microRNA	Regulation in current study	Human (Homo sapiens)	Mouse (Mus musculus)	Additional information & PubMed search
hsa-let-7g	↑ (day 10)	↔(6)	↑ C2C12 (3,8)	
hsa-miR-1	↑ (time course)	↑ (4,6)	↑ primary (3, 8), ↑ C2C12 (2, 3, 5, 8, 9)	MyomiR
hsa-miR-7	↓ (day 8)	↔(6)		
hsa-miR-10a	↑ (time course)	↔(6)	↑ primary (3)	
hsa-miR-10b	↑ (time course)	\uparrow (4), ↔ (6)	↑ primary (3), ↑ C2C12 (5)	
hsa-miR-17	↓ (day 8)	↔(6)	↓ primary (3), ↓ C2C12 (5, 8)	
hsa-miR-20a	\downarrow (day 8)	↔(6)	↓ primary (3, 8), ↓ C2C12 (5, 8, 9)	
hsa-miR-20b*	↑ (day 8)	N/A	↓ primary (3), ↓ C2C12 (5, 8, 9)	Opposite to mouse
hsa-miR-21	↓ (day 6, day 8, day 10)	↑ (4, 6)	↑ C2C12 (2)	Contradict previous studies
hsa-miR-23b	↑ (day 8)	↑ (6)	↑ primary (3), ↑ C2C12 (2)	
hsa-miR-26a	↑ (time course)	↑ (4, 6)	↑ primary (3), ↑ C2C12 (2, 5, 9)	
hsa-miR-26b	↑ (time course)	↔(6)	↑ primary (3), ↑ C2C12 (5)	

Table S2. microRNAs found to be differentially regulated during myogenicdifferentiation in the current study compared to selected publications.

hsa-miR-27a	↓ (day 4, day 8, day 10)	↑ (4, 6)	↑ primary (3, 8)	Contradict previous studies
hsa-miR-28-5p	↑ (time course)	↑ (6)		
hsa-miR-29b	↓ (day 4, day 8, day 10)	N/A	↑ primary (3), ↓ C2C12 (5)	Partial agreement mouse studies
hsa-miR-30a	↑ (day 10)	↑ (6)	↑ primary (3), ↑ C2C12 (8, 9)	
hsa-miR-30b	↑ (time course)	\leftrightarrow (6)	↑ primary (3, 8)	
hsa-miR-30c	↑ (time course)	↑ (4, 6)	↑ primary (3)	
hsa-miR-30d	↑ (time course)	↑ (6)	↑ primary (3, 8), ↑ C2C12 (2)	
hsa-miR-30e*	↑ (time course)	↑ (6)		
hsa-miR-31	\downarrow (time course)	↓(6)	↓ primary (3), ↓ C2C12 (5)	
hsa-miR-31*	↓ (day 4, day 6, day 8)	N/A	↓ C2C12 (5)	
hsa-miR-34a	↑ (day 10)	↔ (6)	↑ primary (3, 8), ↑ C2C12 (8)	
hsa-miR-34b	↑ (day 6, day 10)	↔ (6)		
hsa-miR-98	↑ (day 10)	↑ (6)	↑ primary (3)	
hsa-miR-99a	↑ (time course)	↓ (6)	↑ primary (8), ↑ C2C12 (2, 5, 8)	Contradict human, validate mouse

hsa-miR-99b	\uparrow (time course)	\leftrightarrow (6)		
hsa-miR-101	↑ (time course)	↑ (6)	↑ C2C12 [#] (5)	
hsa-miR-103	↑ (time course)	↑ (6)	↑ primary (3), ↑ C2C12 (2)	
hsa-miR-106a	↓ (day 4, day 8)	↑ (4)	↓ primary (3, 8), ↓ C2C12 (5, 8)	Contradict human, validate mouse
hsa-miR-107	\uparrow (time course)	↑ (6)	↑ primary (3)	
hsa-miR-125a- 5p	↑ (time course)	↔(6)		
hsa-miR-125b	↑ (time course)	↔(6)	↓ C2C12 (5, 9)	Opposite to mouse
hsa-miR-125b- 1*	\downarrow (day 4)	N/A	↓ primary (3), ↓ C2C12 (5)	
hsa-miR-127- 3p	↑ (time course)	↔(6)		
hsa-miR-129*	↑ (time course)	N/A	↑ primary (3)	
hsa-miR-130b	\downarrow (day 8)	\leftrightarrow (6)	↓ C2C12 (5)	
hsa-miR-133a	↑ (time course)	↑ (4, 6)	↑ primary (8), ↑ C2C12 (2, 5, 9)	MyomiR
hsa-miR-133b	↑ (time course)	↑ (6)	↑ primary (3, 8), ↑ C2C12 (2, 5, 8, 9)	MyomiR
hsa-miR-138	\downarrow (time course)	N/A	↑ primary (3, 8)	Opposite to mouse
hsa-miR-138-1*	↑ (day 8)	N/A	↑ primary (8)	

hsa-miR-140-3p	\uparrow (time course)	↑(6)	↑ primary (3, 8)	
hsa-miR-143	↓ (day 8)	\leftrightarrow (6)	↑ primary (3, 8), ↑ C2C12 (2, 8, 9)	Opposite to mouse
hsa-miR-145	↑ (day 6, day 8)	↔(6)	↑ primary (8), ↑ C2C12 (2, 8, 9)	
hsa-miR-148b	↑ (time course)	\leftrightarrow (6)	↑ primary (3)	
hsa-miR-151-3p	↑ (day 10)	↔ (6)	↓ C2C12 (10)	Opposite to mouse, decreased expression in C2C12 (10).
hsa-miR-151- 5p	↑ (time course)	N/A		
hsa-miR-152	↑ (day 10)	↑ (6)	↑ primary (3), ↑ C2C12 (5, 9)	
hsa-miR-155	↓ (day 6, day 8)	↓ (6)	↓ primary (3), ↓ C2C12 (5, 8)	
hsa-miR-181a	↑ (time course)	N/A	↑ primary (3, 8), ↑ C2C12 (5, 8)	
hsa-miR-183	↑ (day 8)	↓ (6)	↑ primary (3), ↓ primary (8), ↓ C2C12 (8)	Partial agreement with previous studies
hsa-miR-185	↑ (day 10)	\leftrightarrow (6)	↑ primary (3)	
hsa-miR-186	↑ (day 10)	↔(6)	↓ C2C12 (8)	Opposite to mouse

hsa-miR-191	\uparrow (time course)	\leftrightarrow (6)	↑ primary (3, 8)	
hsa-miR-193a- 3p	↓ (day 4, day 8, day 10)	↑ (6)		Contradict previous human study
hsa-miR-193a- 5p	↑ (time course)	N/A		
hsa-miR-196a*	↑ (time course)	N/A		
hsa-miR-196b	↓ (day 8)	↔(6)	↓ primary (8), ↓ C2C12 (8)	
hsa-miR-197	\downarrow (time course)	↔(6)	↑ primary (3)	Opposite to mouse
hsa-miR-b-3p	↑ (time course)	\leftrightarrow (6)	↑ C2C12 (9)	
hsa-miR-199a- 5p	↑ (time course)	↑ (1), ↔ (6)	↑ C2C12 (9)	Increase during human differentiation (1).
hsa-miR-199b- 5p	↑ (day 10)	↔(6)		
hsa-miR-206	↑ (time course)	↑(6)	↑ primary (8), ↑ C2C12 (2, 5, 8, 7)	MyomiR
hsa-miR-222	↓ (day 6)	↑ (4), ↓ (6)	↓ C2C12 (5, 9)	Partial agreement with previous studies
hsa-miR-299- 5p	↑ (day 6)	↔(6)		
hsa-miR-300	↓ (day 8)	N/A		

hsa-miR-301a	↓ (day 4)	\leftrightarrow (6)	↑ primary (3), ↓ C2C12 (5)	Partial agreement mouse studies
hsa-miR-320c	↑ (day 10)	↓¤ (6)	↑ primary¤ (8)	Contradict human, validate mouse
hsa-miR-320d	↑ (day 10)	↓¤ (6)	↑ primary¤ (8)	Contradict human, validate mouse
hsa-miR-331-3p	\uparrow (time course)	↑ (6)		
hsa-miR-335	↑ (time course)	↑ (7), ↔ (6)	↑ primary (3), ↑ C2C12 (5, 7, 9)	Increased during human and C2C12 differentiation (7).
hsa-miR-337-3p	↑ (day 10)	\leftrightarrow (6)	↑ primary (3)	
hsa-miR-339-5p	↓ (day 10)	↓(6)	↑ primary (3, 8), ↑ C2C12(8)	Opposite to mouse
hsa-miR-342-3p	↑ (time course)	↔(6)	↓ C2C12 (8)	Opposite to mouse
hsa-miR-361-5p	\uparrow (time course)	↑ (6)	↑ primary (8)	
hsa-miR-365	↑ (time course)	↔(6)	↑ primary (3, 8)	
hsa-miR-374a	↑ (day 10)	↑ (6)		
hsa-miR-374b	↑ (time course)	N/A		

hsa-miR-376c	↓ (day 8)	N/A	↑ primary (3)	Opposite to mouse
hsa-miR-409-3p	↑ (day 6, day 10)	N/A	↑ primary (8)	
hsa-miR-423-3p	↑ (time course)	↔(6)	↓ C2C12 (8)	Opposite to mouse
hsa-miR-423-5p	\uparrow (time course)	N/A	↑ primary (8)	
hsa-miR-432	\downarrow (time course)	↔(6)		
hsa-miR-491- 3p	↑ (time course)	N/A		
hsa-miR-498	↑ (day 8)	N/A		
hsa-miR-549	↑ (day 8)	↔(6)		Primate specific
hsa-miR-550	↑ (day 8)	↓ (6)		Contradict previous human study, primate specific
hsa-miR-551b	↑ (time course)	↔(6)		
hsa-miR-574- 3p	↑ (time course)	N/A		
hsa-miR-600	↑ (time course)	\leftrightarrow (6)		Primate specific
hsa-miR-620	↓ (day 6, day 8)	N/A		Primate specific
hsa-miR-625	↑ (day 8)	N/A		Primate specific
hsa-miR-634	↑ (day 10)	N/A		

hsa-miR-654-	↑ (day 6)	N/A		
3p				
hsa-miR-665	↑ (day 2)	N/A	↑ C2C12 (5)	
hsa-miR-765	\downarrow (time course)	N/A		Primate specific
hsa-miR-887	↑ (day 10)	N/A		Primate specific
hsa-miR-1246	↑ (day 2)	N/A		
hsa-miR-1264	↓ (day 10)	N/A		
miR-1275	\downarrow (time course)	N/A		Primate specific
hsa-miR-1287	\downarrow (time course)	N/A		
hsa-miR-1290	↑ (day 2)	N/A		Primate specific
hsa-miR-1297	↑ (day 10)	N/A		

The 103 miRNAs found to be differentially regulated in the current study were compared to expression profiling results from selected publications (using large scale data acquisition, e.g. microarrays or low density qPCR cards). Comparison was made to studies looking at skeletal muscle cell differentiation using either cells of human (ref 4 and 6) or mouse (ref 2, 3, 5, 8, and 9) origin. For mouse, comparison was made to data from both primary muscle cells (primary) and C2C12 (C2C12). Directional changes are reported using arrows, increased expression is marked by ↑ and decreased expression is marked by↓. For comparison to human data, unchanged expression is marked by \leftrightarrow and whenever expression data is absent it is marked by N/A. Novelty of the findings of the current data is reported, where miRNA symbol in **bold** indicate that the miRNA never previously been linked to differentiation and miRNA symbol in *italic* indicate that the miRNA been found to be regulated during mouse, but not human, skeletal muscle cell differentiation. Also, as a comparison to published literature a search in PubMed was made for each miRNA never previously being indicated to be linked to differentiation (in mouse and/or human). The search in PubMed was performed with the miRNA name and the phrase "Skeletal muscle AND differentiation". Any results influencing the novelty of findings from the PubMed search are reported in the column "Additional information & PubMed search".

[#] mmu-miR-101a increased in ref 5.

^{^m} Probe annotated as detecting miR-320 (no differentiation between isoforms).

References:

- Alexander MS, Kawahara G, Motohashi N, Casar JC, Eisenberg I, Myers JA, Gasperini MJ, Estrella EA, Kho AT, Mitsuhashi S, Shapiro F, Kang PB, and Kunkel LM. MicroRNA-199a is induced in dystrophic muscle and affects WNT signaling, cell proliferation, and myogenic differentiation. *Cell Death Differ*. 20:9, 2013.
- Chen JF, Mandel EM, Thomson JM, Wu Q, Callis TE, Hammond SM, Conlon FL, and Wang DZ. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nat Genet* 38:2, 2006.
- Chen Y, Gelfond J, McManus LM, and Shireman PK. Temporal microRNA expression during in vitro myogenic progenitor cell proliferation and differentiation: regulation of proliferation by miR-682. *Physiol Genomics* 43:10, 2011.
- Ciarapica R, Russo G, Verginelli F, Raimondi L, Donfranscesco A, Rota R, and Giordano A. Deregulated expression of miR-26a and Ezh2 in rhabdomyosarcoma. *Cell Cycle* 8:1, 2009.
- 5. Dey BK, Gagan J, and Dutta A. miR-206 and -486 induce myoblast differentiation by downregulating Pax7. *Mol Cell Biol* 31:1, 2011.
- 6. Dmitriev P, Barat A, Polesskaya A, O'Connell MJ, Robert T, Dessen P, Walsh TA, Lazar V, Turki A, Carnac G, Laoudj-Chenivesse D, Lipinski M, and Vassetzky YS. Simultaneous miRNA and mRNA transcriptome profiling of human myoblasts reveals a novel set of myogenic differentiation-associated miRNAs and their target genes. *BMC Genomics* 14: 265, 2013.
- Greco S, De Simone M, Colussi C, Zaccagnini G, Fasanaro P, Pescatori M, Cardani R, Perbellini R, Isaia E, Sale P, Meola G, Capogrossi MC, Gaetano C, and Martelli F. Common micro-RNA signature in skeletal muscle damage and regeneration induced by Duchenne muscular dystrophy and acute ischemia. *FASEB J*. 23:10, 2009.
- Marzi MJ, Puggioni EM, Dall'Olio V, Bucci G, Bernard L, Bianchi F, Crescenzi M, Di Fiore PP, and Nicassio F. Differentiation-associated microRNAs antagonize the Rb-E2F pathway to restrict proliferation. *J Cell Biol* 199:1, 2012.

- Sun Y, Ge Y, Drnevich J, Zhao Y, Band M, and Chen J. Mammalian target of rapamycin regulates miRNA-1 and follistatin in skeletal myogenesis. *J Cell Biol* 189:7, 2010.
- Wei H, Li Z, Wang X, Wang J, Pang W, Yang G, and Shen QW. microRNA-151-3p Regulates Slow Muscle Gene Expression by Targeting ATP2a2 in Skeletal Muscle Cells. J *Cell Physiol.*, 2014 [Epud ahead of print].