·综 述·

MicroRNA 调控猪骨骼肌发育的研究进展

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摘 要：骨骼肌的生长发育是影响猪肉产量和品质的重要因素，其受遗传和营养等众多因素的精细调控。MicroRNA (miRNA)是一种长度约为 22 nt 的非编码 RNA，通过与靶基因的 mRNA 3′UTR 序列结合，调控其转录后的表达发挥作用。近年来，大量的研究表明 miRNA 参与机体的生长发育、生殖、疾病等多种生命过程。本文对 miRNA 在猪骨骼肌发育调控中的作用进行了综述，以期为猪的遗传改良提供参考和借鉴。

关键词：miRNA；猪；骨骼肌；发育调控

Porcine skeletal muscle development regulated by MicroRNA: a review

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Abstract: The growth and development of skeletal muscle is an important factor affecting pork production and quality, which is elaborately regulated by many genetic and nutritional factors. MicroRNA (miRNA) is a non-coding RNA with a length of about 22 nt, which binds to the 3′UTR sequence of the mRNA of the target genes, and consequently regulates its post-transcriptional expression level. In recent years, a large number of studies have shown that miRNAs are involved in various life processes such as growth and development, reproduction, and diseases. The role of miRNAs in the regulation of porcine skeletal muscle development was

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reviewed, with the hope to provide a reference for the genetic improvement of pigs.
Keywords: miRNA; pig; skeletal muscle; developmental regulation

随着我国经济发展和人民生活改善，国民对肉类品质的要求越来越高。骨骼肌是生物体内最大的运动和代谢器官，同时也是机体最重要的组成部分，其重量约占机体总重量的40%–45%[1]。成肌细胞经历增殖、分化、融合形成肌管，最终形成成熟的肌纤维[2]。肌生成过程受到非编码 RNA、肌源性调节因子家族、生肌增强因子 2 家族、表观遗传学修饰和信号通路等多种因素调节。其中肌源性调节因子家族(myogenic regulatory factor family, MRFs)，包括肌源性分化 1 (myogenic differentiation 1, MyoD)，肌细胞生成素(myogenin, MyoG)，肌源性因子 5 (myogenic factor 5, Myf5)和肌肉特异性调节因子 4 (muscle-specific regulatory factor 4, MRF4, 或 Myf6)；表观遗传学修饰，包括组蛋白修饰、DNA 甲基化修饰等；非编 RNA，包括 microRNAs (miRNAs)、miRNA 长非编码 RNA (miRNA long non-coding RNAs, lncRNAs)和环状 RNA (circular RNA, circRNAs)。miRNAs 是一种长度约为 18–24 个核苷酸的物种间高度保守的小分子非编码 RNA[3]，广泛存在于动植物中。miRNA 通过互补配对结合其目标基因的 3′UTR 序列，从而使 mRNA 降解或抑制靶基因的翻译，进而在转录后水平调控靶基因的表达[4]。近年来，大量研究表明 miRNA 在猪骨骼肌发育过程中发挥着重要作用。本团队一直从事猪骨骼肌生长发育的研究，且在 miRNA 影响骨骼肌发育中做了很多研究。本文对 miRNA 在猪骨骼肌发育调控中的作用进行综述，旨在进一步了解与猪肉质性状相关的 miRNA 调控机制，并为猪肉品质改良奠定理论基础。

1 骨骼肌发育调控

1.1 骨骼肌的生长发育

骨骼肌是动物运动、能量储存和代谢的重要器官[5]，同时也是人类食物蛋白质的重要来源。骨骼肌起源于原肠胚，经历中胚层瞬时组织，再到中胚层的前部区域形成体节，在体节内肌源性祖细胞和骨骼肌成肌细胞不断生长分化，最终形成分布在躯干和四肢的骨骼肌[6]。骨骼肌的形成是多步骤的过程，在复杂的调控网络下，经历增殖分化和融合等多个阶段使多个单核成肌细胞形成多核肌细胞，最终成为成熟的肌纤维[7]。

1.2 骨骼肌生长发育的调控因子及机制

骨骼肌的生长发育受到多种因素的调控，其中转录因子在这一过程中发挥着重要作用。随着对骨骼肌生长发育机制的研究，目前已经发现多种转录因子调控骨骼肌的生长发育过程。其中，包括细胞周期素依赖性激酶(cyclin-dependent kinases, CDKs)家族、配对盒(paired box proteins, Pax)基因家族、肌原性调节因子(myogenic regulator factors, MRFs)以及成肌增强因子家族(myocyte enhancer factors, MEF2)等。

细胞周期素依赖性激酶家族 CDK 目前发现有 21 种。其中，调控细胞周期的 CDK 通过在细胞周期循环进行中活化，结合不同类型的细胞周期蛋白(cyclin)形成复合物，使细胞周期有序进行[8]。CDK4/6 可以与细胞周期蛋白 D (cyclin D)形成复合物，使视网膜母细胞瘤(retinoblastoma, RB)基因磷酸化，随之转录因子 E2F 大量释放，促进与细胞周期相关基因的转录，细胞进入 S 期[9]。

Pax 家族有 *Pax1–Pax9*，共 9 个基因，其中

以 Pax3 和 Pax7 为主，参与肌细胞的增殖[10]、分化[11]、迁移和肌肉再生[12]等生物学过程。Pax3 在肌生成过程中位于调控网络的上游，通过活化 *MyoD* 基因在肌肉形成中发挥重要作用[13]。Pax7 主要在肌源性前体细胞中表达，研究表明敲除 *Pax7* 基因，肌细胞不能进行正常的增殖、分化和融合，影响肌肉损伤后的再生修复[14]。

肌源性调节因子(myogenic regulatory factor family, MRFs)家族，主要包括成肌分化因子(myogenic differentiation 1, MyoD 或 MyoD1)、生肌因子 5 (myogenic factor 5, Myf5)、生肌调节因子 4 (myogenic regulatory factor 4, MRF4)和肌细胞生成素(myogenin, MyoG)。其中，MyoD 和 Myf5 主要在骨骼肌发育前期发挥作用，促进肌细胞的增殖分化，最终形成肌细胞[15-16]；MRF4 主要参与肌细胞分化过程[17]；MyoG 影响成肌细胞分化成肌管，且 MyoG 基因缺失后，小鼠会因为缺乏肌纤维出生后死亡[18-20]。成肌增强因子家族(MEF2)，由 MEF2A、MEF2B、MEF2C 和 MEF2D 构成。其中 MEF2A 与 MyoD 协同作用，激活与骨骼肌发育相关的基因[21]；MEF2B 在早期发育的骨骼肌中表达[22]；在 C2C12 成肌细胞中，分化时期的 MEF2C 启动子转录活性高于增殖期的转录活性[23]，MEF2D 可以促进成肌细胞的分化[24]。此外，MEF2 在肌纤维类型变化中也发挥着重要的作用[25]，可促进 I 型肌纤维的形成[26]。

近些年来，越来越多的研究表明 miRNA 在骨骼肌发育的调控中也发挥着重要的作用，直接或间接与上述肌肉发育调控因子相互作用，共同构成骨骼肌发育的复杂的分子调控网络。

2 miRNA 的生成及作用机制

2.1 miRNA 的生成

miRNA 的生物合成是一个较为复杂的过程，其在细胞核中转录产生初级 miRNA (pri-miRNA)，此时的 pri-miRNA 具有不完全配对茎环、侧翼序列和终端环[27]。随后，在核酸内切酶 Drosha 和双链 RNA 结合蛋白复合物 DGCR8 的作用下，pri-miRNA 被剪切成为长度约 70 nt 的前体 miRNA (pre-miRNA)[28]。产生的 pre-miRNA 在核转运蛋白 5 的作用下，通过核孔从细胞核运送到细胞质，细胞质中的第二类 RNaseIII核酸内切酶 Dicer 将 pre-miRNA 剪切成长度约为 22 nt 的双链 miRNA[29-31]。最后，产生的双链 miRNA 被装载到 Argonaute (AGO)蛋白上，产生 2 条单链 miRNA。其中一条被降解，另一条形成含有 RNA 诱导沉默复合体(RNA-inducing silencing complex, RISC)的单链 miRNA[32-33]。

2.2 miRNA 的作用机制

成熟的 miRNA 5′端有 2–8 个核苷酸，在 Ago 蛋白的参与下与靶基因的 mRNA 3′UTR 序列互补结合，形成 RNA 诱导沉默复合体 RISC[34]。RISC 是基因转录后沉默的关键基因，miRNA 与靶向 mRNA 结合后，介导靶 mRNA 的降解或抑制其翻译，从而调控相关基因转录后的表达水平[35-37]。

3 miRNA 在猪骨骼肌发育调控中的应用

3.1 miRNA 调控猪骨骼肌细胞的增殖分化

骨骼肌卫星细胞作为肌肉干细胞在出生后骨骼肌的生长发育中起着不可或缺的作用。近年来，越来越多的研究表明 miRNA 可以影响猪骨骼肌细胞的增殖分化，为进一步揭示猪骨骼肌生长发育的不同调控机制具有重要意义。miR-1、miR-133 和 miR-206 是肌肉特异性 miRNA，在肌生成过程中发挥着重要的作用[38]。研究表明，miR-1 和 miR-206 可以靶向 *Pax7* 基因从而抑制骨骼肌卫星细胞增殖并促进成肌细

胞的分化[39]，此外，miR-1 通过靶向 CNN3 调节猪骨骼肌的生长发育[40]。非肌肉特异性的 miRNA 在骨骼肌中也发挥着重要的作用。Ye 等[41]利用 miRNA 表达谱芯片和 RNA-seq，分析了巴马小型猪和长白猪的垂体前叶的差异表达基因，首次鉴定了一个在猪体内高度表达的 miRNA，命名为 Y-56。Song 等[42]对 Y-56 进一步研究发现其在猪肌肉组织中高表达，并通过靶向胰岛素样生长因子-1 受体(insulin-like growth factor-1 receptors, IGF-1R)抑制猪骨骼肌细胞的增殖。而 miR-199b 通过靶向 JAG1 抑制猪肌肉卫星细胞增殖[43]，miR-27a 抑制猪成肌细胞的分化过程[44]。越来越多的 miRNA 被发现在肌生成过程中发挥着双重功能。在猪骨骼肌卫星细胞中用 miR-22 抑制剂处理后，CCND1、CCNB1 和 P21 下调，MyoG 和 MyHC 表达上调，表明 MicroRNA-22 抑制猪骨骼肌中的增殖并促进卫星细胞的分化[45]。对不同年龄的荣昌猪背最长肌进行高通量测序发现 miR-323-3p 在不同年龄荣昌猪中表达不同，进一步研究发现 miR-323-3p 过表达抑制成肌细胞增殖并促进分化[46]。本团队研究发现，Lnc-ORA 与 microRNA-532-3p 和 IGF2BP2 相互作用抑制 C2C12 成肌细胞的分化[47]。此外，通过对 miR-106a-5p 在 C2C12 成肌细胞系中的研究，发现其直接与 PIK3R1 的 3′UTR 结合，抑制 PI3K/AKT 信号传导，从而抑制 C2C12 成肌细胞的分化[48]。这为调控猪肌肉生长发育提供了新的靶基因。综上所述，miRNA 在猪骨骼肌细胞增殖分化过程中发挥着重要作用(图 1，表 1)。

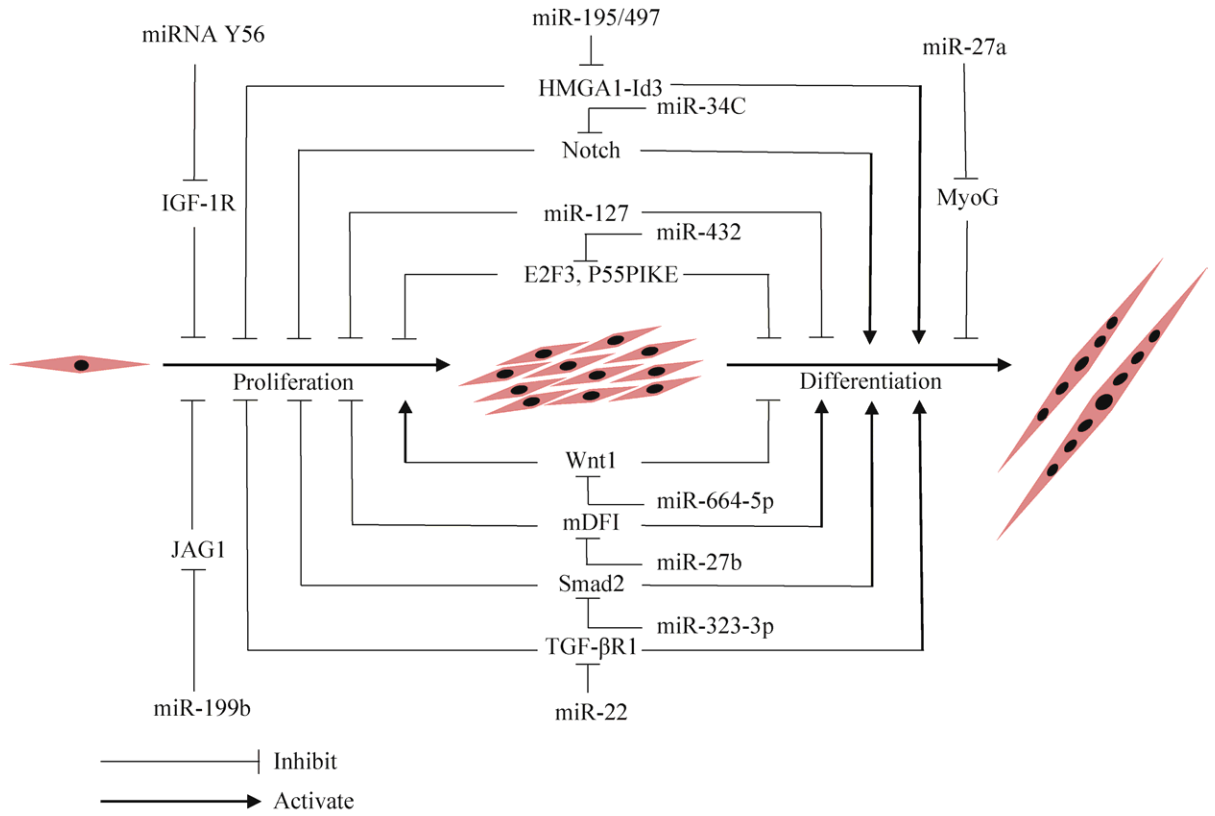

图 1　miRNAs 在猪骨骼肌细胞增殖和分化中的作用

Figure 1　The role of various miRNAs in regulating the proliferation and differentiation of porcine skeletal muscle cells.

表 1 与猪骨骼肌细胞增殖和分化相关的 miRNAs

Table 1 miRNAs associated with proliferation and differentiation of porcine skeletal muscle cells

miRNA	Proliferation	Differentiate	Target	References
miRNA Y-56	Inhibition	Unknown	IGF-1R	[42]
miR-199b	Inhibition	Unknown	JAG1	[43]
miR-27a	Unknown	Inhibition	MyoG	[44]
miR-22	Inhibition	Activate	TGF-βR1	[45]
miR-323-3p	Inhibition	Activate	Smad2	[46]
miR-27b	Inhibition	Activate	MDFI	[49]
miR-664-5p	Activate	Inhibition	Wnt1	[50]
miRNA-195/497	Inhibition	Activate	HMGA1-Id3	[51]
miR-34c	Inhibition	Activate	Notch1	[52]
miR-127	Inhibition	Inhibition	Unknown	[53]
miR-432	Inhibition	Inhibition	E2F3, P55PIK	[54]

3.2 miRNA 调控猪骨骼肌肌纤维类型转化

骨骼肌是异质性器官，由肌纤维组成。根据肌球蛋白重链(myosin heavy chain, MyHC)的不同形态，肌纤维可分为 4 种类型，分别是 I 型、IIa 型、IIx 型、IIb 型[55]。其中 MyHC I 为慢速氧化型、MyHC IIa 为快速氧化型。MyHC IIx 和 MyHC IIb 分别是中间型和快速糖酵解型。与 MyHC IIb 型纤维相比，MyHC I 型纤维具有较高的线粒体和肌红蛋白含量，较低的肌球蛋白 ATP 酶活性和糖酵解能力。而具有较大纤维尺寸的 MyHC IIb 型纤维含有较高的糖原，并显示出更快的收缩速度[56-57]。提高 MyHC I 型纤维可改善猪肉的颜色，减少 PSE 肉的发生比例，更受消费者喜欢。肌肉纤维类型的组成受到多种因素的影响，其中 miRNA 被认为在调控骨骼肌纤维类型的转变过程中起着重要作用。对以杜洛克猪为父本，梅山猪为母本的杂交后代的快肌(股二头肌)和慢肌(比目鱼肌)进行 miRNA 测序发现，共有 63 个差异表达基因，包括 22 个上调的 miRNA 和 41 个下调的 miRNA。其中，ssc-miR-499 在慢肌中的表达高于在快肌中的表达[58]。Zhang 等[59]发现亮氨酸增加慢速 MyHC 蛋白水平并降低快速 MyHC 蛋白水平，并且亮氨酸还可以通过抑制 miR-27a 的表达和功能从而促进猪骨骼肌纤维类型从快肌纤维转变为慢肌纤维。本团队比较了荣昌猪(RC)和大白猪(LW)的 miRNA 表达谱发现，与 LW 猪相比，RC 猪的背最长肌(LD)具有更高比例的慢肌纤维和更高表达的 miR-152，这表明 miR-152 可能参与肌纤维类型的组成和肌生成，进一步研究发 miR-152 通过靶向 UCP3 促进慢肌纤维的形成[60]。另外，本团队还发现 miR-151-3p 可以靶向 ATP2a2 调节慢肌的表达使肌纤维类型发生转化[61]。综上所述，miRNA 通过靶向其目标基因，促使猪肌纤维类型改变(表 2)，进而改善猪肉品质。

表 2 与猪骨骼肌肌纤维类型转化相关的 miRNAs

Table 2 miRNAs associated with porcine skeletal muscle muscle fiber type transformation

miRNA	Function	Target	References
miR-499	Promote slow muscle fiber expression	Unknown	[58]
miR-27a	Promote fast-twitch to slow-twitch muscle fibers	Unknown	[59]
miR-152	Promote slow muscle fiber expression	UCP3	[60]
miR-208b	Decreased number of slow-twitch fibers	SOX-6	[62]
miR-23a	Reduce the proportion of slow-twitch fibers	MEF2C	[63]

3.3 miRNA 调控猪肉质性状变化

猪肉的 pH、肌内脂肪含量、新鲜度、剪切力、滴水损失、肌纤维类型、乳酸及糖酵解是影响猪肉品质的重要指标。近年来，越来越多的研究表明 miRNA 在调控猪肉质性状的过程中发挥着重要作用。Shen 等[64]发现 miR-152 在糖酵解肌纤维中的表达低于氧化肌纤维。双荧光素酶测定发现 miR-152 靶向肌肉丙酮酸激酶(muscle pyruvate kinase, PKM)发挥作用。在猪骨骼肌细胞中过表达 miR-152 抑制了 *PKM* 基因的表达并减少细胞中乳酸的产生，抑制 miR-152 表现相反的结果。表明 miR-152 可能通过靶向 *PKM* 基因的表达来影响肌肉 pH 值从而使猪肉品质发生变化(图 2)。Wei 等[65]对较高滴水损失和较低滴水损失的个体背最长肌进行 RNA-Seq，共有 73 个差异表达的 miRNA，通过表达模式分析进一步发现 miRNA-499 和 miRNA-22 是影响滴水损失性状的潜在基因。Daza[66]对 174 头大白猪和皮特兰猪的 F2 代背最长肌进行 miRNA 表达谱分析，发现 miR-874 与宰后 24 h 肌肉 pH 显著相关。肌内脂肪是影响猪肉品质的重要因素。因此，鉴定调节肌肉中脂肪沉积与代谢的相关基因，对于改善肌肉品质具有重要意义。在本团队的研究中，通过对猪脂肪组织进行 miRNA 测序分析，发现 miR-146a-5p 是猪 IMF 成脂的潜在调节剂。进一步的研究表明，在猪原代脂肪细胞中 miR-146a-5p 通过直接靶向 SMAD 家族成员 4 (SMAD4)减弱 TGF-β 信号传导，从而抑制细胞增殖。此外，miR-146a-5p 通过靶向 TNF 受体相关因子 6 (TNF receptor correlated factor 6, TRAF6)来削弱下游的 AKT/mTORC1 信号传导，从而抑制肌内前体脂肪细胞的分化。由此表明，MiR-146a-5p 分别通过减弱 TGF-β 和 AKT/mTORC1 信号传导来靶向 SMAD4 和 TRAF6 抑制猪肌肉内脂肪形成[67]。此外，本团队发现多个 miRNA 可以调控成脂相关过程，影响肌肉脂肪的沉积(表 3)。这些研究为改善猪肉品质，提供了多个候选基因。

3.4 miRNA 影响地方品种猪肌肉的生长发育

miRNA 在猪肌肉生长发育的时空过程中发挥着重要作用。本团队对八眉猪的胚胎期(65 d)、出生时(0 d)和 10 月龄 3 个不同生长阶段的背最长肌进行 RNA-seq 整合分析，通过 mRNA-miRNA 互作网络及 RT-qPCR 分析发现成年猪与刚出生相比，刚出生时 miR-128、miR-133a-3p、miR-26a 和 miR-378 基因上调，miR-217 基因下调。此外，与胚胎期 65 d 相比，成年时期 miR-23a 和 miR-206 上调，miR-20b、miR-181c、miR-362 和 miR-127 下调[78]。此外本团队对地方品种荣昌猪在断奶(35 日龄)和屠宰时期(287 日龄)的背最长肌进行 miRNA-seq 测序，发现 miR-127、miR-299 和 miR-432-5p 在成年猪中的表达显著低于在断奶仔猪中的表达，而 miR-7134-3p 和 miR-664-5p 在成年猪中的表达显著高于在断奶仔猪中的表达[53]。

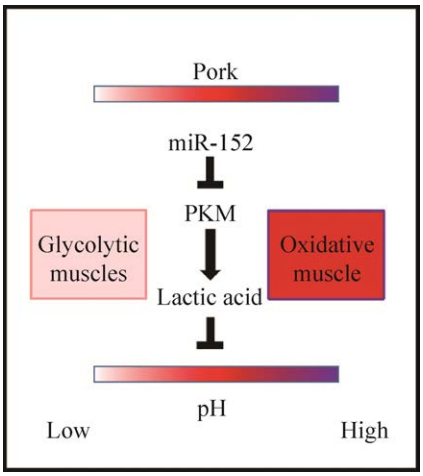

图 2 miR-152 通过靶向丙酮酸激酶调节猪肉 pH 值并影响猪肉品质[64]

Figure 2 miR-152 targets pyruvate kinase to regulate the pork pH and affects pork quality[64].

表 3 与脂肪沉积相关的 miRNAs

Table 3 miRNAs associated with fat deposition

miRNA	Function	Target	References
miR-146a-5p	Inhibits adipogenensis	SMAD4/TRAF6	[67]
miR-99b-5p	Attenuates adipogenesis	SCD1/Lpin1	[68]
miR-199a-5p	Affects porcine preadipocyte proliferation	Cav-1	[69]
miR-214-3p	Promotes 3T3-L1 preadipocyte differentiation	Ctnnb1	[70]
miR-129-5p	Inhibits 3T3-L1 preadipocyte proliferation	G3BP1	[71]
miR-324-5p	Promotes lipid droplet accumulation	KLF3	[72]
miR-370	Represses lipid accumulation	FoxO1	[73]
miR-425-5p	Inhibited adipogenesis	KLF13	[74]
miR-127	Inhibited adipogenesis	MAPK4/HOXC6	[75]
miR-103	Promotes 3T3-L1 cell adipogenesis	MEF2D	[76]
miR-125a	Inhibits porcine preadipocytes differentiation	ERRα	[77]

miRNA 在不同猪种中在猪肌肉生长发育过程中发挥着不同的作用。国内研究团队通过对 8 月龄的五指山猪和长白猪进行 Solexa 测序，并结合生物信息学分析，筛选出与骨骼肌发育相关的差异 miRNA，其中发现 5 个差异 miRNA 富集于胰岛素信号通路(ssc-miR-362、ssc-miR-455-3p、ssc-miR-497、ssc-miR-499-5p 和 ssc-miR-874)[79]。Xi 等[80]通过对通城猪和大白猪的 5 个胚胎期(40、55、63、70、90 d)的背最长肌进行 Solexa 测序，寻找参与骨骼肌发育的不同 miRNA，最终发现 miR-499-5P 可以靶向肌原纤维发生调节因子 destrin 的 3′UTR 肌动蛋白解聚因子(recombinant human destrin, DSTN)调控肌纤维的发育。这为进一步研究我国地方猪种骨骼肌生长的分子机制提供了基础数据。

4 展望

猪骨骼肌的生长发育与产肉性能及肌肉品质等密切相关。虽然人们通过研究发现 miRNA 参与机体的生长发育、生殖、疾病等多种生命过程，并在猪骨骼肌发育调控中发挥重要作用，但是随着单细胞测序、空间转录组学等技术的出现，将为该领域的研究提供新的方向。单细胞测序是一种可以评估细胞群异质性并解析时间维度上细胞类型和基因表达的变化过程的方法，利用单细胞测序技术可以鉴定 miRNA 对肌肉组织的不同肌纤维在不同时间上表达的影响。而空间转录组测序技术在获得基因表达数据的同时最大程度地保留样本的空间位置信息，鉴定 miRNA 对不同肌纤维中特定空间位置基因表达的影响，这两种技术的发展和应用对于解析肌肉生长发育的时空调节网络具有重要意义。另外，外泌体来源的 miRNA 在多种生物学过程中发挥着重要作用。因此，鉴定不同外泌体来源的 miRNA 是否影响肌肉干细胞的增殖、分化及肌纤维的转变等具有重要的研究意义。相信随着相关技术的发展和研究的不断深入，人们对 miRNA 在动物肌肉发育，特别是猪骨骼肌发育调控中的作用和机制将更加清晰。

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