

miR-15a 在眼科疾病中的研究现状及进展

李宇博,王峰,苏颖

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作者单位:(157000)中国黑龙江省哈尔滨市,哈尔滨医科大学附属第一医院眼科

作者简介:李宇博,哈尔滨医科大学在读硕士研究生,研究方向:眼底病学。

通讯作者:苏颖,医学博士,教授,硕士研究生导师,主任医师,科室副主任,研究方向:玻璃体视网膜病、视神经疾病的诊断和治疗. hyingsu@126.com

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摘要

miRNA-15a(miR-15a)是位于13q14基因上的非编码RNA小分子,随着研究的深入,其对全身各组织器官及细胞的生长、凋亡等调控作用越发被重视,成为现今研究较多的miRNA。本文主要针对miR-15a的作用机制及在眼科疾病中的研究现状进行阐述,为进一步研究眼科疾病及治疗提供可借鉴的参考依据。

关键词:miR-15a;眼科疾病;糖尿病视网膜病变;白内障;视网膜血管内皮细胞;视网膜色素上皮细胞

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Research status and progress of miR-15a in ophthalmological diseases

Yu-Bo Li, Feng Wang, Ying Su

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Department of Ophthalmology, the First Affiliated Hospital of Harbin Medical University, Harbin 157000, Heilongjiang Province, China

Correspondence to: Ying Su. Department of Ophthalmology, the First Affiliated Hospital of Harbin Medical University, Harbin 157000, Heilongjiang Province, China. hyingsu@126.com

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Abstract

• miRNA-15a(miR-15a) is a non-coding small molecule RNA located on 13q14 gene. It affects the growth, development, differentiation and apoptosis of all organs and cells of the whole body. As the study progressively deepened, it was found that the role of miR-15a in different tissues and cells was not entirely consistent. Sometimes it plays a role in suppressing cancer, and sometimes it promotes cancer. The signal pathways it

affects are complex and diverse. With the deepening of biological research into cell signaling pathways, miRNA-15a has become a miRNA more extensively studied. But in the ophthalmology, the corresponding research is not much. In this article, we mainly focus on the mechanism of miR-15a and its current research situation in ophthalmic diseases, so as to provide a reference for further study and their treatment.

• **KEYWORDS:** miR-15a; ophthalmic diseases; diabetic retinopathy; cataract; retinal vascular endothelial cells; retinal pigment endothelial cells

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0 引言

miRNA是一种广泛存在于真核生物中的非编码RNA小分子,其大小约为18~25个核苷酸,主要通过作用于信使RNA的3'端非编码区(UTR)从而降解靶RNA或终止翻译^[1]。这一概念最初由Lagos-Quintana等^[2]首次提出。随着对其研究的深入,已发现的miRNA达2000余种,这些miRNA在细胞及组织的增殖、分化及凋亡等方面发挥着重要作用。本文主要针对miRNA-15a(miR-15a)的研究现状进行综述,以期为miR-15a的研究提供可借鉴的参考依据。

1 miR-15a概述

miR-15a为现今研究较多的miRNA之一,最初于2002年被Pekarsky等在慢性淋巴细胞白血病(CLL)中发现^[3-5]。该课题组观察到,部分CLL患者13q14基因上存在小的缺失与移位,经过进一步的分子杂交,最终定位于DLEU2基因外显子2与5之间,这在miRNA的研究中具有标志性的作用,使人们对miRNA的研究更加深入。大量实验研究证明,miR-15a通过多种信号通路对细胞进程进行调控,从而进一步影响着机体组织,如造血系统^[6]、肝脏^[7]、眼^[8-10]、皮肤^[11]等相关疾病的发生发展。miR-15a在不同细胞中的作用机制及产生的结果复杂多变,研究显示,在慢性淋巴细胞白血病^[12]、胃癌^[13]、子宫内膜癌^[14]等疾病中,其能够起到抑癌基因的作用。但另有研究显示,在结肠直肠癌^[15]、卡其波肉瘤^[16]的发生发展中miR-15a具有促癌作用。随着研究的深入,miR-15a的靶基因、靶蛋白及其影响的信号通路被逐渐证实,但由于miRNA调控机制的复杂性,其作用及机制尚有待进一步探索及论证。

2 miR-15a研究现状

国内外关于miR-15a的研究始于2002年,现已证实其在机体各组织器官疾病的发生发展中起着重要的作用。它可作用于多种致癌基因如BCL2、CCNE1^[17-20]、

CCND1^[21]、MCL^[12]等调节细胞周期、细胞凋亡,进而抑制癌症,其缺失可能会促进癌症的发生、侵袭性增强及转移。如在以13q14缺失型最为常见的CLL^[12],其异常表达的27种蛋白质中有8种被认为是miR-15a的可能靶点^[22]。miR-15a的缺失^[1, 4-5]导致致癌基因BCL2基因的过表达^[5],进而导致CLL的发生。相似的情况亦可见于口腔鳞状细胞癌^[6]、慢性肾病^[23-24]等疾病。而在其他肿瘤细胞中亦可见到miR-15a的异常表达,如骨髓中的转移性前列腺癌细胞中,下调miR-15a可通过CDK4~6等致癌基因促进癌症的进展^[20],产生异常的TGF- β 信号,促进肿瘤细胞扩增并诱导上皮间充质纤维化(EMT),使早期上皮肿瘤向侵袭性和转移性肿瘤变化^[25]。相似地,在胃癌细胞中,miR-15a的降低介导了EMT过程^[26]。在上皮细胞和角质细胞^[27]中,过表达miR-15a能够显著降低IL-10R α 的表达,miR-15a的缺失导致IL-10R α 的表达上调^[28],进一步导致黑色素瘤的发生。但在儿童室管膜瘤中,miR-15a在初发癌症细胞中表达降低,复发癌症细胞中表达却升高,这表明,在侵略性更强的室管膜肿瘤中,miR-15a或许为癌基因。同时,在炎症相关性疾病细胞中miR-15a的表达亦有所改变。如动脉粥样硬化患者的脂肪间充质细胞在缺氧条件下miR-15a表达下调,血管内皮生长因子(VEGF)、成纤维细胞生长因子(FGF)表达上调^[29]。研究也证实糖尿病患者中,miR-15a表达异常。但不同的研究团队对此持有不同意见,Zampetaki等^[30]通过qPCR检测发现,糖尿病患者血浆中miR-15a表达下调,而Sun等^[31]却认为,在机体处于高糖环境中的1h内miR-15a的表达上调,随后随着胰岛素分泌增多,miR-15a表达逐渐下调。也有研究认为,糖尿病患者外周血中miR-15a的表达增多并与疾病的严重程度相关,即糖尿病越重,miR-15a在外周血中的含量越高。

3 miR-15a作用机制

由上述研究现状可以发现,miR-15a的作用机制复杂。现已证实,miRNA能够活化目标基因的翻译过程,其首先在细胞核内转录并加工,形成前体miRNA,在胞浆中剪切为成熟miRNA后与mRNA完全或不完全配对来调节基因表达。Calin等^[5]认为,miR-15a位于13q14.3基因上,该基因的缺失会导致miR-15a表达降低。由于不同细胞中miR-15a作用的mRNA及信号通路不完全相同,产生的结果也复杂多变。研究显示,miR-15a可通过作用于BCL2、CCND1、CCNE1、CDK4~6等致癌基因影响癌症的进展^[22],而可能的信号通路包括FGF-2、FGFR1、VEGF^[32]、WNT、SMAD^[33]、TLR5/8、IRAK1、TRAF6^[8]、TNF α 、IL-10R α ^[28]、BDNF^[17]等。miR-15a的增多或减少通过这些信号通路影响下游蛋白如炎症因子IL-1 β ^[10]、IL-6^[10]、TGF- β ^[34]、肿瘤坏死因子 α (TNF α)、血管内皮生长因子A(VEGF-A)、脑源性神经营养因子(BDNF)^[35-36]等调控炎症反应及细胞凋亡等过程。

4 miR-15a在眼科疾病中的研究现状

miR-15a在眼科疾病发生发展中的作用机制研究起步较晚且较少,国外仅见少量文献报道,且研究方向主要集中于miR-15a与糖尿病视网膜病变。前文已述,miR-15a在糖尿病中表达异常,Kamalden等^[37]发现,糖尿病视网膜病变中增多的miR-15a与视网膜细胞无关,在

糖尿病视网膜血管内皮细胞(REC)中,miR-15a显著降低,因此眼科对miR-15a的研究也主要集中于糖尿病视网膜病变中REC上。2012年,Shambhu等在对小鼠晶状体中miRNA受半乳糖饮食影响的研究中发现,半乳糖饮食组的小鼠晶状体中miR-15a表达下调,但并未对其机制进行进一步研究^[38]。2015年,Hirota等最先在实验中通过qPCR检测发现,miR-15a在增殖期糖尿病视网膜病变(PDR)的玻璃体中表达升高。2016年,Ye等^[8]对敲除了miR-15a的小鼠视网膜细胞进行流式细胞仪检测,结果显示CD45⁺白细胞显著增多,而通过对miR-15a敲除小鼠及对照组小鼠骨髓与外周血的检测发现,二者CD45⁺白细胞含量无显著差异。类似地,基因敲除小鼠的REC细胞中IL-1 β 、TNF α 和NK- κ B的含量显著低于对照组小鼠。向体外高糖诱导的人视网膜色素上皮(RPE)细胞中转染miR-15a的拟生物后,Western Blot显示IL-1 β 、TNF α 及NK- κ B的表达下调。研究表明,在缺乏miR-15a的细胞中,IL-1 β 和TNF α 显著表达并促进NK- κ B活化;增多的miR-15a能够通过抑制IL-1 β 和TNF α 信号肽及NK- κ B的磷酸化从而抑制白细胞停滞,最终防止高糖诱导的REC细胞凋亡。2016年,Wang等在对体外高糖诱导的人REC和RPE细胞的研究中发现,miR-15a能够直接抑制其高表达的酸化鞘磷脂酶(ASM)及VEGF-A的活性,调节视网膜神经酰胺的表达、骨髓循环血管原细胞(CAC)释放,迁移与归巢至视网膜脉管系统,并通过对不同浓度高糖诱导过的RPE细胞进行qPCR与Western Blot检测发现,更高浓度的高糖诱导过的RPE细胞ASM表达更高,而miR-15a抑制作用更明显。miR-15a能够直接与3'UTR结合并抑制VEGF-A与ASM的表达。Wang等^[10]在向体外培养的人REC细胞中转染miR-15a诱导剂与抑制剂时发现,表达上调的miR-15a能够直接抑制ASM、神经酰胺与VEGF-A的合成,阻止视网膜炎炎症反应及血管损伤,促进CAC释放与归巢;表达下调的miR-15a直接影响视网膜,促进ASM与神经酰胺活化、引起持续的低度慢性炎症反应、VEGF-A的高表达并抑制CAC的迁移能力。Ye等^[9]和Gong等^[38]进一步研究发现,过表达的miR-15a也能够通过抑制高糖条件下TGF- β 3/SMAD信号通路降低VEGF的表达,提高REC中紧密连接蛋白(ZO-1)和闭合蛋白含量,最终抑制高糖下内皮细胞的通透性,维护视网膜血管内皮屏障。该研究亦证实了Wang等^[10]研究结果。国内学者对高糖诱导后的HARPE-19细胞系进行检测也发现,miR-15a表达下调,而ASM表达上调;过表达的miR-15a抑制了ASM、VEGF、IL-1 β 、IL-6、TNF α mRNA的表达,该结果与上述研究相同。但Hirota等发现PDR中,miR-15a表达反向升高,这一结果与上述研究结果并不相同,分析认为miR-15a具有细胞及组织特异性,可能与早期内皮细胞内高表达相关,具体机制尚不清晰^[39]。

近年来,miR-15a对白内障进展的影响也逐渐被发现。2015年,Li等^[40]对60例年龄相关性白内障患者和20例正常晶状体上皮细胞进行研究,使用实时PCR检测hsa-miR-15a-5p、hsa-miR-15a-3p及其靶基因bcl-2和mcl-1的表达发现,hsa-miR-15a-5p和hsa-miR-15a-3p在正常晶状体上皮细胞中呈低水平表达,但在皮质、核性

或后囊下白内障患者的相应细胞中呈高表达 ($P < 0.01$), 靶基因 *bcl-2* 和 *mcl-1* 在正常晶状体上皮细胞中可检测到表达,但在所有类型的白内障患者中其水平显著降低 ($P < 0.01$)。这说明 miR-15a 可能通过抑制抗凋亡基因 *bcl-2* 和 *mcl-1* 的表达促进年龄相关性白内障的发病。Tian 等^[41] 和 Abdullah 等^[42] 研究亦证实这一点。2019 年, Li 等^[43] 进一步证实, miR-15a 可通过调节 *bcl-2* 和 E2F3 触发凋亡并抑制人晶状体上皮细胞 (HLE-B3 细胞) 的增殖。

5 展望

miR-15a 在细胞生长、增殖、分化等方面具有调节作用,但由于作用于不同细胞时信号通路不同,所起效果也有所不同。目前,miR-15a 在眼科疾病的研究虽然多局限在糖尿病视网膜病变中,但为眼科疾病基础性研究与临床实践相结合方面提供了可借鉴的途径,开拓了眼科疾病研究的视野,从分子学角度进一步验证了糖尿病视网膜病变发生发展与 miRNA 的关系,也提示未来关于眼科疾病的研究中,在关注 miR-15a 靶基因、靶蛋白及其相关信号通路对疾病影响的同时,还要关注靶基因药物对眼科疾病的治疗潜力。

参考文献

- Ambros V. The functions of animal microRNAs. *Nature* 2004; 431(7006): 350-355
- Lagos-Quintana M, Rauhut R, Lendeckel W, et al. Identification of novel genes coding for small expressed RNAs. *Science* 2001; 294(5543): 853-858
- Sampath D, Liu C, Vasani K, et al. Histone deacetylases mediate the silencing of miR-15a, miR-16, and miR-29b in chronic lymphocytic leukemia. *Blood* 2012; 119(5): 1162-1172
- Allegra D, Bilan V, Garding A, et al. Defective DROSHA processing contributes to downregulation of miR-15/16 in chronic lymphocytic leukemia. *Leukemia* 2014; 28(1): 98-107
- Calin GA, Dumitru CD, Shimizu M, et al. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci U S A* 2002; 99(24): 15524-15529
- Cimmino A, Calin GA, Fabbri M, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc Natl Acad Sci U S A* 2005; 102(39): 13944-13949
- Long J, Jiang C, Liu B, et al. MicroRNA-15a-5p suppresses cancer proliferation and division in human hepatocellular carcinoma by targeting BDNF. *Tumour Biol* 2016; 37(5): 5821-5828
- Ye EA, Liu L, Jiang Y, et al. miR-15a/16 reduces retinal leukostasis through decreased pro-inflammatory signaling. *J Neuroinflammation* 2016; 13(1): 305
- Ye EA, Liu L, Steinle JJ. miR-15a/16 inhibits TGF-beta3/VEGF signaling and increases retinal endothelial cell barrier proteins. *Vision Res* 2017; 139: 23-29
- Wang Q, Navitskaya S, Chakravarthy H, et al. Dual Anti-inflammatory and Anti-angiogenic Action of miR-15a in Diabetic Retinopathy. *EBioMedicine* 2016; 11: 138-150
- Mizrahi A, Barzilai A, Gur-Wahnon D, et al. Alterations of microRNAs throughout the malignant evolution of cutaneous squamous cell carcinoma: the role of miR-497 in epithelial to mesenchymal transition of keratinocytes. *Oncogene* 2018; 37(2): 218-230
- Pekarsky Y, Croce CM. Role of miR-15/16 in CLL. *Cell Death Differ*

2015; 22(1): 6-11

- Wang T, Hou J, Li Z, et al. miR-15a-3p and miR-16-1-3p Negatively Regulate Twist1 to Repress Gastric Cancer Cell Invasion and Metastasis. *Int J Biol Sci* 2017; 13(1): 122-134
- Liu XJ, Bai XG, Teng YL, et al. miRNA-15a-5p regulates VEGFA in endometrial mesenchymal stem cells and contributes to the pathogenesis of endometriosis. *Eur Rev Med Pharmacol Sci* 2016; 20(16): 3319-3326
- de Groen FL, Timmer LM, Menezes RX, et al. Oncogenic Role of miR-15a-3p in 13q Amplicon-Driven Colorectal Adenoma-to-Carcinoma Progression. *PLoS One* 2015; 10(7): e0132495
- Wu XJ, Pu XM, Zhao ZF, et al. The expression profiles of microRNAs in Kaposi's sarcoma. *Tumour Biol* 2015; 36(1): 437-446
- Bonci D, Coppola V, Musumeci M, et al. The miR-15a-miR-16-1 cluster controls prostate cancer by targeting multiple oncogenic activities. *Nat Med* 2008; 14(11): 1271-1277
- Liu Q, Fu H, Sun F, et al. miR-16 family induces cell cycle arrest by regulating multiple cell cycle genes. *Nucleic Acids Res* 2008; 36(16): 5391-5404
- Klein U, Lia M, Crespo M, et al. The DLEU2/miR-15a/16-1 cluster controls B cell proliferation and its deletion leads to chronic lymphocytic leukemia. *Cancer Cell* 2010; 17(1): 28-40
- Aqeilan RI, Calin GA, Croce CM. miR-15a and miR-16-1 in cancer: discovery, function and future perspectives. *Cell Death Differ* 2010; 17(2): 215-220
- Liu Z, Cheng C, Luo X, et al. CDK4 and miR-15a comprise an abnormal automodulatory feedback loop stimulating the pathogenesis and inducing chemotherapy resistance in nasopharyngeal carcinoma. *BMC Cancer* 2016; 16: 238
- Calin GA, Cimmino A, Fabbri M, et al. MiR-15a and miR-16-1 cluster functions in human leukemia. *Proc Natl Acad Sci U S A* 2008; 105(13): 5166-5171
- Singh Y, Garden OA, Lang F, et al. MicroRNA-15b/16 Enhances the Induction of Regulatory T Cells by Regulating the Expression of Rictor and mTOR. *J Immunol* 2015; 195(12): 5667-5677
- Nandakumar P, Tin A, Grove ML, et al. MicroRNAs in the miR-17 and miR-15 families are downregulated in chronic kidney disease with hypertension. *PLoS One* 2017; 12(8): e0176734
- Massagué J. TGFbeta in Cancer. *Cell* 2008; 134(2): 215-230
- Wang T, Hou J, Li Z, et al. miR-15a-3p and miR-16-1-3p Negatively Regulate Twist1 to Repress Gastric Cancer Cell Invasion and Metastasis. *Int J Biol Sci* 2017; 13(1): 122-134
- Krüger-Krasagakes S, Krasagakis K, Garbe C, et al. Expression of interleukin 10 in human melanoma. *Br J Cancer* 1994; 70(6): 1182-1185
- Venza I, Visalli M, Beninati C, et al. IL-10Rα expression is post-transcriptionally regulated by miR-15a, miR-185, and miR-211 in melanoma. *BMC Med Genomics* 2015; 8: 81
- Saad A, Zhu XY, Herrmann S, et al. Adipose-derived mesenchymal stem cells from patients with atherosclerotic renovascular disease have increased DNA damage and reduced angiogenesis that can be modified by hypoxia. *Stem Cell Res Ther* 2016; 7(1): 128
- Zampetaki A, Kiechl S, Drozdov I, et al. Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. *Circ Res* 2010; 107(6): 810-817
- Sun LL, Jiang BG, Li WT, et al. MicroRNA-15a positively regulates insulin synthesis by inhibiting uncoupling protein-2 expression. *Diabetes Res Clin Pract* 2011; 91(1): 94-100

32 Yin KJ, Olsen K, Hamblin M, *et al.* Vascular endothelial cell-specific microRNA-15a inhibits angiogenesis in hindlimb ischemia. *J Biol Chem* 2012; 287(32): 27055-27064

33 Musumeci M, Coppola V, Addario A, *et al.* Control of tumor and microenvironment cross-talk by miR-15a and miR-16 in prostate cancer. *Oncogene* 2011; 30(41): 4231-4242

34 Teicher BA. Malignant cells, directors of the malignant process: role of transforming growth factor-beta. *Cancer Metastasis Rev* 2001; 20(1-2): 133-143

35 Cao L, Liu X, Lin EJ, *et al.* Environmental and genetic activation of a brain-adipocyte BDNF/leptin axis causes cancer remission and inhibition. *Cell* 2010; 142(1): 52-64

36 Kaplan DR, Matsumoto K, Lucarelli E, *et al.* Induction of TrkB by retinoic acid mediates biologic responsiveness to BDNF and differentiation of human neuroblastoma cells. Eukaryotic Signal Transduction Group. *Neuron* 1993; 11(2): 321-331

37 Kamalden TA, Macgregor-Das AM, Kannan SM, *et al.* Exosomal MicroRNA-15a Transfer from the Pancreas Augments Diabetic Complications by Inducing Oxidative Stress. *Antioxid Redox Signal* 2017; 27(13): 913-930

38 Gong Q, Li F, Xie J, *et al.* Upregulated VEGF and Robo4 correlate with the reduction of miR-15a in the development of diabetic retinopathy. *Endocrine* 2019; 65(1): 35-45

39 Varma SD, Kovtun S, Hegde K, *et al.* Effect of high sugar levels on miRNA expression. Studies with galactosemic mice lenses. *Mol Vis* 2012; 18: 1609-1618

40 Li Y, Liu S, Zhang F, *et al.* Expression of the microRNAs hsa-miR-15a and hsa-miR-16-1 in lens epithelial cells of patients with age-related cataract. *Int J Clin Exp Med* 2015; 8(2): 2405-2410

41 Tian R, Xu Y, Dou WW, *et al.* Bioinformatics analysis of microarray data to explore the key genes involved in HSF4 mutation-induced cataract. *Int J Ophthalmol* 2018; 11(6): 910-917

42 Abdullah OA, El Gazzar WB, Salem TI, *et al.* miR-15a: a potential diagnostic biomarker and a candidate for non-operative therapeutic modality for age-related cataract. *Br J Biomed Sci* 2019; 76(4): 184-189

43 Li Q, Pan H, Liu Q. MicroRNA-15a modulates lens epithelial cells apoptosis and proliferation through targeting B-cell lymphoma-2 and E2F transcription factor 3 in age-related cataracts. *Biosci Rep* 2019; 39(12): BSR20191773