

microRNA在肝纤维化中的研究进展

马艳华¹, 邢卉春^{1,2} (1. 北京大学北京地坛医院教学医院, 北京 100015; 2. 首都医科大学附属北京地坛医院 肝三科, 北京 100015)

摘要: 微小RNA (microRNA, 简称miRNA) 是长约19~25个核苷酸的内源性非编码单链小RNA, 其通过与mRNA 3'-端非翻译区 (3'-untranslated region, 3'-UTR) 结合进行转录后水平的负性调控。miRNA通过调节肝星状细胞的活化、转分化、迁移、增殖和凋亡, 在肝纤维化过程中发挥重要作用。不同miRNA在肝纤维化过程中发挥着不同的促(抗)纤维化作用, miRNA的作用已逐步引起科研工作者的重视。本文对miRNA在肝纤维化进程中的作用进行综述。

关键词: 肝纤维化; microRNA; 机制

Advances of microRNA on liver fibrosis

MA Yan-hua¹, XING Hui-chun^{1,2} (1. Peking University Ditan Teaching Hospital, Beijing 100015, China; 2. Department of Hepatology Division 3, Beijing Ditan Hospital, Capital Medical University, Beijing 100015, China)

Abstract: microRNA (miRNA) are endogenous single-stranded small non-coding RNA of 19~25 nucleotides that regulate gene expression at post transcription level through binding to the 3'-untranslated region (3'-UTR) of mRNA. miRNA are involved in the process of liver fibrosis by regulating the activation, differentiation, migration, proliferation and apoptosis of hepatic stellate cell (HSC). Different miRNA play different roles on promoting (anti) fibrosis in liver fibrosis which attracted the attention of researchers gradually. In this paper, the roles of miRNA in the process of liver fibrosis were reviewed.

Key words: Liver fibrosis; microRNA; Mechanism

肝纤维化是指在各种致病因子的作用下, 肝星状细胞 (hepatic stellate cell, HSC) 活化, 细胞外基质 (extracellular matrix, ECM) 大量产生且降解减少, 继发正常肝组织破坏为特征的可逆性病变。肝纤维化是多种慢性肝脏疾病的共同病理过程, 而乙型肝炎病毒 (hepatitis B virus, HBV) 是最常见的致病因素, 全球有约1/3人口曾感染HBV, 其中约2.4亿人感染后转为慢性感染, 而乙型肝炎肝纤维化是慢性乙型肝炎逐步进展至肝硬化的重要阶段, 每年有大量患者在肝硬化基础上发生原发性肝癌^[1]。如未经有效诊断和治疗会导致患者反复住院, 生活质量严重下降甚至死亡。因此早期诊断和治疗肝纤维化非常重要。然而, 肝纤维化的早期诊断并不尽如人意, 肝组织活检、影像学或实验室检查都有一定的局限性和滞后性^[2-5]。随着对表观遗传学的深入研究, 发现miRNA通过与靶基因mRNA

的3'-UTR部分或完全互补结合, 抑制翻译或降解mRNA, 在肝纤维化的发生和发展过程中发挥重要作用^[6]。

1 microRNA 概述

在细胞核内miRNA基因通过RNA聚合酶II介导转录出含有茎环结构的初级转录产物 (pri-miRNA), 随后被RNaseIII Drosha切割为前体miRNA (pre-miRNA)。Pre-miRNA在转运蛋白exportin-5的作用下转运至细胞质, 然后经RNaseIII Dicer进一步切割为成熟的miRNA^[7]。这些成熟的miRNA是一类长约19~25个核苷酸的内源性非编码单链RNA分子, 与其他蛋白质一起组成RNA诱导的沉默复合体 (RNA-induced silencing complex, RISC), 并通过与mRNA的3'-UTR碱基配对引起mRNA的降解或抑制其翻译, 从而在转录后水平发挥负性调控作用, 若两者完全互补配对则降解mRNA; 若不完全互补则抑制靶mRNA转录后水平的表达。研究发现miRNA在外周血中稳定存在, 因此miRNA有可能作为肝纤维化早期诊断的非侵入性生物标志^[6-8]。

2 microRNA 的筛选

研究表明,肝星状细胞的活化是肝纤维化发生的中心环节,尽管HSC仅占肝组织细胞数的5%~8%,但其却是肝纤维化发生的主要原因^[9]。最近研究表明miRNA参与HSC的活化^[10]。Guo等^[11]采用微阵列分析发现,与静止型HSC相比,在活化的大鼠HSC中,有12种miRNA表达上调,9种表达下调(倍数变化 > 2.0 , $P < 0.05$),其中上调的miRNA有miR-874、miR-29c、miR-501、miR-349、miR-325-5p、miR-328、miR-138、miR-143、miR-207、miR-872、miR-140和miR-193,下调的miRNA有miR-15b、miR-16、miR-341、miR-20b-3p、miR-375、miR-122、miR-146a、miR-92b和miR-126。Maubach等^[12]用基因芯片技术分析小鼠HSC发现,与静止型HSC相比,活化的HSC中有16种miRNA表达上调($P < 0.05$),包括let-7b、let-7c、let-7e、miR-125b、miR-132、miR-143、miR-145和miR-152等;26种表达下调($P < 0.05$),包括miR-10a、miR-122a、miR-125a、miR-126、miR-146a、miR-150、miR-151、miR-16和miR-195等。同样,在人类HSC活化过程中,miRNA的表达量也发生了改变。Chen等^[13]对静息和活化状态下HSC中的miRNA进行比较发现,与静息的HSC相比,活化的HSC中有31种miRNA表达发生显著改变,其中17种miRNA在HSC活化过程中表达增加,分别是miR-221、miR-301a、miR-222、miR-193、miR-31、miR-143、miR-345-5p、miR-152、miR-199a-5p、miR-218、miR-125b-5p、miR-214、miR-34c、miR-34b、miR-199a-5p、miR-425和miR-145;14种miRNA表达下调,分别是miR-101a、miR-335、miR-150、miR-126-5p、miR-126-3p、miR-877、miR-139-5p、miR-192、miR-450a、miR-497、miR-338、miR-10a-5p、miR-378和miR-195。

除在肝星状细胞活化过程中对miRNA表达量改变进行分析,也有学者在肝纤维化组织中进行了分析。Roderburg等^[14]通过微阵列分析发现,与对照组相比,小鼠肝纤维化模型组有31种miRNA表达出现显著改变($P < 0.01$)。其中10种miRNA表达上调,分别是miR-199a-5p、miR-302c、miR-199a-3p、miR-223、miR-199b、miR-125b-5p、miR-221、miR-34c、miR-24和miR-670;21种miRNA表达下调,分别是miR-29b、miR-29a、miR-101a、miR-30c、miR-434-3p、miR-183、miR-22、miR-340-5p、miR-206、miR-877、miR-705、miR-714、miR-341、miR-29c、miR-30b、miR-365、miR-677、

miR-96、miR-148a、miR-704和miR-193。

同样,在乙型肝炎肝纤维化患者中,随着纤维化进展,miRNA表达水平也发生改变。Zhang等^[15]用微阵列分析了50例慢性乙型肝炎患者不同肝纤维化分期外周血miRNA的表达情况,发现与S0期相比,在S1~S4期表达量改变超过2倍以上的miRNA共140种。其中S1期有28种上调,20种下调;S2期有49种上调,48种下调;S3期有57种上调,27种下调;S4期有40种上调,16种下调。总体分析发现,有12种miRNA在各期的表达量改变均在2倍以上,其中上调的有10种,分别是miR-2861、miR-235-3p、miR-3620-3p、miR-3656、miR-371a-5p、miR-4646-5p、miR-4651、miR-4695-5p、miR-4800-5p和miR-638;下调的2种为miR-497-5p和miR-486-3p。在肝纤维化过程中这些miRNA的具体作用机制将从以下几个方面进行介绍。

2.1 miRNA的促纤维化作用 miRNA的异常表达可能通过改变HSC相关的信号转导通路而在肝纤维化过程中发挥作用。肝纤维化过程中上调的miRNA的抑制剂有可能作为抗纤维化的新型药物。Li等^[16]发现miR-33a在肝纤维化患者中显著高表达,miR-33a靶向于过氧化物酶体增殖激活受体 α (peroxisome proliferator activated receptors, PPAR- α)来促进HSC的增殖、转化以及细胞外基质的产生;另一研究发现,转化生长因子 $\beta 1$ (transforming growth factor $\beta 1$, TGF- $\beta 1$)可诱导miR-33a的表达,同时miR-33a通过靶作用于Smad7又可反过来刺激由TGF- $\beta 1$ 诱导的肝星状细胞的活化^[17];而miR-33a抑制剂能够显著抑制转化生长因子 β (TGF- β)介导的I型胶原的表达及蛋白激酶B (protein kinase B, PKB, Akt)的磷酸化。另有研究显示,与健康对照组相比,肝纤维化患者血清中miR-125-5p的表达水平显著增加^[18]。同时,Zheng等^[19]在小鼠肝纤维化模型中发现,miR-125-5p可靶作用于低氧诱导因子-1 (hypoxia inducible factor-1, HIF-1),进而促进HSC的活化和增殖。

此外,miR-21在活化的HSC、肝纤维化模型肝组织和血清中的表达均上调,进一步研究发现,miR-21通过调控其直接靶点快速发育生长因子同源蛋白2 (sprouty homolog 2, SPRY2),提高细胞外调节蛋白激酶 (extracellular regulated protein kinases, ERK) 通路活性,进而促进HSC的生物学活性^[20]。Ji等^[21]研究发现,在一定程度上,miR-27a和miR-27b通过影响类维生素X受体 α (retinoid X receptor α , RXR α)的表达,促进细胞DNA的合成

以及HSC的分化。Hu等^[22]通过基因芯片和定量PCR发现,在大鼠、小鼠以及人的原代HSC中,miR-31的表达随HSC的活化而升高。荧光素酶报告基因和Western实验发现miR-31通过靶作用于缺氧诱导因子(hypoxia-inducible factor, FIH-1)调节HSC的活化及迁移。这些研究提示miR-31/FIH1信号转导通路与肝纤维化紧密相连,可能通过参与TGF- β /Smad3信号转导通路对肝星状细胞进行调控。miR-214在肝星状细胞活化过程中、大鼠肝纤维化模型、HBV相关肝纤维化患者肝组织标本以及TGF- β 刺激后的肝星状细胞中表达显著升高,并与肝纤维化严重程度呈正相关,miR-214可能通过下调人缺氧诱导因子-1 α 抑制剂(human hypoxia-inducible factor-1 alpha inhibit, HIF1AN)的表达促进肝星状细胞的活化和迁移^[23]。此外,还有一些miRNA通过负性调控作用下调肝纤维化抑制因子而发挥促肝纤维化作用。

2.2 miRNA的抗纤维化作用 在肝纤维化过程中,某些miRNA的表达下调,且其类似物具有一定的抗纤维化作用。越来越多的数据表明,miR-29在肝纤维化调控中发挥着重要作用,有可能作为监测肝纤维化的生物化学指标。

关于miR-29的作用机制,有研究发现miR-29在人和小鼠的肝纤维化组织以及活化的HSC中显著下调,mi-29b类似物导入小鼠体内后能够抑制CCl₄所致的小鼠肝纤维化,并伴随着 α -平滑肌肌动蛋白(α -smooth muscle actin, α -SMA)、I型胶原和金属蛋白酶组织抑制剂(tissue inhibitor of metalloproteinase, TIMP-1)表达量的下调。miR-29在活化的HSC中过表达,能够通过负性调节细胞周期蛋白D1(cyclinD1)和p21的表达将细胞周

期阻滞在G₁期,进而抑制细胞的生长和分化。此外,miR-29能够诱导caspase-9和核糖聚合酶(poly ADP-ribose polymerase, PARP)介导的HSC凋亡。miR-29通过与下游效应器磷脂酰肌醇-3激酶受体1(phosphoinositide kinases receptor 1, PIK3R1)以及AKT1的3'-UTR结合抑制PIK3/AKT通路,从而抑制HSC的活化、诱导HSC凋亡,最终起到抑制肝纤维化的作用^[24]。Kwiecinski等^[25]研究发现,在HSC中过表达miR-29类似物可抑制胰岛素样生长因子(insulin-like growth factors, IGF-1)和血小板源性生长因子C(platelet derived growth factor, PDGF-C)的表达,进而抑制HSC的增殖和分化。TGF- β 能够阻断TGF- β 信号转导通路,从而有效抑制肝纤维化。Tu等^[26]证实miR-101能够靶作用于TGF- β 受体1(TGF β receptor 1, T β R1)及T β R1转录激活因子Kruppel-样因子6(Kruppel-like factor 6, KLF6),进而抑制TGF- β 信号转导通路。在TGF- β 1诱导的HSC活化过程中,miR-146a的表达量降低。miR-146a通过靶作用于Smad4,调节TGF- β 1诱导的HSC的分化。过表达的miR-146a能够减少 α -SMA的表达^[27]。Li等^[28]发现,在小鼠肝纤维化模型中miR-483的表达量降低,在HSC中过表达miR-483-5p和miR-483-3p的前体可以抑制血小板源性生长因子 β (platelet derived growth factor, PDGF- β)和组织金属蛋白酶抑制剂2(tissue inhibitor of metalloproteinase 2, TIMP2)的表达水平,进而抑制TGF- β 引起的肝星状细胞活化及抑制肝纤维化。在肝纤维化过程中,这些miRNA发挥其负性调控作用,可以通过下调肝纤维化促进因子而发挥抗肝纤维化作用。目前已发现的在肝纤维化过程中发生变化的miRNA见表1。

表1 已发现的肝纤维化过程中发生变化的miRNA

miRNA	表达	靶基因	miRNA	表达	靶基因
miR-21	上调	SPRY2 ^[20]	miR-17-5p	上调	Smad7 ^[29]
miR-27	上调	RXR α ^[21]	miR-214	上调	Twist-1 ^[23]
miR-31	上调	FIH1 ^[22]	miR-15b/16	下调	Bcl2 ^[11]
miR-33a	上调	PPAR- α ^[16]	miR-29b	下调	PI3K/AKT ^[24]
miR-30c/193	上调	SNAIL ^[30]	miR-101	下调	T β R1 ^[26]
miR-125-5p	上调	HIF1 ^[19]	miR-145	下调	ZEB2 ^[31]
miR-126	上调	NF- κ B ^[32]	miR-146	下调	Smad4,wnt ^[27, 33]
miR-133a	上调	_[34]	miR-150	下调	c-myb ^[35]
miR-199/200	上调	_[36]	miR-194	下调	rac1 ^[35]
miR-221/222	上调	CDKN1B ^[37]	miR-483	下调	PDGF- β , TIMP2 ^[28]

注:“-”为文中未描述

综上, 血浆miRNA种类极多、作用复杂, 某些miRNA可能是极具应用前景的评价肝纤维化的生物标志物。目前越来越多的学者也致力于这方面的研究。随着广泛、大样本的临床验证, 对miRNA与肝纤维化相关性的认识将更加深入。

参考文献

- [1] Egresi A, Lengyel G, Hagymási K. Options for non-invasive assessment of liver fibrosis based on clinical data[J]. *Orv Hetil*, 2015, 156(2):43-52.
- [2] Rockey DC, Caldwell SH, Goodman ZD, et al. Liver biopsy[J]. *Hepatology*, 2009, 49(3):1017-1044.
- [3] Mani H, Kleiner DE. Liver biopsy findings in chronic hepatitis B[J]. *Hepatology*, 2009, 49(5 Suppl):S61-S71.
- [4] Piccinino F, Sagnelli E, Pasquale G, et al. Complications following percutaneous liver biopsy. A multicentre retrospective study on 68,276 biopsies[J]. *J Hepatol*, 1986, 2(2):165-173.
- [5] Montalto G, Soresi M, Carroccio A, et al. Percutaneous liver biopsy: a safe outpatient procedure? [J]. *Digestion*, 2001, 63(1):55-60.
- [6] CHEN X, BA Y, MA L, et al. Characterization of microRNA in serum: a novel class of biomarkers for diagnosis of cancer and other diseases[J]. *Cell Res*, 2008, 18(10):997-1006.
- [7] Kitab B, Alj HS, Ezzikouri S, et al. MicroRNA as important players in host-hepatitis B virus interactions[J]. *J Clin Transl Hepatol*, 2015, 3(2):149-161.
- [8] Sarkar N, Chakravarty R. Hepatitis B virus infection, microRNA and liver disease[J]. *Int J Mol Sci*, 2015, 16(8):17746-17762.
- [9] Tacke F, Weiskirchen R. Update on hepatic stellate cells: pathogenic role in liver fibrosis and novel isolation techniques[J]. *Expert Rev Gastroenterol Hepatol*, 2012, 6(1):67-80.
- [10] HE Y, HUANG C, ZHANG SP, et al. The potential of microRNA in liver fibrosis[J]. *Cell Signal*, 2012, 24(12):2268-2272.
- [11] GUO C, PAN Q, LI D, et al. miR-15b and miR-16 are implicated in activation of the rat hepatic stellate cell: An essential role for apoptosis[J]. *J Hepatol*, 2009, 50(4):766-778.
- [12] Maubach G, Lim MC, Chen J, et al. miRNA studies in vitro and in vivo activated hepatic stellate cells[J]. *World J Gastroenterol*, 2011, 17(22):2748-2773.
- [13] CHEN C, WU CQ, ZHANG ZQ, et al. Loss of expression of miR-335 is implicated in hepatic stellate cell migration and activation[J]. *Exp Cell Res*, 2011, 317(12):1714-1725.
- [14] Roderburg C, Urban GW, Bettermann K, et al. Micro-RNA profiling reveals a role for miR-29 in human and murine liver fibrosis[J]. *Hepatology*, 2011, 53(1):209-218.
- [15] ZHANG Q, XU M, QU Y, et al. Analysis of the differential expression of circulating microRNA during the progression of hepatic fibrosis in patients with chronic hepatitis B virus infection[J]. *Mol Med Rep*, 2015, 12(4):5647-5654.
- [16] LI ZJ, OU-YANG PH, HAN XP. Profibrotic effect of miR-33a with Akt activation in hepatic stellate cells[J]. *Cell Signal*, 2014, 26(1):141-148.
- [17] HUANG CF, SUN CC, ZHAO F, et al. miR-33a levels in hepatic and serum after chronic HBV-induced fibrosis[J]. *J Gastroenterol*, 2015, 50(4):480-490.
- [18] ZHENG J, ZHOU Z, XU Z, et al. Serum microRNA-125a-5p, a useful biomarker in liver diseases, correlates with disease progression[J]. *Mol Med Rep*, 2015, 12(1):1584-1590.
- [19] LI G, LI J, LI C, et al. MicroRNA-125a-5p Contributes to Hepatic Stellate Cell Activation through Targeting FIH1[J]. *Cell Physiol Biochem*, 2016, 38(4):1544-1552.
- [20] ZHAO J, TANG N, WU K, et al. MiR-21 simultaneously regulates ERK1 signaling in HSC activation and hepatocyte EMT in hepatic fibrosis[J]. *PLoS One*, 2014, 9(10):e108005.
- [21] JI J, ZHANG J, HUANG G, et al. Over-expressed microRNA-27a and 27b influence fat accumulation and cell proliferation during rat hepatic stellate cell activation[J]. *FEBS Lett*, 2009, 583(4):759-766.
- [22] HU J, CHEN C, LIU Q, et al. The role of the miR-31/FIH1 pathway in TGF-beta-induced liver fibrosis[J]. *Clin Sci (Lond)*, 2015, 129(4):305-317.
- [23] Iizuka M, Ogawa T, Enomoto M, et al. Induction of microRNA-214-5p in human and rodent liver fibrosis[J]. *Fibrogenesis Tissue Repair*, 2012, 5(1):12.
- [24] WANG J, CHU ES, CHEN HY, et al. microRNA-29b prevents liver fibrosis by attenuating hepatic stellate cell activation and inducing apoptosis through targeting PI3K/AKT pathway[J]. *Oncotarget*, 2015, 6(9):7325-7338.
- [25] Kwiecinski M, Elfimova N, Noetel A, et al. Expression of platelet-derived growth factor-C and insulin-like growth factor I in hepatic stellate cells is inhibited by miR-29[J]. *Lab Invest*, 2012, 92(7):978-987.
- [26] TU X, ZHANG H, ZHANG J, et al. MicroRNA-101 suppresses liver fibrosis by targeting the TGFβ signalling pathway[J]. *J Pathol*, 2014, 234(1):46-59.
- [27] HE Y, HUANG C, SUN X, et al. MicroRNA-146a modulates TGF-beta1-induced hepatic stellate cell proliferation by targeting SMAD4[J]. *Cell Signal*, 2012, 24(10):1923-1930.
- [28] LI F, MA N, ZHAO R, et al. Overexpression of miR-483-5p/3p cooperate to inhibit mouse liver fibrosis by suppressing the TGF-β stimulated HSCs in transgenic mice[J]. *J Cell Mol Med*, 2014, 18(6):966-974.
- [29] YU F, GUO Y, CHEN B, et al. MicroRNA-17-5p activates hepatic stellate cells through targeting of Smad7[J]. *Lab Invest*, 2015, 95(7):781-789.
- [30] Roy S, Benz F, Vargas Cardenas D, et al. miR-30c and miR-193 are a part of the TGF-beta-dependent regulatory network controlling extracellular matrix genes in liver fibrosis[J]. *J Dig Dis*, 2015, 16(9):513-524.
- [31] ZHOU DD, WANG X, WANG Y, et al. MicroRNA-145 inhibits hepatic stellate cell activation and proliferation by targeting ZEB2 through Wnt/beta-catenin pathway[J]. *Mol Immunol*, 2016, 75:151-160.
- [32] FENG X, TAN W, CHENG S, et al. Upregulation of microRNA-126 in hepatic stellate cells may affect pathogenesis of liver fibrosis through the NF-κB pathway[J]. *DNA Cell Biol*, 2015, 34(7):470-480.
- [33] DU J, NIU X, WANG Y, et al. MiR-146a-5p suppresses activation and proliferation of hepatic stellate cells in nonalcoholic fibrosing steatohepatitis through directly targeting Wnt1 and Wnt5a[J]. *Sci Rep*, 2015, 5:16163.
- [34] Roderburg C, Luedde M, Vargas Cardenas D, et al. miR-133a mediates TGF-beta-dependent derepression of collagen synthesis in hepatic stellate cells during liver fibrosis[J]. *J Hepatol*, 2013, 58(4):736-742.
- [35] Venugopal SK, Jiang J, Kim TH, et al. Liver fibrosis causes downregulation of miRNA-150 and miRNA-194 in hepatic stellate cells, and their overexpression causes decreased stellate cell activation[J]. *Am J Physiol Gastrointest Liver Physiol*, 2009, 298(1):G101-G106.
- [36] Murakami Y, Toyoda H, Tanaka M, et al. The Progression of Liver Fibrosis Is Related with Overexpression of the miR-199 and 200 Families[J]. *Plos One*, 2011, 6(1):e16081.
- [37] Ogawa T, Enomoto M, Fujii H, et al. MicroRNA-221/222 upregulation indicates the activation of stellate cells and the progression of liver fibrosis[J]. *Gut*, 2012, 61(11):1600-1609.

收稿日期: 2016-09-05