

热休克蛋白 27 (HSP27) 信号来促进尖细胞形成。因此, BMP6 通过调控 ALK3-p38MAPK-HSP27 信号和 ALK2-Smad1/5 信号来维持干细胞、尖细胞的平衡, 这种平衡有利于 CNV 的发生发展。然而, BMP2 却只能通过激活 ALK3-p38MAPK-HSP27 信号来促进尖细胞形成^[10]。

2.1.2 其他信号通路 实验证明^[11], TGF-β 激活的 ALK1-Smad 信号可促进血管出芽; 而其激活的 ALK5-Smad 信号维持新生血管稳定。当 TGF-β1-ALK5-Smad2 信号通路激活时, 其通过改变血管生成过程中一些关键受体的表达量从而影响干细胞、尖细胞形成, 进而影响 CNV 的发生发展。相反, TGF-β-ALK1-Smad 信号通路可通过间接抑制 TGF-β-

ALK5-Smad 信号通路来促进血管出芽^[11], 从而促进 CNV 的发生发展。此外, 尖细胞高表达神经纤毛蛋白-1 (Nrp-1), 而 Nrp-1 可通过抑制 TGF-β 和 BMP9/BMP10 激活的 ALK1/ALK5-Smad2/3 信号通路来抑制干细胞形成, 进而促进血管出芽^[12], 从而有利于 CNV 的发生发展。

2.2 Smad 蛋白调控促血管生成和抗血管生成因子的表达 角膜中促血管生成和抗血管生成机制的不平衡影响了 CNV 的发生发展^[13]。Smad 蛋白可能通过血管内皮生长因子 (VEGF) 途径、Ras 同源基因-Rho 相关螺旋卷曲蛋白激酶 (Rho-Rock) 途径、Wnt 途径和 Notch 途径, 参与促血管生成和抗血管生成因子表达的调控, 从而影响着 CNV 的发生发展 (图 1)。

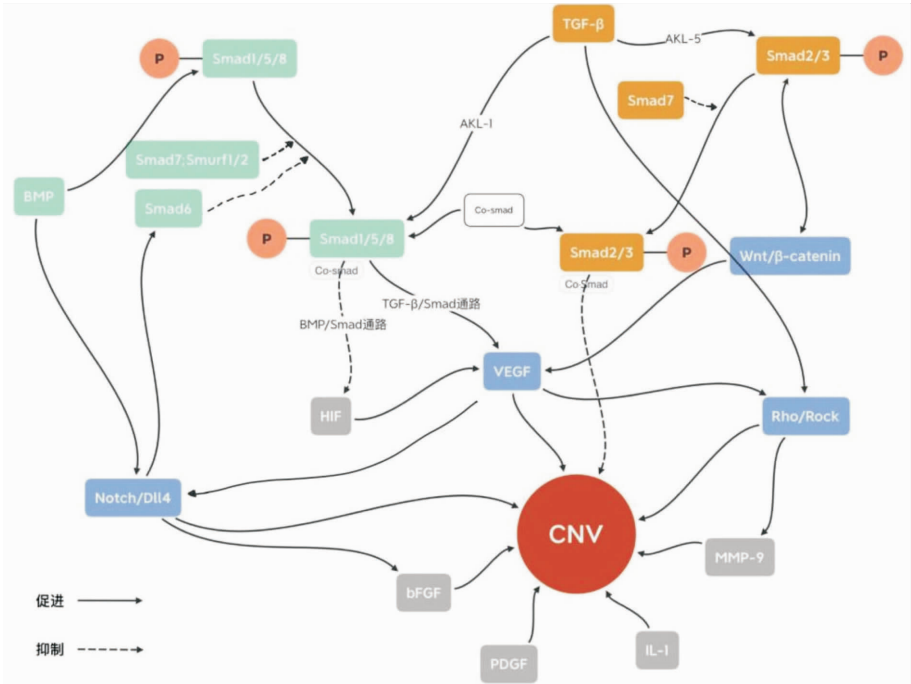


图 1 Smad 蛋白相关信号转导途径在 CNV 发生发展过程中的作用

2.2.1 Smad 蛋白与 VEGF 途径 VEGF 家族包括 VEGF-A、VEGF-B、VEGF-C、VEGF-D、VEGF-E 和胎盘生长因子 (PLGF)。其中, VEGF-A 是最有效的促血管生成因子。在角膜中, 明确发现了 VEGF-A 表达的上调可促进 CNV 的形成^[14], 其机制为 VEGF 结合其受体 VEGFR2 后, 可激活多种细胞内信号, 如 MAPK、磷脂酰肌醇 3 激酶-蛋白激酶 B 和一氧化氮信号, 进而促进血管内皮细胞增殖、迁移^[15-16]。目前有研究证明, 抗 VEGF 途径的药物可以有效抑制 CNV 的形成。此外, VEGF 基因的启动子区域受 TGF-β/Smad 通路的影响, 这说明 TGF-β/Smad 是 VEGF 的上游通路^[17]。有研究证明, TGF-β1-ALK1-Smad1/5-PLGF/VEGFR1-VEGF-A/VEGFR2 信号通路能够促进血管生成, 但是, TGF-β-ALK5-Smad2/3 信号通路却抑制血管生成^[18]。由此可见, TGF-β/

Smad 通路对血管生成有双重作用, 可能和参与通路的 ALK 及 Smads 的种类有关。此外, 在肿瘤微血管环境中, BMP 介导的 Smad1/5/8 信号通路可使缺氧诱导因子降解, 从而对缺氧诱导的 VEGF 表达产生负调控作用, 然而此结论是否适用于角膜血管还不清楚^[19]。对于 I-Smads 来说, 有研究发现, Smad7 能下调 VEGF 的表达, 从而发挥抑制 CNV 发生发展的作用^[20]。

2.2.2 Smad 蛋白与 Wnt 途径 配体蛋白质 Wnt 和膜蛋白受体结合可激活 Wnt 信号转导途径, 经典的 Wnt 信号转导途径主要由 β-catenin 介导^[21]。研究证明, Wnt/β-catenin 信号转导途径能直接促进 VEGF 和其他促血管生长因子的表达, 进而促进新血管的形成^[22]。此外, R-Smads 蛋白可以与 Wnt/β-catenin 途径协同激活靶基因转录, 如 VEGF 基因, 其

中,Smad3 可以促进 β -catenin 进行核易位,从而促进 Wnt/ β -catenin 信号的转导^[23];同样,Wnt 信号可通过抑制糖原合成酶激酶-3 β 的活性,从而抑制 Smad1 和 Smad3 多泛素化和降解,进而促进 Smad 蛋白发挥作用^[23]。另外,当 β -catenin 信号转导受抑制后,TGF- β 诱导的 Smad3 激活减少,进一步提示我们 Wnt/ β -catenin 途径可促进 TGF- β /Smad 信号转导^[24]。由于 TGF- β /Smad 信号同 Wnt/ β -catenin 途径可相互刺激,因此,Smad7 不仅可通过抑制 TGF- β /Smad 信号转导间接抑制 Wnt/ β -catenin 途径,还能与 β -catenin 结合以促进 β -catenin 降解,从而直接抑制 β -catenin 信号转导,这为 CNV 的治疗提供了广阔的思路,如过氧化物酶体增殖物激活受体 γ 激动剂可能通过刺激 Smad7,以抑制 TGF- β /Smad 信号通路和 Wnt/ β -catenin 途径,从而抑制 VEGF 等血管因子的生成,进而抑制 CNV 的发生发展^[25]。

2.2.3 Smad 蛋白与 Rho/Rock 途径 Rho/Rock 信号转导途径与血管内皮细胞的迁移密切相关^[26]。研究发现,Rho/Rock 途径能上调促血管生成因子,如基质金属蛋白酶-9 和骨桥蛋白的表达,进而促进血管生成^[27]。Smad 蛋白可通过两种途径与 Rho/Rock 途径相互作用:其一,VEGF 是 Rho/Rock 途径的上游信号^[28],因此,Smad 蛋白可能通过 VEGF 间接地影响 Rho/Rock 途径;其二,RhoA 蛋白是 TGF- β 1 的下游因子^[29],TGF- β 1/Smad 信号通路可以促进 Rho/Rock 信号途径中的 RhoA、RhoC 和 Rock1 蛋白的表达,而 TGF- β /Smad 抑制剂则抑制它们的表达,同样,Rho/Rock 信号抑制剂下调 Smad2 蛋白的表达^[30]。因此,TGF- β /Smad 信号通路还可以直接与 Rho/Rock 途径相互作用来调控 CNV 的发生发展。

2.2.4 Smad 蛋白与 Notch 途径 在人体中,Notch 信号转导途径主要包括五种 Notch 配体(Delta-like1、3、4 即 Dll1、3、4;Jagged1 和 Jagged2)和四种 Notch 受体(Notch1-4)^[31]。Notch 配体与受体结合后激活 Notch 信号,Notch 胞内段(NICD)被裂解并释放,然后从细胞膜转运至细胞核^[32]。在人角膜上皮细胞中,Notch 信号通路不仅可直接参与调节血管内皮细胞的增殖和分化,还协助碱性成纤维细胞生长因子诱导角膜血管平滑肌细胞和内皮细胞增殖^[33]。然而,Notch 信号还可参与调节 Smad6 的表达,从而抑制内皮细胞对 BMP 的反应性,继而抑制新血管形成^[34]。因此,Notch 信号对 CNV 可能起着双重作用。有研究发现,TGF- β 激活的 Smad3 可与 NICD 发生作用,从而促进 Notch 信号靶基因的表达^[23]。此外,TGF- β /Smad 信号通路诱导产生的 VEGF 是 Notch 信号的上游通路^[26],在血管内皮细胞中,二者形成 VEGF-VEGFR-Dll4-Notch-VEGFR 调节反馈通路^[35],这表明,Smad 蛋白还可能通过 VEGF 间接作用于 Notch 信号。另外,TGF- β 抑制剂可以下调

Notch 信号通路中的 RNA 和蛋白质的表达^[36],进一步说明 TGF- β /Smad 信号通路能促进 Notch 信号转导。而且,BMP/Smad 通路也可以直接与 Notch 信号靶基因的调控序列结合,进而促进 Notch 信号转导^[23]。

3 前景展望

虽然早有研究发现,Smad 蛋白在促血管生成因子和抗血管生成因子的表达调控中具有关键作用^[37]。但是近些年来,Smad 蛋白直接调控 CNV 发生发展的研究相当少。本文主要总结了 Smad 蛋白对茎细胞、尖细胞形成的调控以及 Smad 蛋白与 VEGF 途径、Wnt 途径、Rho/Rock 途径和 Notch 途径的相互作用,从而推测 Smad 蛋白可能通过这些信号转导途径参与 CNV 发生发展的调控,但是其具体机制尚未明确,尚需进一步研究,以为 CNV 的治疗提供新的可能靶点。

参考文献

- [1] SHARIF Z, SHARIF W. Corneal neovascularization: updates on pathophysiology, investigations & management [J]. *Rom J Ophthalmol*, 2019, 63(1): 15-22.
- [2] XU F, LIU C, ZHOU D, ZHANG L. TGF- β /SMAD pathway and its regulation in hepatic fibrosis [J]. *J Histochem Cytochem*, 2016, 64(3): 157-167.
- [3] MACIAS M J, MASSAGUÉ J. Structural determinants of Smad function in TGF- β signaling [J]. *Trends Biochem Sci*, 2015, 40(6): 296-308.
- [4] HATA A, CHEN Y G. TGF- β signaling from receptors to smads [J]. *Cold Spring Harb Perspect Biol*, 2016, 8(9): a022061.
- [5] ZHANG Y. Non-Smad signaling pathways of the TGF- β family [J]. *Cold Spring Harb Perspect Biol*, 2017, 9(2): a022129.
- [6] YETKIN-ARIK Y B. Endothelial tip cells in vitro are less glycolytic and have a more flexible response to metabolic stress than non-tip cells [J]. *Sci Rep*, 2019, 9(1): 10414.
- [7] KOON Y L, ZHANG S, RAHMAT M B, KOH C G, CHIAM K H. Enhanced Delta-Notch lateral inhibition model incorporating intracellular notch heterogeneity and tension-dependent rate of Delta-Notch binding that reproduces sprouting angiogenesis patterns [J]. *Sci Rep*, 2018, 8(1): 9519.
- [8] NEDVETSKY P I. cAMP-dependent protein kinase a (PKA) regulates angiogenesis by modulating tip cell behavior in a notch-independent manner [J]. *Development*, 2016, 143(19): 3582-3590.
- [9] KERR G, SHELDON H, CHAIKUAD A, ALFANO I, VON DELFT F, BULLOCK A N, et al. A small molecule targeting ALK1 prevents Notch cooperativity and inhibits functional angiogenesis [J]. *Angiogenesis*, 2015, 18(2): 209-217.
- [10] BENN A, HIEPEN C, OSTERLAND M, SCHÜTTE C, ZWILSEN A, KNAUS P. Role of bone morphogenetic proteins in sprouting angiogenesis: differential BMP receptor-dependent signaling pathways balance stalk vs. tip cell competence [J]. *FASEB J*, 2017, 31(11): 4720-4733.
- [11] JARAD M, KUCZYNSKI E A, MORRISON J, VILORIA-PETIT A M, COOMBER B L. Release of endothelial cell associated VEGFR2 during TGF- β modulated angiogenesis in vitro [J]. *BMC Cell Biol*, 2017, 18(1): 10.
- [12] ASPALTER M I. Alk1 and Alk5 inhibition by Nrpl controls vascular sprouting downstream of Notch [J]. *Nat Commun*, 2015, 6: 7264.
- [13] ABDELFATTAH N S, AMGAD M, ZAYED A, HUSSEIN H, EL-BAKY E N. Molecular underpinnings of corneal angiogenesis: advances over the past decade [J]. *Int J Ophthalmol*, 2016, 9(5): 768-779.
- [14] LIU S, ROMANO V, STEGER B, KAYE S B, HAMILL K J, WILLOUGHBY C E. Gene-based antiangiogenic applications

- for corneal neovascularization [J]. *Surv Ophthalmol*, 2018, 63(2):193-213.
- [15] LIU X, WANG S, WANG X, LIANG J, ZHANG Y. Recent drug therapies for corneal neovascularization [J]. *Chem Biol Drug Des*, 2017, 90(5):653-664.
- [16] VOICULESCU O B, ALEXANDRESCU C. Corneal neovascularization and biological therapy [J]. *J Med Life*, 2015, 8(4):444-448.
- [17] ASSIS P A, DE FIGUEIREDO-PONTES L L, LIMA A S, LEÃO V, CÂNDIDO L A, PINTÃO C T, *et al.* Halofuginone inhibits phosphorylation of SMAD-2 reducing angiogenesis and leukemia burden in an acute promyelocytic leukemia mouse model [J]. *J Exp Clin Oncol*, 2015, 34(1):65.
- [18] OH M K, KIM I S. Involvement of placental growth factor up-regulated via TGF- β 1-ALK1-Smad1/5 signaling in prohypertrophic-induced angiogenesis [J]. *PLoS One*, 2019, 14(4):e0216289.
- [19] SEYSTAHL K, TRITSCHLER I, SZABO E, TABATABAI G, WELLER M. Differential regulation of TGF- β -induced, ALK-5-mediated VEGF release by SMAD2/3 versus SMAD1/5/8 signaling in glioblastoma [J]. *Neuro Oncol*, 2015, 17(2):254-265.
- [20] HAQUE R, IU VONE P M, HE L, CHOI K S C, NGO A, GOKHALE S, *et al.* The microRNA-21 signaling pathway is involved in prorenin receptor (PRR)-induced VEGF expression in ARPE-19 cells under a hyperglycemic condition [J]. *Mol Vis*, 2017, 23:251-262.
- [21] NGUYEN V, HOUGH R, BERNAUDO S, PENG C. Wnt/ β -catenin signalling in ovarian cancer: insights into its hyperactivation and function in tumorigenesis [J]. *J Ovarian Res*, 2019, 12(1):122.
- [22] WANG Y, WU Z, TIAN J, MI Y, REN X, KANG J, *et al.* Intermedin protects HUVECs from ischemia reperfusion injury via Wnt/ β -catenin signaling pathway [J]. *Ren Fail*, 2019, 41(1):159-166.
- [23] LUO K. Signaling cross talk between TGF- β /Smad and other signaling pathways [J]. *Cold Spring Harb Perspect Biol*, 2017, 9(1):a022137.
- [24] TAIYAB A, HOLMS J, WEST-MAYS J A. β -Catenin/Smad3 interaction regulates transforming growth factor- β -induced epithelial to mesenchymal transition in the lens [J]. *Int J Mol Sci*, 2019, 20(9):2078.
- [25] VALLÉE A, LECARPENTIER Y, GUILLEVIN R, VALLÉE J N. Interactions between TGF- β 1, canonical WNT/ β -catenin pathway and PPAR γ in radiation-induced fibrosis [J]. *Onco*
- cotarget*, 2017, 8(52):90579-90604.
- [26] ZHANG J, WANG S, HE Y, YAO B, ZHANG Y. Regulation of matrix metalloproteinases 2 and 9 in corneal neovascularization [J]. *Chem Biol Drug Des*, 2020, 95(5):485-492.
- [27] ROSHANDEL D, ESLANI M, BARADARAN-RAFI A, CHEUNG A Y, KURJI K, JABBEHDARI S, *et al.* Current and emerging therapies for corneal neovascularization [J]. *Ocul Surf*, 2018, 16(4):398-414.
- [28] VAN DYKEN P, LACOSTE B. Impact of metabolic syndrome on neuroinflammation and the blood-brain barrier [J]. *Front Neurosci*, 2018, 12:930.
- [29] LIU L J, YAO F J, LU G H, XU C G, XU Z, TANG K, *et al.* The role of the Rho/ROCK pathway in Ang II and TGF- β 1-induced atrial remodeling [J]. *PLoS One*, 2016, 11(9):e0161625.
- [30] JI H, TANG H, LIN H, MAO J, GAO L, LIU J, *et al.* Rho/rock cross-talks with transforming growth factor- β /Smad pathway participates in lung fibroblast-myofibroblast differentiation [J]. *Biomed Rep*, 2014, 2(6):787-792.
- [31] CAMPBELL D P, DOETZLHOFFER A. Canonical notch signaling plays an instructive role in auditory supporting cell development [J]. *Sci Rep*, 2016, 6:19484.
- [32] COLOMBO M, GALLETTI S, GARAVELLI S, PLATONOVA N, PAOLI A, BASILE A, *et al.* Notch signaling deregulation in multiple myeloma: a rational molecular target [J]. *Oncotarget*, 2015, 6(29):26826-26840.
- [33] XIE F. Notch signaling pathway is involved in bFGF-induced corneal lymphangiogenesis and hemangiogenesis [J]. *J Ophthalmol*, 2019, 2019:9613923.
- [34] MOUILLESSEAU P K. Notch regulates BMP responsiveness and lateral branching in vessel networks via SMAD6 [J]. *Nat Commun*, 2016, 7:13247.
- [35] ZAKHARI J S, ZABONICK J, GETTLER B, WILLIAMS S K. Vascuogenic and angiogenic potential of adipose stromal vascular fraction cell populations in vitro [J]. *In Vitro Cell Dev Biol Anim*, 2018, 54(1):32-40.
- [36] WANG Y, SHEN R W, HAN B, LI Z, XIONG L, ZHANG F Y, *et al.* Notch signaling mediated by TGF- β /Smad pathway in concanavalin A-induced liver fibrosis in rats [J]. *World J Gastroenterol*, 2017, 23(13):2330-2336.
- [37] NAKAGAWA T, LI J H, GARCIA G, MU W, PIEK E, BÖTTINGER E P, *et al.* TGF- β induces proangiogenic and antiangiogenic factors via parallel but distinct Smad pathways [J]. *Kidney Int*, 2004, 66(2):605-613.

Research progress of Smad protein in regulating the occurrence and development of corneal neovascularization

ZENG Ao^{1,2}, YAN Yu^{1,2}, WANG Shurong¹, ZHANG Yan¹, HE Yuxi¹

1. Department of Ophthalmology, the Second Hospital of Jilin University, Changchun 130041, Jilin Province, China

2. Bethune College of Medicine, Jilin University, Changchun 130021, Jilin Province, China

Corresponding author: HE Yuxi, E-mail: heyuxihot@163.com

[Abstract] Corneal neovascularization (CNV) is a serious blinding lesion closely associated with the occurrence and development of various ocular surface diseases. In the process of CNV, many proteins participate in the regulation. Studies have shown that Smad protein can influence the occurrence and development of CNV through multiple signaling pathways. This paper summarizes the research progress of Smad protein in regulating the occurrence and development of CNV.

[Key words] Smad protein; corneal neovascularization; transforming growth factor β ; bone morphogenetic protein; stem cells; tip cells