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**VEGFc 通过 Notch 信号通路和 MMP13 促进大鼠角膜新生血管形成**

**VEGFc promotes corneal angiogenesis in rat through Notch signaling pathway and MMP13**

马 婧

指导教师姓名: 刘祖国 教授

陈永雄 教授

专 业 名 称:

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# **VEGF<sub>c</sub> promotes corneal angiogenesis in rat through Notch signaling pathway and MMP13**

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By  
Jing Ma  
( Physiology )

Dissertation Supervisors:  
Prof. Zuguo Liu and Prof. Chen Yong Xiong

Xiamen, China

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## 摘要

正常角膜透明无血管，是眼的最重要屈光介质之一。角膜新生血管的形成是多种局部和全身性疾病所导致的角膜病理性变化，它不仅会使角膜失去透明性，影响视力，甚至致盲，而且会导致角膜移植时发生免疫排斥反应。角膜新生血管形成的确切机制至今尚不清楚。因此，角膜新生血管是眼科医生面临的一个十分棘手的难题，如何治疗新生血管也是当前眼科研究的一个热点。本论文旨在通过对血管内皮生长因子c (VEGFc) 激活角膜基质细胞和上皮细胞 Notch 信号通路及基质金属蛋白酶-13 (MMP13) 抑制 I 型胶原产生的研究，探讨角膜新生血管形成的新机制，为角膜新生血管的临床治疗和新药物的开发提供理论依据。

以往角膜新生血管形成机制的研究，都聚焦在血管内皮细胞在促血管因子及相关信号通路的作用下，通过降解细胞外基质、增殖和迁移的“出芽”生长过程，而角膜基质细胞及上皮细胞在促血管因子及相关信号通路的作用下，细胞外基质的产生被抑制，从而促进新生血管形成的机制被完全忽视。研究证明，VEGFc 无论是完整的多肽前体，还是经蛋白水解的成熟体，都能促进新生血管的形成。然而，VEGFc 的受体有 VEGF 受体 2 (VEGFr2) 和 VEGF 受体 3 (VEGFr3)，VEGFc 的主要作用被认为是通过 VEGFr3，促进新生淋巴管的形成；VEGFc 通过 VEGFr3 促进新生血管形成的机制目前仍不清楚。由于 Notch 能通过启动转录因子 Hes1，调节 VEGFr3 的表达，加强 VEGFc 的功能；MMP13 能通过降解细胞外基质中的胶原纤维，促进新生血管的形成。因此，VEGFc 促进新生血管形成的机制，有可能是通过 Notch 信号通路及 MMP13 的途径。然而，目前有关 VEGFc 通过 Notch 信号通路及 MMP13 促进新生血管形成的机制尚未见报道，角膜基质细胞和上皮细胞在 VEGFc 的作用下，通过 Notch 信号通路及 MMP13 抑制细胞外基质的产生的机制也未被研究。因此，本课题以碱烧伤诱导角膜新生血管形成的大鼠模型和体外培养的大鼠角膜基质细胞和上皮细胞，通过 RT-PCR、蛋白免疫印记、原位杂交和免疫组织化学染色等检测方法，研究 VEGFc 通过 Notch4、Hes1 转录因子和 MMP13 的表达，促进角膜新生血管形成的机制。

碱烧伤诱导角膜新生血管形成大鼠模型的结果显示, VEGFc、VEGFr3、Notch4、MMP13 和 Hes1 蛋白和 mRNA 的表达都随新生血管的增多而增加, Col1A2 蛋白和 mRNA 的表达随新生血管的增多而减少; VEGFc 和 MMP13 mRNA 除表达在新生血管的内皮细胞外, 也显著表达在角膜基质细胞和上皮细胞; MMP13 选择性抑制剂 444283 能恢复 Col1A2 蛋白的表达, 减少角膜新生血管的形成; 而 VEGFa 蛋白的表达并不随新生血管的增多而增加, VEGFr2 蛋白的表达则随新生血管的增多而减少。表明 VEGFc、VEGFr3、Notch4、MMP13、Hes1 和 Col1A2 参与了角膜新生血管的形成, 而 VEGFa 和 VEGFr2 与角膜新生血管的形成并无关联。体外培养的角膜基质细胞和上皮细胞的实验结果显示, VEGFc 能促进 VEGFr3 Notch4、Hes1、MMP13 的表达, 抑制 Col1A2 的表达; VEGFc 抑制 Col1A2 的表达, 是通过上调 Notch4/Hes1 和/或 MMP13/Hes1 表达的途径, 能分别被  $\gamma$ -secretase inhibitor DAPT 和 MMP13 选择性抑制剂 444283 所阻断。体外培养的角膜基质细胞和上皮细胞的实验结果表明, 在角膜新生血管形成过程中, VEGFc 能分别通过上调 Notch4/Hes1 和/或 MMP13/Hes1 表达的途径, 抑制 Col1A2 的产生, 从而促进角膜新生血管的形成。

本课题研究的结果表明, 在新生血管形成过程中, 促血管生成因子及相关信号通路除可通过血管内皮细胞降解细胞外基质、增殖和迁移的“出芽”机制外, 还可通过抑制角膜基质细胞及上皮细胞细胞外基质的产生, 促进新生血管的形成。因此, 恢复角膜基质细胞及上皮细胞细胞外基质的产生, 是治疗角膜新生血管的新途径。MMP13 选择性抑制剂 444283 能用于角膜新生血管的治疗。

**关键词:** 血管内皮生长因子 c; 血管内皮生长因子受体 3; Notch4; Hes1; 基质金属蛋白酶 13; Col1A2

**ABSTRACT**

Normal cornea without blood vessels are transparency, it is one of the most important refractive media of eye. The formation of corneal neovascularization is corneal pathological changes which is caused by a variety of local and systemic disease, it will not only loses the corneal transparency, affects vision and causes blindness, but also lead to corneal transplantation immune rejection. The exact formational mechanism of corneal neovascularization is still unclear. Corneal vascularization is an eye doctor has been facing a very difficult problem, how to treat angiogenesis is currently a hotspot in the research of ophthalmology. This paper aims to study the role of vascular endothelial growth factor C (VEGF<sub>C</sub>) is to activate Notch signal pathway and matrix metalloproteinase -13 (MMP13) for inhibiting the production of type I collagen in corneal stromal cells and epithelial cells, to discuss the new mechanisms of corneal neovascularization, to provide theoretical basis for the development of clinical treatment and new drug for corneal neovascularization. Previous studies of corneal neovascularization mechanism are focused on the vascular endothelial cells affected by angiogenic factors and related signal pathway forming the "budding" growth process for neovascularization through extracellular matrix degradation, proliferation, migration, but the mechanism-the extracellular matrix produced by the stromal cells and epithelial cells is suppressed by angiogenic factors and related signaling pathway, thereby promotes the formation of neovascularization -was completely ignored. Research shows, VEGF<sub>C</sub> whether complete polypeptide precursor, or the mature protein by hydrolysis, can promote the formation of new blood vessels. However, VEGF<sub>C</sub> receptors are VEGF receptor 2 (VEGFR<sub>2</sub>) and VEGF receptor 3 (VEGFR<sub>3</sub>), the main function of VEGF<sub>C</sub> promote the formation of new lymphatic vessel by VEGFR<sub>3</sub>; the mechanisms of VEGF<sub>C</sub> through VEGFR<sub>3</sub> promoting angiogenesis is still unclear. Because Notch can activate the transcription factor Hes1, regulate the expression of VEGFR<sub>3</sub>, strengthen the function of VEGF<sub>C</sub>; MMP13 promote the formation of new blood vessels by

degrading the extracellular matrix collagen fibers, Therefore, the mechanism of VEGFc promoting neovascularization is likely through Notch signaling pathway and MMP13. However, at present the mechanism of VEGFc through the Notch signaling pathway and MMP13 promoting angiogenesis has not been reported, the mechanism of corneal stromal and epithelial cells in the presence of VEGFc inhibiting extracellular matrix production by Notch signal pathway and MMP13 has not been studied . Therefore, this paper aims to study the mechanism of VEGFc promoting corneal angiogenesis through Notch4 , the transcription factor Hes1 and MMP13 by the corneal neovascularization induced by alkali burn in the rat and the cultivation of rat cornea stromal cells and epithelial in vitro, by RT-PCR, Western blot, in situ hybridization and immunohistochemistry staining detection method.

Alkali burn induced corneal neovascularization in rats model results show, the protein and mRNA expression of VEGFc, VEGFr3, Notch4, MMP13 and Hes1 increase along with the increase of neovascularization , the protein and mRNA expression of Col1A2 decrease along with increasing neovascularization; VEGFc and MMP13 mRNA expression are in endothelial cells of angiogenesis and also expressed in corneal stromal and epithelial cells; MMP13 selective inhibitors 444283 can restore Col1A2 protein expression , reduce the formation of corneal neovascularization; while the expression of VEGFa protein doesnot increase along with increased angiogenesis , and the expression of VEGFr2 protein decrease along with increasing neovascularization. It is showed that VEGFc, VEGFr3, Notch4, MMP13, Hes1 and Col1A2 is involved in the formation of corneal neovascularization and the formation of VEGFa and VEGFr2 are no correlation with the corneal neovascularization. In vitro, cultured corneal stromal cells and epithelial cells experimental results show, VEGFc can promote the expression of VEGFr3 Notch4, Hes1, MMP13 and inhibit the expression of Col1A2; the inhibition of expression of Col1A2 by VEGFc, is through the up-regulation of Notch4/Hes1 and / or MMP13/Hes1 expression, respectively by  $\gamma$ -secretase inhibitor DAPT and selective inhibitor of MMP13 444283 blocking. Results in vitro showed that, during the corneal neovascularization process, VEGFc can respectively through the

up-regulation of Notch4/Hes1 and / or MMP13/Hes1 expression, inhibit the production of Col1A2, thereby promoting the formation of corneal neovascularization.

The research results show that, in the angiogenesis process, angiogenic factors and related signaling pathway, not only through the "budding" mechanism of vascular endothelial cells degrading extracellular matrix , proliferation and migration , but also through the inhibition of extracellular matrix expression produced by corneal stromal cells and epithelial cells, promote the formation of corneal neovascularization. Therefore, the restoration of extracellular matrix produced by corneal stromal cells and epithelial cells, is a new way for the treatment of corneal neovascularization. Selective inhibitors of MMP13 444283 can be used for the treatment of corneal neovascularization.

**Key words:** Vascular endothelial growth factor c (VEGFc) , VEGFr3, Notch4 , Hes1, matrix metalloproteinases 13 (MMP13), Col1A2

## 第一章 前言

### 1.1 研究背景

角膜本身无血管,毛细血管网仅围绕角膜缘。如果维持角膜无血管的平衡因素被破坏,毛细血管超越角膜缘侵入透明区 1—2mm,即可视为角膜新生血管形成 (corneal neovascularization CNV) [1]。角膜新生血管不是一种独立的角膜疾病,而是一种病理改变。尽管角膜新生血管形成有利于病原微生物的清除和组织修复,但临床上难以治愈,严重影响角膜的透明性,最终可导致角膜组织的结构破坏和视力损害,是致盲的重要原因之一[2]。

尽管 Campbell 等人[1]在 1949 年就开始了新生血管形成机制的研究,但迄今为止新生血管形成及调控的确切机制仍不清楚。研究表明,角膜中存在促血管形成因子(如成纤维细胞生长因子、血管内皮生长因子、低氧诱导因子)与抗血管形成因子(血管生成抑制因子、内皮抑制素、色素上皮衍生因子)之间的平衡,当炎症、感染、外伤、变性等发生时,这种平衡被破坏,倾向于促血管形成因子的作用,从而促进新生血管的形成[3]。在促血管形成因子中,血管内皮生长因子因具有促进内皮细胞增生和迁移的特异性而成为最重要的促新生血管形成的细胞因子[4-6],被认为通过 Notch、NF- $\kappa$ B、MAPK、PI3k 等信号通路调节新生血管的形成[7-9]。

#### 1.1.1 血管内皮生长因子家族研究进展

VEGF 家族为多肽类生长因子,包括 VEGFa, VEGFb, VEGFc, VEGFd 和胎盘生长因子 (PLGF)。VEGF 是主要的促血管生成因子,其生物学作用包括促进血管生成,增加血管通透性,维持血管完整性及调节造血作用,亦称为血管通透因子或血管调理素[10, 11]。VEGF 广泛分布于人和动物体内的脑、肾、肝、脾、肺、眼等许多组织[12],正常眼视网膜色素上皮细胞、血管内皮细胞、周细胞、角膜上皮细胞、角膜基质细胞、炎症细胞均可产生较低水平的 VEGF[13-17]。VEGF 通过其特异性受体 VEGFr 发挥生物学功能。VEGFr 家族有 3 种不同的酪氨酸激酶受体,分别是 VEGFr1, VEGFr2, VEGFr3 [18-19]。这 3



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