肌细胞特异性microRNAs生物学效应研究进展

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摘要 microRNAs(miRNAs)是一类含有20~22个核苷酸的非编码单链小分子RNA, 发挥转录后水平负调控基因表达和翻译的作用, 具有生物学功能多样性, 可作为多种疾病诊断和预后重要分子标志。该文介绍了肌细胞特异性miRNAs, 如miR-1、miR-133、miR-145、miR-206基因等染色体分布、序列、组织表达丰度、主要通路, 并对肌细胞特异性miRNAs在气管平滑肌、血管平滑肌、心肌等细胞中的生物学效应研究进展进行综述。

关键词 肌细胞特异性microRNAs; 生物学效应; 气管平滑肌; 血管平滑肌; 心肌; 骨骼肌

The Progress on Biological Effect of Muscle-specific MicroRNAs

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Abstract miRNAs are a class of small non-coding, single stranded tiny molecule RNA (containing 20-22 nucleotides) that negatively regulates gene expression and translation at post-translational level. They have diverse biological functions and can be used as significant molecule markers for disease diagnosis and prognosis. In this review, muscle-specific miRNAs were introduced, such as the distribution of chromosome, sequence, tissue expression abundance and main pathway of miR-1, miR-133, miR-145 and miR-206. Their biological effects in airway smooth muscle, vascular smooth muscle and cardiac muscle were also discussed.

Keywords muscle-specific microRNA; biological effect; airway smooth muscle; vascular smooth muscle; cardiac muscle; skeletal muscle

microRNAs(miRNAs)是一类非编码单链小分子RNA, 长度约为20~22个核苷酸, 广泛存在于真核细胞中, 发挥转录后水平负调控基因的表达和翻译的作用[1-2]。自1993年在线虫中发现第一个命名为lin-4的miRNA以来[3], 目前研究人员在植物、动物、昆虫以及病毒等206个生物物种中陆续发现了超过28 645种miRNAs(数据更新自miRBase Release 21: 2014.6, http://www.mirbase.org)[4], 从而形成一个庞大的基因调控家族, 其中人类有1 881条, 小鼠有1 193条, 大鼠有495条。

多数miRNAs是通过Drosha和Dicer两种RNA酶III加工后形成。Drosha将原始长链转录本加工成含有60~70个碱基的茎环结构前体(包括相反的两个臂“−3p”和“−5p”), 然后由Dicer将其剪切为成熟的miRNAs[5-6]。miRNAs与Argonaute蛋白质结合形成复合体, 通过不完全配对靶向信使RNA(mRNA)从

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而阻遏其翻译[7]。作为模板的−3p臂和−5p臂碱基虽然来自同一个茎环结构前体, 但序列不同, 故形成的miRNAs序列也不同, 靶向mRNA也不同, 其具体表达量由物种、组织、不同生理病理状况决定[8-9]。

miRNAs在细胞增殖、分化、衰老、凋亡等生物学过程和肿瘤、心血管、呼吸系统等疾病发生发展过程中占据重要地位[10-11]。整体调控层面, miRNAs通过特异性降解或沉默mRNA, 可调控人类基因组中超过5 300个基因的表达[12], 达到人类基因总数的30%~60%[13-14]。个体调控层面, 单个miRNA在体内可与蛋白质形成核糖核酸蛋白复合体(protein-RNA complexes)[15], 通过与靶基因mRNA 3′端非编码区(3′-untranslated region, 3′UTR)完全或不完全互补配对结合, 封闭靶蛋白翻译并直接调控约200个目标基因[16]。在调控方式上, miRNAs存在不同的基因表达负调控形式, 除了最常见的转录本降解, 还有扣留(sequestering)、翻译抑制等[17], 并可能参与对基因表达正调控, 如转录和翻译活化作用[18]。研究证明, 异常miRNAs表达和众多人类疾病相关, 可作为诊断和预后的重要分子标志之一[19-20]。

1 肌细胞特异性miRNAs及染色体定位

miRNAs的分布具有组织特异性, 如肌细胞特异性miRNAs只针对肌细胞(包括平滑肌、心肌、骨骼肌)相关基因进行转录后表达调控, 影响肌细胞增殖及分化, 属于进化上保守的肌细胞调控系统[21]。目前研究最多且特异性较好的肌细胞特异性miRNAs包括miR-1、miR-133、miR-145、miR-206等。在亚型和染色体分布上, miR-1(miR-1-1、miR-1-2)含22个核苷酸, 其中miR-1-1基因分布在人20号染色体(20q13.33), miR-1-2基因分布在人18号染色体(18q11.2)[22]; miR-133包括133a和133b2种亚型[23], 其中miR-133a(miR-133a-1、miR-133a-2)含22个核苷酸, miR-133a-1基因分布在人18号染色体(18q11.2), miR-133a-2基因分布在人20号染色体(20q13.33), miR-133b共21个核苷酸, 其基因分布在人6号染色体(6p12.2)[22]。miR-145共22个核苷酸, 其基因分布于人5号染色体(5q32); miR-206共22个核苷酸, 其基因分布于人6号染色体(6p12.2)[24]。上述肌细胞特异性miRNAs序列信息见表1。

就肌细胞分布和生物学功能而论, miR-1主要分布于心肌、骨骼肌, 在肌细胞发育、分化上起关键作用[25-26]。miR-133分布于心肌、骨骼肌, 可调控肌细胞增殖、分化[27-28]。miR-145主要分布于血管平滑肌细胞, 参与平滑肌增殖、表型转化等生理病理学过程[29]。miR-206核苷酸序列和miR-1具有相同的种子区域(UGGAAUGU), 均属于miR-1家族, 两者在肌细胞调控方面有相似之处[30]。表2总结了Genecards

表1 人主要肌细胞特异性miRNAs基本信息

Table 1 The essential information of human major muscle-specific miRNAs

miRNAs	别名 Alternative name	染色体定位 Chromosomal assignment	宿主基因 Host gene	茎环核苷酸数 Number of stem-loop sequences	序列(5′→3′) Sequence (5′→3′)	登记号 Accession number
miR-1-1-3p	MIR1-1 Hsa-mir-1b	20: 62,554,303-62,554,379	*MIR1-1* host gene intron area	71	UGG AAU GUA AAG AAG UAU GUA U	MIMAT0000416
miR-1-2-3p	MIR1-2 Hsa-mir-1d	18: 21,828,996-21,829,106	*MIB1* gene intron area	85	UGG AAU GUA AAG AAG UAU GUA U	MIMAT0000416
miR-133a-1-3p	MIR133A1 Hsa-mir-133a-1	18: 21,825,698-21,825,785	*MIB1* gene intron area	88	UUU GGU CCC CUU CAA CCA GCU G	MIMAT0000427
miR-133a-2-3p	MIR133A2 Hsa-mir-133a-2	20: 62,564,912-62,565,013	*MIR1-1* host gene intron area	102	UUU GGU CCC CUU CAA CCA GCU G	MIMAT0000427
miR-133b-3p	MIR133B Hsa-mir-133b	6: 52,148,923-52,149,041	Intergenic region	119	UUU GGU CCC CUU CAA CCA GCU A	MIMAT0000770
miR-145-5p	MIR145 Hsa-mir-145	5: 149,430,646-149,430,733	*CARMN* gene intron area	88	GGA UUC CUG GAA AUA CUG UUC U	MIMAT0004601
miR-206-3p	MIR206 Hsa-mir-206	6: 52,144,331-52,144,442	Intergenic region	86	UGG AAU GUA AGG AAG UGU GUG G	MIMAT0000462

MIB1: mindbomb E3泛素蛋白连接酶1; CARMN: 心脏中胚层增强关联非编码RNA。

MIB1: mindbomb E3 ubiquitin protein ligase 1; CARMN: cardiac mesoderm enhancer-associated non-coding RNA.

表2 主要肌细胞特异性miRNAs下游靶基因和相关通路

Table 2 The downstream target genes and related pathways of major muscle-specific miRNAs

miRNAs	组织表达丰度(从高到低) Expression abundance (from high to low)	靶基因数目 Target genes number	通路数目 Pathway number	通路名称 Pathway name
miR-1	Heart, spleen, ovary, artery, colon	418	3	Heart development
				Transcription factors regulate miRs related to cardiac hypertrophy
				miRs in muscle cell differentiation
miR-133	Skeletal muscle, skin, ovary, prostate, cortex, tibial nerve, leukocyte	310	3	miRs in cardiomyocyte hypertrophy
				Transcription factors regulate miRs related to cardiac hypertrophy
				miRs in muscle cell differentiation
miR-145	Highly expressed inmotor system, nerve system, digestive system, endocrine system, etc	176	3	Heart development
				miRNAs involved in DNA damage response
				Serum response factor and miRs in Smooth muscle differentiation and proliferation
miR-206	Lung, skeletal muscle, artery	417	2	Glial cell differentiation
				miRs in musce cell differentiation

组织表达丰度、通路信息来源genecards: http://www.genecards.org; 预测靶基因数目来源miRBase: http://www.mirbase.org。

Tissue expression abundance and pathway source: genecards, http://www.genecards.org; Target genes number source: miRBase, http://www.mirbase.org.

和miRBase数据库中上述肌细胞特异性miRNAs组织表达丰度、预测靶基因数目和相关通路。

2 肌细胞特异性miRNAs在平滑肌细胞中的生物学效应

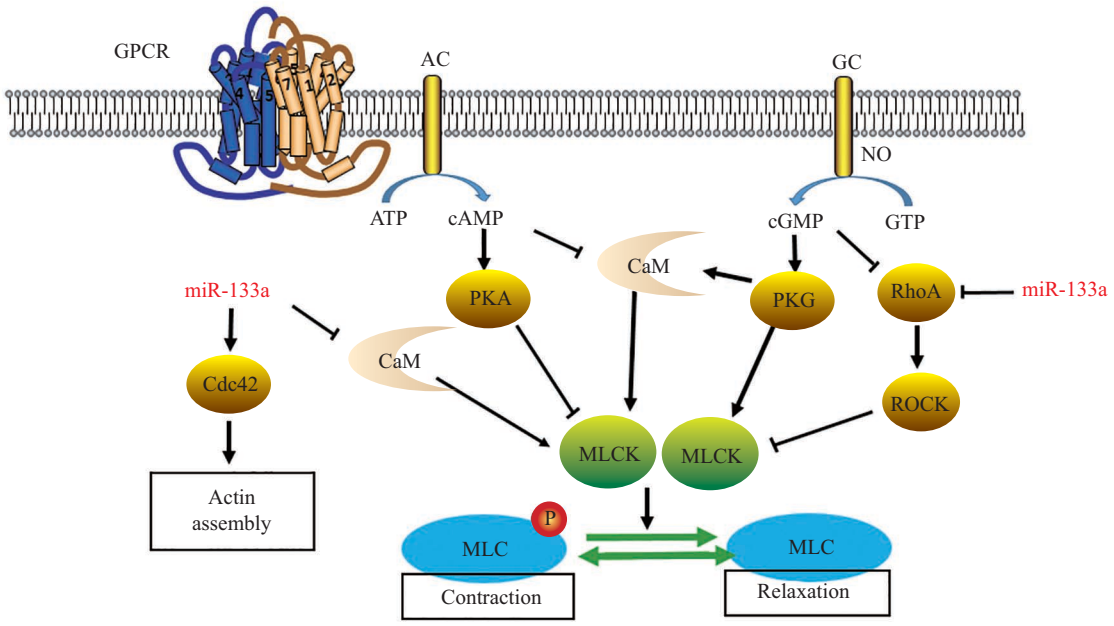
2.1 肌细胞特异性miRNAs在气管平滑肌细胞中的生物学效应和呼吸系统相关疾病

在调控气管平滑肌细胞收缩舒张效应方面, 研究发现, miR-133a能通过降低人气管平滑肌细胞中收缩相关基因*RhoA*表达, 减轻ROCK(Rho激酶)对肌球蛋白轻链磷酸酶(myosin light chain phosphatase, MLCP)抑制, 从而降低肌球蛋白轻链(myosin light chainn, MLC)磷酸化水平, 舒张气管平滑肌细胞[31], 这为哮喘治疗药物研发提供了全新的策略。在上述过程中, 白细胞介素-13(interleukin-13, IL-13)能在干预后第3、6 h显著降低气管平滑肌细胞miR-133a的表达且呈现剂量依赖关系(最佳剂量为100 ng/mL)[31]。miR-133下游靶基因除了RhoA之外, 还包括钙调蛋白(calmodulin, CaM)、细胞分裂周期蛋白42(cell division cycle protein 42, Cdc42)等[32]。图1总结了miR-133a对气管平滑肌细胞收缩舒张效应可能的调节机制。

与miR-133功能相反, miR-145可通过增加α肌动蛋白聚集促进气管平滑肌收缩[33]。干扰素β(interferon β, IFNβ)和IFNγ能显著增加miR-145在上述气管平滑肌收缩舒张生物学效应中的作用, 而表皮细胞生长因子(epidermal growth factor, EGF)单独或联合IFN应用均对miR-145表达无显著作用[33]。

在调控气管平滑肌细胞免疫、炎症效应方面, 研究发现, miR-145表达降低可显著抑制嗜酸粒细胞炎症、Th2相关细胞因子分泌和气道高反应性, 并可促进气管平滑肌细胞增殖和迁移, 调节细胞胞外基质蛋白及收缩蛋白表达[34]。其他miRNA对气管平滑肌细胞作用方面, miR-221可通过调节$p21^{WAF1}$和$p27^{kip1}$水平促进气管平滑肌细胞增殖及IL-6释放[35]; miR-25可通过靶向枯否样因子4(kruppel-like factor 4, KLF4)调节炎症介质T淋巴细胞活性调节蛋白(regulated on activation normal T cell expressed and secreted, RANTES)、嗜酸细胞活化趋化因子(eotaxin)和肿瘤坏死因子-α(tumor necrosis factor-α, TNF-α)表达, 从而参与气管平滑肌表型及炎症反应[36]。

疾病相关性方面, 因为能够负性调控气管平滑肌细胞中RhoA蛋白质表达, miR-133被认为和哮喘气道高反应性密切相关[37]。而同样能够靶向RhoA的miRNA还有miR-155、miR-31等[38-39], 后者可能同样在呼吸系统疾病中起关键作用。抑制miR-145也可缓解过敏性疾病的气道高反应性, 这是一种被认为可以相当于激素的新疗法[40]。选择性抑制miR-126可以缓解哮喘多种症状, 降低气道高反应性和黏



GPCR: G蛋白偶联受体; AC: 腺苷酸环化酶; GC: 鸟苷酸环化酶; cAMP: 环磷酸腺苷; cGMP: 环磷酸鸟苷; CaM: 钙调蛋白; PKA: 蛋白激酶A; PKG: 蛋白激酶G; ROCK: Rho激酶; RhoA: ras同源物基因家族成员A; MLC: 肌球蛋白轻链; MLCK: 肌球蛋白轻链激酶; MLCP: 肌球蛋白轻链磷酸酶; Cdc42: 细胞分裂周期蛋白42。

GPCR: G protein coupled receptor; AC: adenylate cyclase; GC: guanylate cyclase; cAMP: cyclic adenosine monophosphate; cGMP: cyclic guanosine monophosphate; CaM: calmodulin; PKA: protein kinase A; PKG: protein kinase G; ROCK: Rho kinase; RhoA: Ras homolog gene family A; MLC: myosin light chain; MLCK: myosin light chain kinase; MLCP: myosin light chain phosphatase; Cdc42: the cell division cycle protein 42.

图1　miR-133a对气管平滑肌细胞收缩舒张效应调节机制

Fig.1　The mechanism of miR-133a regulating the smooth muscle cell in airway

液分泌亢进, 是治疗过敏性哮喘潜在的抗炎治疗新方法[41]。此外, 肌细胞特异性miRNAs, 如miR-1、miR-133、miR-206, 在慢性阻塞性肺病(chronic obstructive pulmonary disease, COPD)患者血清中表达显著增高, 被认为可能和该病骨骼肌功能发育障碍密切相关[34]。

2.2　肌细胞特异性miRNAs在血管平滑肌细胞中的生物学效应和循环系统相关疾病

miRNAs在血管平滑肌增殖、分化及血管损伤修复等方面扮演重要角色[42]。研究发现, miR-133a在血管平滑肌细胞胞质中特异性表达(miR-133b不表达), 直接靶向转录因子SP-1(specificity protein-1)和膜突蛋白, 可用于治疗相关血管疾病[38]。miR-145在正常血管中高表达, 增生型血管中低表达, 可促进血管平滑肌收缩, 调节血管平滑肌分化[43], 如通过抑制转化生长因子-β(transforming growth factor-β, TGF-β)依赖的细胞外基质积聚和纤维化, 促进TGF-β诱导的血管平滑肌分化[39]。研究发现, 抑制miR-145表达能够产生类似L型钙通道表达降低的作用, 而上述效应能够被钙调蛋白激酶II(calmodulin kinase II, CaMK II)抑制剂KN93所阻断, 提示miR-145在血管平滑肌细胞收缩表型分化过程中起关键作用[44]。在小鼠肺动脉平滑肌细胞中, miR-206表达水平与右心室收缩压呈正相关, 下调miR-206能抑制平滑肌细胞凋亡、促进增殖, 并可通过Notch3蛋白调节细胞分化[45]。miR-21可以被骨形态发生蛋白4(bone morphogenetic protein 4, BMP4)激活, 下游作用于程序性细胞死亡因子4(programmed cell death factor 4, PDCD4)来调控血管平滑肌细胞收缩舒张表型[46]。

疾病相关性方面, miR-206被认为是潜在的治疗肺动脉高压(pulmonary hypertension, PAH)新方法之一, 可通过抑制肺动脉平滑肌细胞增殖并促进其凋亡导致血管中层壁变薄, 从而影响PAH病理生理学发展进程[45]。miR-145靶向治疗可明显改善患者动脉粥样硬化症状(atherosclerosis symptoms, AS), 其过表达可稳定斑块, 降低斑块炎症, 限制血管损伤反应中新生内膜形成[47]。

3　肌细胞特异性miRNAs在心肌细胞中的生物学效应

miR-1与miR-133在心肌细胞中特异性表达, 并在肌细胞诱导分化、增殖、凋亡等方面起重要作用[48]。研究发现, miR-1和miR-133等miRNAs能够在体外

直接诱导成纤维细胞重编码为心肌样细胞[49]。进一步实验证实, 含有上述miR-1和miR-133等多种miRNAs的复合物能够有效促进心肌样细胞重编程过程, 从而显著提高心脏功能。这是一种潜在的心肌损伤治疗方法[50]。在小鼠心肌肥大模型中, miR-133表达下降则出现钙调磷酸酶(calcineurin)活性增强, 体内给予钙调磷酸酶抑制剂可提高miR-133水平, 而胞内转染miR-133则能下调钙调磷酸酶表达, 提示两者在心肌肥大病理状态下存在相互抑制作用[51]。研究发现, 在miR-1-2敲除小鼠模型中出现房间隔缺损, 心律失常及肌细胞循环障碍等心脏功能异常病理表现, 提示仅敲除miR-1家族成员miR-1-2就可对心脏发育及修复产生显著影响[52]。研究发现, 高表达的miR-1能够通过调节亚基B56α, 抑制蛋白磷酸酶2A(protein phosphatase 2A, PP2A)生物学功能, 激活心肌细胞兰尼碱受体2(ryanodine receptor 2, RyR2), 促使肌浆网上自发的钙离子释放, 提高胞内钙离子浓度引起细胞收缩[53]。研究发现, miR-133过表达能直接抑制β_1肾上腺素能受体, 通过cAMP-PKA通路, 降低胱冬肽酶-3(caspase-3)表达, 减少心肌细胞凋亡, 最终对心肌起保护作用[54]。

疾病相关性方面, miR-133a在糖尿病并发的心肌纤维化中扮演重要角色, 其过表达可以抑制细胞外调节蛋白激酶1/2(extracellular regulated protein kinases1/2, ERK1/2)和SMAD-2(drosophila mothers against decapentaplegic protein-2)蛋白质磷酸化并显著降低转化生长因子β1等纤维化标志蛋白质水平[55]。在心力衰竭病理生理过程中, 肌细胞特异性miR-1和miR-133可通过抑制蛋白磷酸酶2A(PP2A)活性来增强心肌细胞兰尼碱受体2(RyR2)磷酸化, 影响下游CaMK II和钙离子循环, 从而增加心律失常风险[56]。

4 肌细胞特异性miRNAs在骨骼肌细胞中的生物学效应

miR-1、miR-133a/b和miR-206占骨骼肌细胞全部miRNA表达量的25%, 在肌肉形成、增殖、分化、凋亡等多种生物学过程中起重要调控作用[57]。其中, miR-1和miR-133均由肌细胞增强因子2调控的双反顺子转录本(MEF2-regulated bicistronic transcripts)编码, 在肌肉生长和分化调控过程中保持动态平衡[58]。在对骨骼肌细胞效应调控方面, miR-1和miR-133可通过调节组蛋白去乙酰化酶4(histone deacetylase 4, HDAC4)和血清应答因子(serum response factor, SRF), 直接调控骨骼肌细胞增殖和分化[59]。miR-206在骨骼肌中特异性表达, 且在不同肌纤维中表达量不同, 如在比目鱼肌中表达量为跖肌的7.2倍[60]。miR-206在肌细胞分化过程中显著升高, 调控的两个靶基因分别为间隙连接蛋白43(connexin 43, *Cx43*)和DNA聚合酶αp180催化亚基(polymerase 1, *Pola1*)。miR-206能和*Cx43*基因mRNA的3′端UTR区结合并显著降低后者表达, 减少骨骼肌成肌细胞融合, 从而在肌细胞分化和发育中起重要调控作用[61]。在C_2C_{12}成肌细胞中, 高表达的miR-206能显著降低*Pola1*、肌原性转录因子*Id1-3*(inhibitor of differentiation 1-3)和*MyoR*(myogenic repressor)等多个基因表达, 促进肌细胞体外分化[62]。此外, 研究表明, 在肌细胞生长抑制素(TGF-β超家族成员)缺乏情况下, 成熟miR-206水平会升高, 表明TGF-β超家族成员可以促进和抑制特定miRNA的成熟[50]。

疾病相关性方面, miR-206可通过调节配对盒基因7(pair box transcription factor 7, *Pax7*)、*Notch3*和肌动蛋白结合蛋白(*Coro1a*)等mRNA表达水平来加速肌原性干细胞分化, 促进骨骼肌再生, 从而延缓杜氏肌营养不良疾病(duchenne muscular dystrophy, DMD)的病理发展进程[63]。在骨骼肌肥大病理过程中, miR-1和miR-133a水平显著下降50%, miR-206前体显著增加18.3倍, 而miR-206本身水平没有改变, 原因之一可能是核糖体RNA对Drosha RNA酶III的竞争性抑制[60]。血清miRNAs表达谱研究发现, miR-1、miR-133和miR-23a在复发性横纹肌溶解症模型中显著高表达, 而miR-195在黏多糖贮积症模型中显著增高, 上述miRNAs很可能是潜在的相关疾病生物标志物[64]。

5 总结与展望

肌细胞特异性miRNAs生物学功能多样, 且可以对同一种细胞表现出不同的生物学效应, 如在对气管平滑肌细胞收缩舒张效应调控中, miR-133能舒张气管平滑肌细胞, 而miR-145促进气管平滑肌细胞收缩, 表现出复杂的调控机制。进一步研究需要整合系统生物学、分子生物学等技术方法, 研究肌细胞特异性miRNAs下游靶基因及其关键调控通路, 以期对其有更全面认识并基于某一生物学效应进行系统研究。

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