，提示CTGF/VEGF比值是血管纤维化转换的强有力的预测指标。这与Van Geest等[39]的研究结果一致：在玻璃体腔注射抗VEGF治疗的患者中，CTGF/VEGF比值同样是纤维化的预测指标；CTGF高表达且VEGF低表达是术后纤维化并发症出现的危险因素。Zhang等[40]研究发现：在抗VEGF治疗之后，人脐静脉内皮细胞中CTGF表达上调，提示抗VEGF具有潜在性诱导CTGF表达的上调的作用。

4 CTGF 在 DR 发展中的作用

CTGF在DR的发展阶段起重要作用。在DR病

变早期，CTGF可导致视网膜毛细血管基底膜厚度增加和周细胞减少；同时CTGF由晚期糖基化终末产物或某种生长因子如VEGF和TGF-β诱导。CTGF作为一种TGF激活的下游调节因子，抑制其表达水平或将是一种合理的预防早期DR的治疗方法[10]。

在PDR中，CTGF在纤维血管膜上中可见，主要定位于肌纤维母细胞，其与平滑肌肌动蛋白(smooth muscle actin，SMA)的数量及表达CTGF的肌纤维母细胞数量密切相关[41]。肌纤维母细胞由产生ECM的纤维母细胞激活，与纤维化程度相关[42]。研究[41]表明在纤维血管膜的内皮细胞上可检出CTGF。Hinton等[36]发现：与非PDR患者相比，PDR患者玻璃体内的CTGF的N末端片段水平升高；在合并玻璃体出血的DR患者和非DR患者的玻璃体内，CTGF的N末端片段水平也明显升高。提示CTGF在PDR中起重要作用。Kuiper等[13]的研究显示：CTGF水平和纤维化程度明显相关，但与血管生成无关。CTGF的水平可良好预测病变的纤维化程度。

5 结语

CTGF是参与DR纤维化过程的一种重要的细胞因子。在病变早期，CTGF主要造成视网膜毛细血管基底膜增厚和周细胞减少，若在此阶段针对CTGF治疗，可起到早期预防作用；在病变晚期，CTGF可引导新生血管生长到纤维化的过程，在这一过程中，CTGF与VEGF水平之间的平衡起关键作用，若同时进行抗VEGF和抗CTGF的治疗，可在抗血管生成的同时也阻断纤维化过程，阻止纤维增殖病变的进展，不仅可为DR的治疗提供新思路，而且可为进一步明确CTGF参与调控PDR的分子机制提供依据。

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