miR-21及其在肝脏疾病中的研究进展

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摘要 微小RNA(microRNA, miRNA)是约22个核苷酸的RNA, 其通过结合信使RNA(messenger RNA, mRNA), 在转录后水平调节基因表达。因此, miRNA的失调广泛地影响着细胞过程, 包括细胞增殖和分化。miR-21在几乎所有类型的癌症中上调, 且促进癌细胞增殖, 迁移和存活, 因此被归类为致癌性miRNA(oncogenes miRNA, onco-miR)。miR-21在肝细胞癌(hepatocellular carcinoma, HCC)、非酒精性脂肪性肝病(non-alcoholic fatty liver disease, NAFLD)和肝纤维化的发生中都发挥着重要作用。该文就miR-21在肝脏疾病中的最新进展作一总结, 以期更好应用于临床诊断和治疗。

关键词 miR-21; 肝细胞癌; 肝纤维化; 非酒精性脂肪性肝病

The Research Development of miR-21 in Liver Diseases

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Abstract MicroRNAs are about 22 nucleotide that regulate gene expression at the post-transcriptional level by binding messenger RNA transcripts. Dysregulation of miRNAs affects a wide range of cellular processes including cell proliferation and differentiation. It is up-regulated in almost all types of cancers, promotes cancer cell proliferation, migration, and survival and therefore was classified as an onco-miR.It plays an important role in the occurrence of hepatocellular carcinoma (HCC), non-alcoholic fatty liver disease (NAFLD) and liver fibrosis. This article summarizes the latest developments in miR-21 in liver diseases in order to better apply to clinical diagnosis and treatment.

Keywords miR-21; hepatocellular carcinoma; liver fibrosis; non-alcoholic fatty liver disease

在过去的研究中, 人们发现非编码RNA(non-coding RNA, ncRNA)在调节肿瘤的发展和生长中起着关键作用。ncRNA可以分为两类: 短链和长链非编码RNA。其中miRNA属于短链非编码RNA分子, 其通过靶向各种转录物的3′-非翻译区(3′-untranslated region, 3′-UTR)来影响mRNA稳定性与翻译, 从而影响总蛋白的水平[1]。据估计, 每种miRNA可以靶向几个到几百个mRNA, 有大约30%的mRNA受到miRNA的调节, 控制着至少30%的蛋白质的表达[2]。miR-21在乳腺癌, 肺癌和宫颈癌以及肝细胞癌(hepatocellular carcinoma, HCC)中表达水平均上调[3]。HCC作为世界上最常见的癌症之一, 其死亡率在中国癌症死亡率中排名第二[4]。因此确定新的生物标志物对于早期诊断和预测HCC预后至关重要。

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在其他非癌性肝脏疾病中, 如非酒精性脂肪性肝病(non-alcoholic fatty liver disease, NAFLD), 其作为全球主要的慢性肝功能障碍疾病, 患病率随着肥胖, 糖尿病和代谢综合征的流行而增加, 然而目前仍缺乏在不引起严重组织损伤的情况下去除脂肪肝细胞的治疗策略[5], 通过研究miR-21在其中的发生机制可能为治疗NAFLD寻找到新的治疗策略。此外, miR-21在肝纤维化、肝炎等肝脏疾病中均有研究。因此, 我们可以大胆预测, miR-21参与着肝脏疾病的发生发展, 且在其中起着不可或缺的作用。

1 miR-21简介

在大鼠和小鼠中, *rno-mir-21*基因分别位于染色体10和21上, 而在人类中, 它位于染色体17q23.2上。miRNA-21基因的转录产物由位于重叠蛋白编码基因内含子的保守启动子转录[6]。在许多种类的细胞中, miR-21都起着抗凋亡和促生长的作用[7]。它的高表达常常是癌细胞的特征, 并且反映病理细胞的生长或细胞应激。例如, rno-mir-21在心脏肥大和血管内膜损伤的实验小鼠中表达量上调[8]。miR-21的诱导又与细胞去分化相关, 这些研究可能证明低水平的miR-21暂时性的促进细胞分化和生长, 而高水平的miR-21可能发挥致癌作用。在免疫系统中, 已经发现miR-21调节T细胞免疫[9]。在正常生理情况下, T辅助细胞(helper thyroid cell, Th cell)1和Th2细胞通过相互调节彼此的分化和功能而以相对稳定的状态存在, miR-21在活化的树突细胞中被诱导并直接靶向促进Th1的mRNA的p35亚单位以编码白细胞介素-12(interleukin-12; IL-12), 并且已经发现在miR-21缺陷小鼠中, 树突细胞分泌IL-12的量增加[10]。Lu等[11]已经发现了在miR-21影响下Th1生长加强。除了树突细胞来源的miR-21在免疫系统中发挥作用外, Murugaiyan等[12]发现T细胞源性的miR-21抑制Sprouty同源物(Sprouty homolog, Spry1)的表达从而促进Th2分化, Spry1是一种丝裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK)途径的抑制剂。此外, 已经发现miR-21在狼疮患者的$CD4^+$ T细胞以及狼疮倾向的狼疮小鼠中过表达, 表明与自身免疫疾病有很强的相关性[10]。

2 miR-21的靶基因

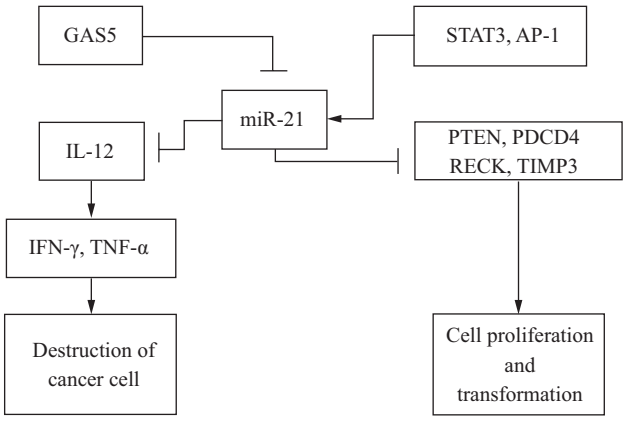
miR-21的靶基因相对较少, 包括磷酸酶张力蛋白同源物基因(phosphatase and tensin homology deleted on chromosome ten, *PTEN*)、程序性细胞死亡蛋白4(programmed cell death protein 4, PDCD4)、原肌球蛋白1(tropomyosin1, TPM1)、伴有Kazal域的富含半胱氨酸的逆转诱导蛋白(reversion-inducing-cysteine-rich protein with kazal motifs, RECK)、金属蛋白酶组织抑制剂(metalloproteinase inhibitor 3, TIMP3)、Fas配体(Fas ligand, FasL)等, 这些基因相同特点是对肿瘤有着抑制作用。例如, Gaudelot等[13]发现, 在结直肠癌中miR-21的表达水平上调, 其作用于靶基因*PTEN*, 使肿瘤产生对5-氟尿嘧啶(5-Fluorouracil, 5-FU)的耐药性, 从而促进肿瘤的增值, 侵袭和转移。Sekar等[14]发现, 在胃癌中miR-21通过靶基因*PDCD4*调节肿瘤发生发展。Kowshik等[15]发现, 在乳腺癌中miR-21使RECK下调, 从而促进肿瘤细胞血管生成、侵袭和迁移。Zhang等[16]同样发现, 在乳腺癌中miR-21靶向作用于TIMP3, 使TIMP3抑制, 从而促进肿瘤细胞的增殖。因此, miR-21在肿瘤中高表达特性及其靶基因可作为肿瘤诊断和治疗研究的分子靶标。

3 miR-21在肝脏疾病中的研究进展

3.1 miR-21与肝细胞癌

HCC是一种上皮源性的肿瘤, 通常发生于有肝病的患者[17]。目前, HCC的诊断主要依赖于影像和血清标志物如甲胎蛋白(alpha fetoprotein, AFP)。AFP同时也是目前中国最常用的HCC诊断生物标志物。然而, 由于缺乏肿瘤特异性和敏感性, AFP在检测肝癌方面存在一定的局限性[18]。因此, 迫切需要具有高特异性和灵敏度的新型生物标志物, 以便于早期阶段可准确地检测HCC。Sun等[19]研究发现, 与测量HCC患者血清和远端肝组织中的miR-21含量相比, 血清细胞外囊泡和肝癌组织中miR-21的表达水平显著增加, 因此联合测量血清细胞外囊泡和肝癌组织中miR-21和AFP可能有助于增强HCC的诊断。

Meng等[20]发现, miR-21可抑制肿瘤抑制因子如PTEN的表达水平。Yin等[21]发现, IL-12是miR-21的直接靶标, IL-12作为细胞因子具有抗肿瘤的治疗活性, 研究已经显示其抑制肿瘤发生并可诱导已形成的肿瘤的消退。而IL-12的主要作用是通过诱导NK细胞和T细胞的增殖从而促进对癌细胞的有效杀伤, 并增强细胞毒性T淋巴细胞(cytotoxic lymphocyte,



IFN-γ: 干扰素γ; TNF-α: 肿瘤坏死因子α。⊥: 抑制; ↓: 促进。
IFN-γ: Interferon γ; TNF-α: tumor necrosis factor α. ⊥: inhibition; ↓: promotion.

图1 miR-21在肝细胞癌中的主要作用机制

Fig.1 The main mechanism of miR-21 in hepatocellular carcinoma

CTL)的产生和活性[22]。Hu等[23]的研究发现, miR-21是HCC中生长停滞特异性5(growth arrest-specific transcript 5, GAS5)的直接靶标, GAS5表达下调可使miR-21在HCC组织中上调。Koenig等[24]发现, 炎性细胞因子通过信号传导与转录激活因子3(signal transducer and activator of transcription 3, STAT3)和激活子蛋白-1(activator protein, AP-1)刺激miR-21的转录, miR-21反过来直接靶向抑制肿瘤抑制因子如PTEN、PDCD4、RECK和TIMP3。Zhang等[25]证明, miR-21维持着$CD24^+$祖细胞的存活以及祖细胞与癌细胞之间的相互作用。此外, miR-21的高表达并不是所有HCC患者预后不良的监测因子[25]。总之, 大部分研究表明, 抗miR-21可靶向作用于肿瘤起始细胞以及肿瘤微环境, 因此, 抗miR-21用于HCC预防和治疗, 具有很大的空间(图1)。

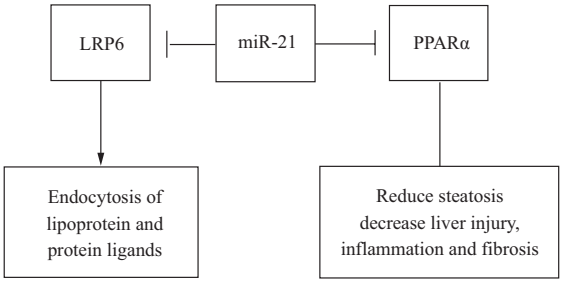
3.2 miR-21与NAFLD

NAFLD是一种复杂的临床病理综合征, 其特征在于肝脏中脂肪的过度积累。包括简单的脂肪变性、非酒精性脂肪性肝炎及与其相关的肝硬化[26]。最新研究表明, miR-21与NAFLD的发展有密切的关系, 并且NAFLD的发生发展与循环miR-21、miR-34a、miR-122和miR-451之间的关联均已被证实[27]。LDL受体基因家族编码至少13种结构相关的细胞表面受体, 这些受体在不同的器官, 组织和细胞类型中发挥不同的生物学功能[28]。低密度脂蛋白(low-density lipoprotein, LDL)受体是跨膜细胞表面蛋白, 参与受体介导的脂蛋白和蛋白质配体的内吞作用。而低密度脂蛋白受体相关蛋白6(low-density lipoprotein receptor-related protein 6, LRP6)则是Wnt信号途径的受体, 在经典的Wnt/β-连环蛋白信号途径中参与LDL的内吞作用[29]。Li等[5]发现, miR-21抑制$HepG_2$细胞中LRP6的表达水平, 从而诱导脂质产生。同时, Loyer等[30]实验发现抑制miR-21可恢复过氧化物酶体增殖剂激活受体α(peroxisome proliferator-activated receptor α, PPARα)的活性, 从而明显减少脂肪变性。Benhamouche-Trouillet等[31]发现, 肝脏中miR-21的降低可明显改善葡萄糖耐量、胰岛素敏感性及预防肝脏脂肪变性和脂肪酸摄取情况, miR-21可能是以细胞特异性方式参与NAFLD进展的各种步骤,如早期肝细胞中脂质积聚和脂肪变性, 或后期非实质细胞中的炎症和纤维化。因此, miR-21可能成为诊断和治疗NAFLD的有用生物标志物, 抑制miR-21可能是NAFLD未来的治疗策略(图2)。

3.3 miR-21与肝纤维化

肝纤维化是肝脏的慢性损伤过程, 其特征在于肝星状细胞(hepatic stellate cell, HSC)的活化, 细胞外基质的过度积累和肝结构的扭曲[32]。肝纤维化是进一步发展为肝硬化, 肝功能衰竭和肝细胞癌的重要环节[33-34]。流行病学表明, 发达国家约45%的死亡是由纤维化疾病引起的[35]。近年来, 尽管许多重要研究使我们对肝纤维化有了更好的了解, 但尚未发现任何药物对肝纤维化具有明确的作用。因此, 迫切需要寻找和开发能够阻止肝纤维化进展的有效且耐受良好的药物。

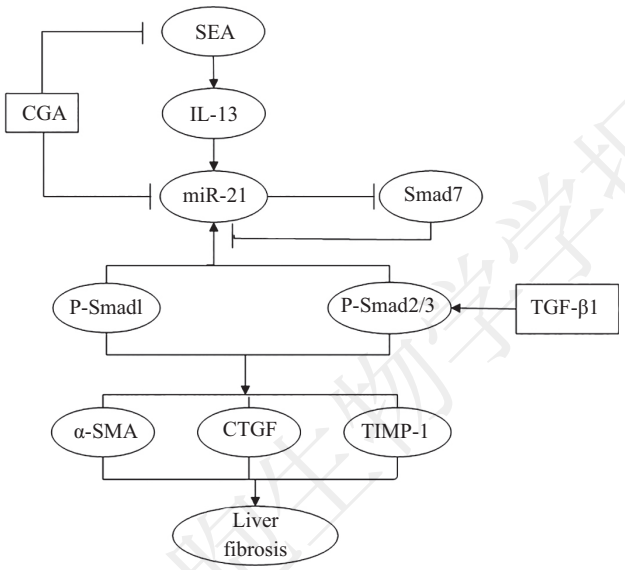
在肝纤维化过程中, 转化生长因子β1(transforming growth factor-β1, TGF-β1)是主要促纤维化细胞因子[36]。



⊥: inhibition; ↓: promotion.

图2 miR-21在非酒精性脂肪性肝病中的主要作用机制

Fig.2 The main mechanism of miR-21 in NAFLD



α-SMA: α平滑肌肌动蛋白; CTGF: 结缔组织生长因子。⊥: 抑制; ↓: 促进。

α-SMA: α Smooth Muscle Actin; CTGF: connective tissue growth factor. ⊥: inhibition; ↓: promotion.

图3 miR-21在肝纤维化中的主要作用机制(根据参考文献[36-40]修改)

Fig.3 The main mechanism of miR-21 in liver fibrosis (modified from reference[36-40])

TGF-β-Smad通路是其重要的信号转导通路, 在该信号通路中, TGF-β1可以激活其下游信号通路Smad 2/3, 通过与HSC上的受体结合来介导纤维化。而miR-21通过Smad2/3磷酸化正调节胶原蛋白的产生, 同时又可被Smad7负反馈调节[37]。与TGF-β作为起始信号不同的是, 在由血吸虫病诱导的肝纤维化的研究中, Chuah等[38]发现, 肝纤维化主要由可溶性虫卵抗原(soluble egg antigen, SEA)引起, SEA诱导产生白细胞介素13(interleukin 13, IL-13), 而不是TGF-β作为起始信号。在Wu等[39]的研究中, 检测健康个体和肝纤维化不同阶段患者的血清miR-21水平, 同时检测正常或肝纤维化人肝组织的miR-21水平, 结果显示, 肝纤维化患者的miR-21水平升高与肝纤维化的严重程度和活动度有关。miR-21可以作为一个重要标准物来诊断肝纤维化及其预后, 也可以作为治疗的靶点来缓解与治疗肝纤维化, 比如Yang等[40]研究发现绿原酸(chlorogenic acid, CGA)可通过直接抑制miR-21来调节TGF-β1/Smad7信号通路来抑制肝纤维化(图3)。

3.4 其他

最近的研究表明, 在乙肝病毒性肝炎中, HBV基因组由部分双链DNA构成, 其编码HBV核心, 逆转录酶/聚合酶, 包膜和乙型肝炎病毒调节蛋白(Hepatitis B virus X protein, HBx)[41], 其中HBx是人肝细胞中HBV复制必不可少的。HBx通过激活细胞信号转导途径以调节转录, 增殖和凋亡过程。HBx可导致miRNA的差异表达, 如miR-520[27]、miR-145、miR-222、miR-21[43]和miR-146[44]。其中miRNA-21基因是由HBx诱导的非常重要的miRNA基因, 其促进细胞增殖[45]和转化[46], 但相关机制尚未完全阐明。

同样在丙肝肝炎(hepatitis C virus, HCV)中, Chen等[47]发现, miR-21在HCV阳性肝活检样品中过表达, 其机制可能是与miR-21启动子中的AP-1结合, 导致髓样分化因子(myeloid differentiation factor88, MyD 88)和白细胞介素-1受体相关激酶1(IL-1 receptor associated kinase 1, IRAK1)的沉默, 从而引起病毒复制。

4 展望

miR-21在众多的肝脏疾病的发生中担任着重要的角色, 可用于肝脏疾病的诊断和治疗, 具有重要的临床价值。miRNA的调控网络非常复杂, miR-21在具体疾病中的作用机制也尚不完善, 但我们可以预见, 随着对miR-21研究的不断加深, miR-21在疾病中的具体调控机制将被慢慢发现, 其作为肝脏疾病的新型治疗靶标有着远大的前景。

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