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综 述

调控褐色脂肪细胞分化的 microRNAs

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摘要: MicroRNA(miRNA)是近年来在真核生物中发现的一类长约 22nt 的内源性非编码 RNA，在动物中主要通过抑制靶 mRNA 翻译，在转录后水平调控基因表达。动物体内有两种类型的脂肪组织：褐色和白色脂肪，白色脂肪以甘油三脂形式贮存能量，而褐色脂肪利用甘油三酯产生能量。褐色脂肪因其对肥胖的拮抗作用而对研究肥胖等代谢疾病具有重要意义，大量研究表明 miRNA 在褐色脂肪细胞分化中扮演着重要角色，其自身也受到多种转录因子和环境因子调控，这个复杂的调控网络维持了体内脂肪组织稳态。文章主要综述了 miRNA 在褐色脂肪细胞分化中的最新研究进展，以期为利用 miRNA 进行肥胖、糖尿病等相关疾病及其并发症的治疗提供新思路。

关键词: microRNA；褐色脂肪细胞；分化

MicroRNAs in the regulation of brown adipocyte differentiation

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Abstract: MicroRNAs (miRNAs), a class of endogenous non-coding RNA about 22 nucleotide long, regulate gene expression at the post-transcription level by inhibiting the translation or inducing the degradation of their target mRNAs in organisms. There are two types of adipose tissues: brown and white. White adipose tissues store energy in the form of triglycerides (TGs), while brown adipose tissues catabolize TGs to generate energy. Brown adipose tissues are of great importance to the research of obesity and related metabolic diseases due to their function of preventing people from obesity. A lot of studies have revealed that miRNAs play crucial roles in regulating brown adipocyte differentiation and are modulated by lots of transcription factors and environmental factors, which form a complex regulatory network maintaining the homeostasis of adipose tissues. In this review, we summarize the latest studies of miRNAs in brown adipocyte differentiation, which might provide new strategies for the treatment of obesity and other related diseases.

Keywords: microRNA; brown adipocyte; differentiation

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近年来，随着人们生活水平的提高，高脂肪食物的过量摄入和久坐的生活习惯，导致超重和肥胖人群的数目越来越大[1]。肥胖引起了一系列相关慢性疾病如二型糖尿病、心肌肥大和心脑血管疾病等，已成为威胁人类健康的重大隐患[2, 3]。肥胖最明显的一个特点就是白色脂肪组织(White adipose tissue, WAT)过度集聚，其细胞水平的表现就是白色脂肪细胞体积增大(肥大)和数目增加(增生)[4]。哺乳动物体内还有另外一种类型的脂肪组织，即褐色脂肪组织(Brown adipose tissue, BAT)，研究人员一度认为BAT仅仅在小型哺乳动物和初生婴儿中存在，随着年龄的增长，成人体内的BAT维持能量平衡的功能逐渐减少甚至消失，然而，近年来成人体内功能性BAT的发现使其又重新成为研究的热门。BAT数目的增加仅仅导致能量消耗的增加而不引起其他组织功能障碍，并且对肥胖有拮抗作用，其功能缺失可能会导致肥胖和胰岛素抵抗，遗传学抑制或者手术切除褐色脂肪的小鼠表现出摄食过量和肥胖现象[5~7]。BAT由褐色脂肪细胞、丰富的血管和神经组成，最新的研究表明微RNA(microRNA，miRNA)在褐色脂肪细胞分化中起了重要的调控作用。研究褐色脂肪细胞分化中的miRNA及其作用机制对于解决肥胖等疾病有着至关重要的意义，本文对褐色脂肪细胞分化中的miRNA研究进行综述，为利用miRNA进行肥胖等相关疾病的预防和治疗提供新思路。

1　脂肪细胞的种类和功能

哺乳动物体内主要有 3 种类型的脂肪细胞 :白色脂肪细胞、褐色脂肪细胞和米色脂肪细胞，这 3 种类型脂肪细胞颜色与功能各异，共同维持着体内的能量代谢平衡。WAT主要分布在皮下、肌肉、腹部和内脏等部位，由白色脂肪细胞组成，能量过剩引起的白色脂肪过度沉积导致了超重和肥胖。白色脂肪细胞中有一个大的脂滴，细胞质中线粒体数量较少，能分泌很多脂肪细胞因子如脂联素、瘦素、抵抗素等，可以将体内过剩的能量以甘油三酯的形式贮存下来，起着贮存能量和分泌的作用。BAT主要分布在人体的肩胛骨间、颈背部、腋窝、纵隔及肾脏周围，由褐色脂肪细胞组成，与体质量指数和静息代谢率有关[8]。经典的褐色脂肪细胞含有很多小的脂滴，细胞质中充满着大量的线粒体，因此看上去是褐色的。线粒体内膜上存在特异性高表达的线粒体解偶联蛋白 1(Mitochondrial brown fat uncoupling protein 1, UCP1)，这是一种褐色脂肪细胞特有的功能蛋白，能消除线粒体内膜两侧的跨膜质子浓度梯度，解除线粒体呼吸过程中电子传递与氧化磷酸化之间的偶联，减缓氧化磷酸化过程，阻碍三磷酸腺苷(ATP)的产生，使能量以热量的形式释放出来，从而增加能量的消耗。在寒冷环境下，褐色脂肪细胞利用甘油三酯产生和消耗热量[9]，使机体免受寒冷的侵袭。除了白色脂肪细胞和经典的褐色脂肪细胞之外，还有一种可以诱导的褐色脂肪细胞(又称为米色脂肪细胞)，它兼具白色及褐色脂肪细胞的特征，但又不同于两者，既能储存能量，又能消耗能量。米色脂肪细胞不像典型的褐色脂肪细胞显著激活*UCP1* 基因表达，而是一种热源脂肪细胞，在未受刺激情况下其*UCP1* 基因表达水平很低，但经过环磷酸腺苷(cAMP)处理之后，其*UCP1* 表达量与褐色脂肪细胞相比没有显著差异，功能类似经典褐色脂肪细胞。

2　microRNA 调控褐色脂肪细胞分化的机制

miRNA是在多种真核细胞和病毒中发现的一类长约 21~22nt的内源性非编码单链RNA，通过特异性碱基互补的方式与靶基因信使RNA(mRNA)的3'-UTR结合，抑制靶mRNA翻译或诱导其降解，从而在转录后水平调控基因的表达[10~12]。miRNA的产生包括以下几个过程：(1)编码miRNA的基因在RNA聚合酶 II 的作用下转录形成初级转录本pri-miRNA；(2)Pri-miRNA在Drosha/DGCR8 复合体[13]的切割下形成miRNA前体pre-miRNA；(3)Pre- miRNA被Exportin5 从细胞核转运到细胞质；(4)Pre- miRNA被Dicer酶加工成双链成熟miRNA，随后双链解旋，形成单链成熟miRNA。miRNA的产生过程受到了许多转录因子调控，随后成熟单链miRNA形成沉默诱导复合体RISC，抑制或者降解靶mRNA。自从第一个miRNA–lin-4[14]在线虫中被发现以来，越来越多的miRNA被鉴定出来，目前miRbase (http://www.mirbase.org/)收录的miRNA条目已达 28 645条(2014 年 6 月)，并且数目在逐年增长。miRNA参与了各种生命过程的调控，包括细胞的增殖、分化和凋亡等，在生物体生长、发育和疾病发生等过程中扮演着重要角色[15,16]。

广义的褐色脂肪细胞来源于*PAX7/MYF5*+/-(Paired box 7/Myogenic factor5 positive/ negative)前体祖细胞。在信号的刺激下，前体祖细胞首先经历细胞系定型确定褐色向分化，然后经历克隆扩增、生长停滞和终末分化 3 个步骤形成了褐色脂肪细胞。在这一过程中起决定性作用的是锌指转录因子PR结构域包含子 16(PR domain containing 16, *PRDM16*), *PRDM16* 通过与编码CCAAT-增强子结合蛋白β(CCAAT/enhancer-binding protein beta, C/EBPβ)的基因形成一个转录复合体开关从而调控*PAX7/MYF5*+/-前体向褐色脂肪细胞分化[17, 18]。此外，*UCP1*、过氧化物酶体增殖物激活受体α(Peroxisome proliferator-activated receptor alpha, *PPARα*)、过氧化物酶体增殖物激活受体γ(Peroxisome proliferator-activated receptor gamma, *PPARγ*)、激活受体γ共活化剂 1α(Peroxisome proliferator-activated receptor gamma coactivator 1-alpha, *PGC1α*)和激活受体γ共活化剂 1β(Proliferator-activated receptor gamma coactivator 1 beta, *PGC1β*)是褐色脂肪标志性基因，在褐色脂肪细胞分化中行使重要功能，其表达量常作为检测褐色脂肪细胞分化的指标。许多研究表明，miRNA 在白色脂肪细胞分化中起到了重要的调控作用(详见表 1)，其作用的实质是对调节白色脂肪细胞分化的 pRB-E2F、MAPK、SMAD/TGFβ、WNT

表 1　调控白色脂肪细胞分化的 microRNA

微 RNA	靶基因	作用方式	前体脂肪细胞模型	物种	参考文献
Let7	*HMGA2*	+	3T3L1	M	[19]
miR-15a	*DLK1*	–	3T3L1	M	[20]
miR-17-5p	*BMPR2*、*BMP2*	+	ADSCs	H	[21]
miR-17-92	*RB2/P130*	+	3T3L1	M	[22]
miR-21	*TGFBR2*	+	C3H10T1/2	H	[23]
miR-22	*HDAC6*	–	ADSCs	H	[24]
miR-27(a、b)	*PPARγ*、*PHB*	–	ADSCs、3T3L1	H、M	[25~27]
miR-30	*RUNX2*	+	ADSCs	H	[28]
miR-33b	*EBF1*	–	PSPA	P	[29]
miR-103	*RAI14*	+	PSPA	P	[30]
miR-106a	*BMP2*	+	ADSCs	H	[21]
miR-135a-5p	*APC*	–	3T3L1	M	[31]
miR-137	*CDC42*	–	ADSCs	H	[32]
miR-138	*EID1*	–	ADSCs	H	[33]
miR-143	*ERK5*、*MAP2K5*、*PTN*、*ORP8*	+	3T3L1、ADSCs	H、M	[34~36]
miR-146b	*SIRT1*	+	3T3L1	M	[37]
miR-199a	*LIF*	+	MSCs	H	[38]
miR-210	*TCF7L2*	+	3T3L1	M	[39]
miR-222	*ERα*		3T3L1	M、H	[40]
miR-223	*PKNOX1*			M	[41]
miR-224	*EGR2*、*ACSL4*	–	3T3L1	M	[42]
miR-363	*E2F3*	–	ADSCs	R	[43]
miR-375	*ERK*	+	3T3L1	M	[44]
miR-378		+	3T3L1	M	[45]
miR-448	*KLF5*	–	3T3L1	M	[46]
miR-486-5p	*SIRT1*	–	ADSCs	H	[47]
miR-519d	*PPARα*	–	Visceral pre-adipocytes	H	[48]
miR-561	*HSD11B1*	–	A549、HepG2	H	[49]
miR-579	*HSD11B1*	–	A549、HepG2	H	[49]

注：+代表促进，–代表抑制，H 代表人，M 代表小鼠，R 代表大鼠，P 代表猪，空白表示暂不清楚。

等信号通路中的一个或多个组件进行靶向调节。miRNA 对褐色脂肪细胞分化亦起到了重要的调节作用，其实质是直接靶向作用 *PRDM16-C/EBPβ* 转录复合体或对调控该复合体表达的上游信号通路的基因进行作用，引起该复合体的表达量变化，进而影响褐色脂肪细胞分化。

3　调控褐色脂肪细胞分化的 microRNA

3.1　miR-133—褐色脂肪细胞分化的负调控因子

Trajkovski等[50]对寒冷刺激(8 ℃，24 h)后的C57Bl/6N小鼠和室温饲养的小鼠褐色脂肪组织进行了miRNA表达谱分析，发现miR-133 在寒冷刺激后发生了最显著的下调，同时与褐色脂肪细胞分化相关的基因*PRDM16*、*UCP1*、*PPARα*和*PPARγ*的表达量上调。生物信息学分析表明，*PRDM16* 基因的3'-UTR区含有与miR-133 的 2~8 种子序列相同的片段，可能是其靶基因，随后的荧光素酶报告实验证实了这一点。后续细胞和个体水平验证表明，在经过肾上腺素刺激或者冷刺激之后，小鼠脂肪组织中*MEF2* 家族表达下调，进而导致miR-133 的表达下调，使其对*PRDM16* 的抑制作用减弱，促进下游基因*UCP1*、*PPARα*、*PPARγ*的表达，进而促进褐色脂肪组织*PAX7/MYF5*+/-前体祖细胞向成熟褐色和米色脂肪细胞分化。无独有偶，Yin等[51]利用细胞系追溯实验和细胞克隆方法证明，褐色脂肪细胞起源于胚胎发育时期的肌源性先祖细胞。小鼠体内成熟的骨骼肌干细胞不仅可以分化成肌细胞，也具有分化成成褐色脂肪细胞的潜能，控制这一过程的决定性因素是一种在骨骼肌干细胞中高表达的肌源性miR-133，它可以直接靶向抑制*PRDM16* 的表达，增强生肌作用。寒冷刺激或者肌肉再生中miR-133 表达被抑制，促进了骨骼肌干细胞向褐色脂肪细胞分化。肌肉再生中抑制miR-133 可以促进非偶合呼吸、葡萄糖摄取和产热作用，增加机体能量消耗提高糖耐量，阻碍饮食性肥胖的发展。miR-133 作为褐色脂肪细胞分化中的负调控因子为肥胖提供了一个重要的治疗靶点。

3.2　miR-155—双稳态负反馈调节回路

Chen等[52]利用深度测序方法比较了褐色脂肪细胞前体和分化后成熟细胞的miRNA表达谱，聚焦到了 3 个差异表达miRNA：miR-146a、miR-155 和miR-223，然后构建 3 个慢病毒载体转染细胞，并利用油红O染色测定脂质积累，发现只有携带miR-155载体转染的细胞发生了明显脂质减少。miR-155 在褐色脂肪组织中富集，在褐色脂肪细胞前体增殖时高表达，但是在诱导分化时表达量下降，它的表达受到*TGFβ1/SMAD*抑制脂肪细胞分化信号通路的一个下游作用元件转化生长因子β1(Transforming growth factor beta1, *TGFβ1*)调节。荧光素酶报告实验表明*C/EBPβ*是miR-155 的靶基因，miR-155 可以靶向抑制*C/EBPβ*，反过来，*C/EBPβ*也会抑制miR-155表达。构建的转基因敲除小鼠(miR-155$^{-/-}$)中对*C/EBPβ*的抑制被解除，褐色脂肪细胞分化增强，并且促进白色脂肪前体细胞“褐色化”[53]。反之，过表达miR-155 的转基因小鼠褐色脂肪组织质量下降并出现了褐色脂肪组织功能障碍，其褐色脂肪细胞多呈现未分化前体状态。由此可见，miR-155 和其直接靶基因*C/EBPβ*形成了一个双稳态负反馈调节回路，巧妙地调节了褐色脂肪细胞增殖和分化的时序。

3.3　miR-193b-365—四元调控环

Sun等[54]利用miRNA芯片比较了小鼠附睾白色脂肪、肩胛褐色脂肪和背最长肌的miRNA表达谱，发现miR-193b-365 基因簇在褐色脂肪中特异性表达，该基因簇位于 16 号染色体长约 5 kb的区域，形成一个双顺反子转录本。在褐色脂肪前体细胞中，miR-193b 或miR-365 的阻断可以明显增强Runt相关转录因子 1(Runt-related transcription factor 1, *RUNX1T1*)表达，削弱褐色脂肪细胞的脂肪形成，而肌肉生成却被诱导增强。在C2C12 成肌细胞中过表达miR-193b 或miR-365 阻遏了肌肉生成的整个过程。荧光素酶报告实验表明，*RUNX1T1*、细胞粘附相关/致癌基因调控蛋白(Cell adhesion molecule-related/down-regulated by oncogenes, *CDON*)和胰岛素样生长因子结合蛋白 5(Insulin-like growth factor-binding protein 5, *IGFBP5*)是miR-193b的直接靶基因，*RUNX1T1* 是褐色和白色脂肪细胞分化的抑制因子，而*CDON*和*IGFBP5* 是促生肌因子。

miR-193b通过靶向抑制*RUNX1T1* 基因，从而减弱了其对*PAX7/MYF5*+前体细胞向褐色脂肪细胞分化过程的抑制作用。miR-193b靶向负调控*CDON*和*IGFBP5* 的表达，抑制了*PAX7/MYF5*+前体细胞的成肌分化，从而促进了*PAX7/MYF5*+前体细胞向褐色脂肪细胞分化。*PRDM16* 可以通过*PPARα* 促进miR-193b的表达，miR-193b靶向抑制*RUNX1T1*，*RUNX1T1* 又抑制了*PRDM16* 和*PPARα*的表达，四者形成了一个以miR-193b为中心的四元环状调节回路，调控褐色脂肪细胞分化。Feuermann等[55]在进行小鼠的体内实验时却得到了相反的结果，正常小鼠和沉默miR-193b-365-1 基因座的转基因小鼠褐色脂肪组织小RNA测序(smallRNA-seq)和转录组测序(RNA-seq)结果表明，只有miR-133a发生显著下调，与功能相关的基因包括*PRDM16* 和*UCP1* 表达水平都没有发生显著改变。随后进行的β肾上腺素刺激和冷暴露处理的实验结果表明处理和对照组之间没有显著差异，无论miR-196b存在与否，褐色脂肪组织的功能都没有受到影响，与功能相关的miRNA和mRNA表达水平也没有发生变化。两个研究小组得到的结果相反，这可能是两个实验的方法(体外、体内)以及控制miR-193b-365-1 表达的手段不同导致了二者研究结果的差异。

3.4 miR-196a—褐色化的正调控者

Mori等[56]发现：在冷暴露或β肾上腺素刺激后，小鼠白色脂肪组织中miR-196a的表达显著上调，而在小鼠白色脂肪中过表达miR-196a诱导了米色脂肪细胞的出现，说明miR-196a对*PAX7/MYF5*-前体祖细胞褐色向分化有促进作用。miR-196a转基因小鼠表现出能量消耗增加和抵抗肥胖的现象，说明诱导出现的米色脂肪细胞具有代谢功能。在*PAX7/MYF5*-前体祖细胞褐色向分化中，miR-196a抑制了其靶基因同源框蛋白Hox-C8[57](Homeobox protein Hox-C8, *HOXC8*)的转录后表达水平，利用染色质免疫沉淀(Chromatin Immunoprecipitation, ChIP)法分析小鼠基因组，发现HOXC8 蛋白结合*C/EBPβ*基因富集，荧光素酶报告实验发现HOXC8 蛋白的同源突变体*HDM*缺乏DNA 结合能力，表明*HOXC8* 协同脱乙酰化酶 3(Histone deacetylase 3, *HDAC3*)作用，调控*C/EBPβ*3′端序列进而抑制其表达。因此，米色脂肪生成过程中miR-196a 上调表达抑制*HOXC8* 基因表达，导致*C/EBPβ*去阻遏，诱导了白色脂肪组织中功能性米色脂肪的发生。miR-196a作为褐色化的正调控者，诱导了前体祖细胞褐色向 分化。

3.5 其他影响褐色脂肪细胞分化的 microRNA

miR-106b-93 簇位于 7 号染色体上，属于miR-17家族，已有研究表明该家族的其他成员如miR-17-92[22]促进了白色脂肪细胞的分化。Wu等[59]发现miR-106b-93 也是褐色脂肪细胞分化中的负调控因子，敲除miR-106b和miR-93 显著诱导了褐色脂肪组织特异性基因如*UCP1*、*PRDM16* 的表达且加速褐色脂肪细胞中脂滴积聚，过表达miR-106b和miR-93 抑制了这些基因的表达，高脂饲料诱导的肥胖小鼠褐色脂肪组织中的miR-106b和miR-93都出现了上调，这些现象都表明了miR-106b-93 对褐色脂肪细胞分化具有抑制作用。Lei等[60]发现，冷暴露刺激后小鼠的褐色脂肪组织和皮下白色脂肪组织中的miR-27 下调表达，体外褐色脂肪细胞前体分化中也发现了同样的下调。进一步研究表明，miR-27 直接靶向调控了整个褐色脂肪调控网络元件：*PRDM16*、*PPARα*、末端水解酶B(Ubiquitin carboxyl-terminal hydrolase B, *CREB*)和*PGC1β*，miR-27 通过对*PPARγ*的直接作用和对*PGC1α*的间接作用，抑制了皮下白色脂肪前体和人工培养细胞的褐色向分化。Karbiener等[61]鉴定出miR-26a和miR-26b是米色脂肪细胞分化中的关键调控者，过表达miR-26a和miR-26b会加速积聚人多能脂肪源干细胞(Human multipotent adipose-derived stem cells, hMADS)分化过程中脂质的积累，抑制miR-26a和miR-26b则会阻止脂质积累。miR-26 显著诱导了与能量耗散和线粒体形成相关的通路，利用靶基因预测、转录组学和RNA干扰方法证明miR-26 通过靶向抑制解聚素-金属蛋白酶结构域包含蛋白 17 基因(Disintegrin and metalloproteinase domain-containing protein 17, *ADAM17*)介导了米色脂肪细胞分化。此外，miR-182[62]、miR-203[62]、miR-378[63]和miR-455[64]也在褐色脂肪细胞分化中起了重要调控作用，详见表 2。

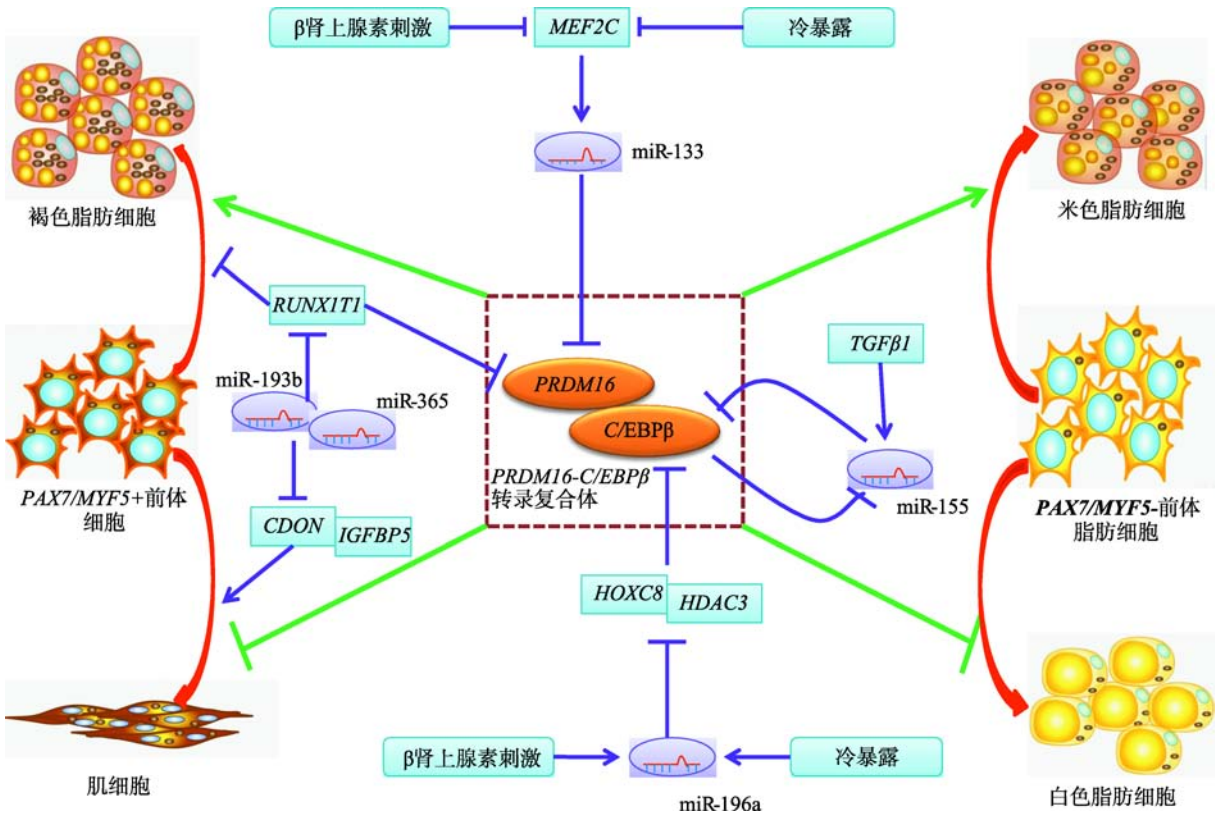

图 1　miR-133、miR-155、miR-196a和miR-193b-365 调控褐色脂肪细胞分化的分子机制(参考文献[58]绘制)

表 2　调控褐色脂肪细胞分化的 microRNA

微 RNA	靶基因	作用方式	前体细胞模型	物种	参考文献
miR-26	*ADAM17*	+	hMADS	H	[61]
miR-27	*PRDM16*、*PPARα*, *CREB*、*PGC1β*、*PPARγ*	–	*PAX7/MYF5*-	M	[60]
miR-106b-93		–	*PAX7/MYF5*+	M	[59]
miR-133	*PRDM16*	–	*PAX7/MYF5*+/-	M	[50,51, 65]
miR-155	*C/EBPβ*	–	*PAX7/MYF5*+/-	M	[52]
miR-193b-365	*RUNX1T1*	+	*PAX7/MYF5*+	M	[54]
miR-196a	*HOXC8*	+	*PAX7/MYF5*-	M	[56]
miR-203		+			[62]
miR-378	*PDE1B*	+	*PAX7/MYF5*+	M	[63]
miR-455		+		M	[64]

注：+代表促进，–代表抑制， H 代表人，M 代表小鼠，空白表示暂不清楚。

4　展　望

在生物体内，一个miRNA可能作用于多个靶基因，也可能是多个miRNA调控一个靶基因[66]，这种作用构成一个调控网络，在某些信号的刺激下，从整体上调控有机体的生命活动，因此miRNA的作用是通过其复杂的靶基因协同调节实现的。在脂肪组织中，miRNA通过对*PRDM16-C/EBPβ*转录复合体的直接或间接作用调节了褐色脂肪细胞分化，同时它自身也受到许多上游转录因子[67]、脂肪细胞因子[68~76]、调控蛋白[77, 78]、葡萄糖浓度[79]和饮食[80]的影响，转录因子、环境因素、miRNA和下游靶基因

及其所在的信号通路形成了一个复杂的调控网络，调控了体内褐色脂肪细胞分化。针对肥胖以及肥胖相关并发症的治疗，不应仅局限于miRNA，其上游的转录因子、下游的靶基因、调控蛋白和环境理化因素也具有成为治疗靶标的潜能。对褐色脂肪细胞分化乃至肥胖等的研究应该在这个动态的网络中寻找答案，二代测序技术和生物芯片技术为人们提供了一个强有力的工具。目前，二代测序技术的成本在逐年降低，利用二代测序技术对miRNA进行研究已经成为一个重要的方法，与传统的芯片技术相比，二代测序技术有许多优势，如新miRNA的发现、开放性等都是芯片技术无法比拟的，芯片技术亦有其优势，如成本低廉和表达量测定准确等，在进行miRNA研究时可以根据研究目的灵活选择。对褐色脂肪细胞分化中miRNA的功能研究，也可借鉴Calura等[81]在通路中研究miRNA作用机制的方法，结合上游转录因子、细胞因子、表型相关的靶基因所在通路，甚至代谢组、蛋白质组等，绘制miRNA参与的调控网络，在网络中发现关键的基因或代谢途径。目前，已经开发出了一些治疗肥胖的药物和治疗方案，但肥胖问题的解决依然任重道远[82]。随着调控脂肪细胞分化研究的深入，人们对这个复杂的调控网络的了解越来越透彻，相信在不久的将来一定能够研究出更加有效的治疗肥胖的药物或方法。

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