A new role of miR-29c as a potent inducer of skeletal muscle hypertrophy

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In this issue, the article from Silva and his colleagues reported that miR-29c plays a role in regulation of skeletal muscle mass in mouse muscle and in cultured myotubes. They found that overexpression of miR-29c in the mouse tibialis anterior increased muscle mass, length and force and induced satellite cell proliferation and differentiation. Ectopic expression of miR-29c in C2C12 myoblasts enhanced myotube fusion and expression of myogenic markers. In addition, they reported that miR-29c mimic inhibited the expression of the muscle atrophy-related factors MuRF1, Atrogin-1 and HDAC4 and provided evidence that miR-29c targets MuRF1 transcript by binding directly to its 3'-untranslated region (3'UTR)[1]. These findings highlight the important role of miRNAs in regulation of muscle homeostasis and indicate miR-29c as a possible candidate for future therapeutic application in diseases involving decreased skeletal muscle mass.

MicroRNAs (miRNAs) are short single-stranded RNA molecules that regulate gene expression post-transcriptionally, by base pairing to complementary sequences mainly located in the 3′UTR of their target mRNAs, to induce mRNA cleavage and translational repression. Thousands of miRNAs have been identified and linked to the regulation of fundamental biological functions in mammals[2]. Muscle homeostasis has been shown to be regulated by miRNAs at several levels: muscle development, growth (hypertrophy and atrophy) and interactions with other tissues.

The miR-29 family contains three members—miR-29a, miR-29b, and miR-29c—all of which share a common seed sequence[3] (Figure 1A). Several miR-29 target transcripts have been identified and their regulation has been associated with many physiological and pathological processes. Although most literature reports describe a functional role of miR-29 in various mechanisms involved in cancer pathogenesis, acting either as a tumour promoter or suppressor, depending on the cell context, involvement of this miRNA in muscle differentiation, function and disease has also been reported[3,4]. The function of miR-29 in the regulation of skeletal muscle mass was previously suggested by many observations. The miR-29 family was shown to be part of a regulatory feedback loop involving Polycomb proteins such as YY1 and Rybp that act as suppressors of myogenic differentiation: these factors repress myogenesis by silencing myogenic loci and miR-29 expression, and can be in turn down-regulated by miR-29 through translational repression[4]. miR-29 also attenuates the inhibitory effect of TGF-β in muscle differentiation, repressing HDAC4 translation and interfering with Smad3 signalling, and miR29 expression is under the regulation of TGF-β and Smad3. Interestingly, miR-29 was involved in induction of muscle atrophy by Smad3: as a consequence of miR-29 transcriptional inhibition by Smad3, translation of the miR-29 target PTEN is increased, leading to impairment of the Akt/mTOR signalling pathway that is essential for skeletal muscle trophicity[4]. Most of these studies were performed in myogenic cell cultures and the function of miR-29 in the regulation of skeletal muscle mass *in vivo* remained to be elucidated. Silva and colleagues give an important contribution in this regard.

Indeed, they report that miR-29c-overexpressing mouse muscles showed increased size and length, including a higher number of serial sarcomeres, have no sign of inflammatory infiltration and generate more force, resulting in a positive adaptive response[1]. These *in vivo* findings correlate with increased expression levels of differentiation specific markers and increased size of C2C12-derived myotubes. Down-regulation of MuRF1 and Atrogin1 levels following miR-29c overexpression strongly support an anti-atrophic role of this miRNA (Figure 1B). As opposed to beneficial induction of skeletal muscle hypertrophy by miR-29c, increased levels of miR-29 have been correlated with pathologic hypertrophy of cardiac myocytes and overall cardiac dysfunction through de-repression of Wnt signalling[5], pointing out that the increase of muscle mass may be detrimental in the case of the heart.

Most of the previously described studies did not distinguish among the three different miR-29 members, and it was assumed that, since their seed sequences is identical, they likely share targets and functions. In contrast with this view, the report from Silva and colleagues highlights an opposite role of miR-29c and miR-29b in regulation of muscle mass, being the first a pro-myogenic and anti-atrophic miRNA through down-regulation of atrophy-related genes such as MuRF1, while the second an inducer of atrophy. In agreement with previous studies, the authors show that indeed MuRF1 expression is increased by miR-29b. This antagonistic effect is hard to conciliate at a first glance. Since miR-29b is expressed from bicistronic miR-29 clusters, it is difficult to imagine transcriptional regulation of miR-29b without affecting miR-29a or miR-29c dosage. miR-29b has been recently shown to be regulated by sequestration through binding to tissue specific long non-coding RNAs (lncRNA), one example of which is lncRNA H19, an active modulator of musculoskeletal development that is expressed specifically in muscle tissue after birth[6]. Recently, post-transcriptional degradation of miR-29b has been shown to occur through binding to a genome encoded transcript harbouring a near-perfect and deeply conserved miRNA-binding site[7]. This transcript originated in vertebrates as a lncRNA and evolved to the protein-coding gene NREP in mammals. miR-29b binds to a complementary site located within the 3′UTR of the NREP transcript, and this binding leads to miR-29b destabilization through 3′ trimming, thus restricting its spatial expression in the mouse cerebellum[7]. As miRNAs are often transcribed in clusters from multiple genomic loci and are generally highly stable, miRNA destabilization through endogenous targets is emerging as an effective posttranscriptional mechanism for the selective regulation of specific miRNAs (Figure 1C).

It has become clear that alteration of the expression of many miRNAs is associated with the progression of several skeletal muscle diseases. This implies that miRNAs have an enormous potential as therapeutic targets against numerous disorders, including pathologies of muscle. In particular miR-29 family members have been found dysregulated in muscular dystrophies[4,8]. The first evidence of a role of miR-29 in Duchenne muscular dystrophy (DMD) dates back to 2010, where a strong down-regulation of miR-29 was observed in *mdx* mice, a model of DMD. It was shown that loss of miR-29 in myoblasts promoted their trans-differentiation into myofibroblasts through upregulation of extracellular molecules including collagens and elastin, while restoration of its expression improved dystrophy pathology by both promoting regeneration and inhibiting fibrogenesis, suggesting a link between miR-29 dysregulation and the fibrotic process[4,8]. miR-29b/c were found strongly down-regulated also in muscle biopsies from patients of myotonic dystrophy type 1 (DM1)[8], and, more recently, decreased levels of RISC-complex-associated miR-29c were detected in DM1 biopsies, implying a reduced functionality of the miRNA in target inhibition[9]. Indeed, ASB2, a new target of miR-29c was identified as being upregulated in DM1 biopsies, and, interestingly, this protein is involved in fibrosis and negative regulation of muscle mass. Notably, CRISPR/Cas9-mediated deletion of the DM1 pathogenetic CTG expansions,

rescued normal miR-29c and ASB2 levels, indicating a direct link between the mutant repeats and the miRNA/target expression[9].

Having established that miR-29c plays a crucial role in driving myogenesis, increasing muscle mass and inhibiting muscle atrophy and fibrosis, Silva and colleagues highlight that this miRNA is an ideal candidate to use as a therapeutic tool to regulate these functions in pathological conditions[1]. One major challenge in using synthetic miRNAs for therapeutic purpose is that they are quickly degraded by the abundant ribonuclease present in tissues and in biological fluids. Recently, delivery of therapeutic miRNAs through extracellular vesicles, such as exosomes, has been successfully applied in animal models of disease[10]. The advantage of exosomes, compared to other RNA delivery vehicles, is that they stabilize miRNAs against degradation, and, being natural carriers of many signalling molecules, are less immunogenic, non-cytotoxic, and non-mutagenic to the recipient. In a recent paper, the effect of exosome-mediated miR-29 delivery on muscle wasting and fibrosis was evaluated in a mouse model of chronic kidney disease. The data from this study provide evidence that injection of miR-29-containing exosomes into the muscle of diseased mice reduced muscle atrophy and attenuated kidney fibrosis by a mechanism involving downregulation of YY1 and TGF-β pathway[10]. These results suggest that miR-29 may indeed represent an effective therapeutic tool for diseases where muscle wasting is involved.

Conflict of interest
None.

References

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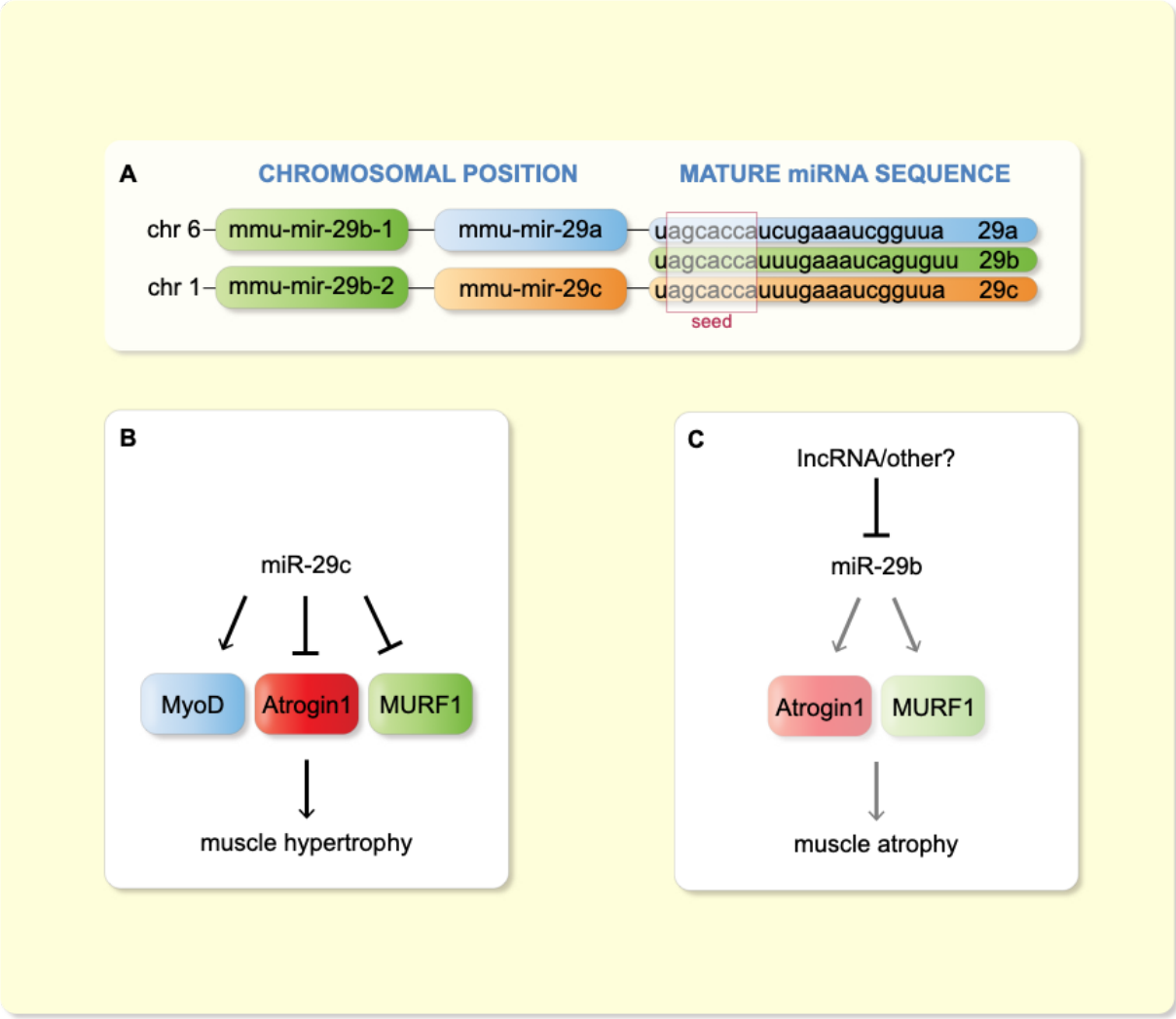

Figure 1. miR-29 family members description and distinct function in skeletal muscle. **(A)** miR-29 members have identical seed sequences (red box) and similar mature miRNA sequences. However, some nucleotide differences contribute to different stability, regulation and intracellular localization. **(B)** miR-29c induces hypertrophy by promoting differentiation through the activation of myogenic regulatory factors, such as MyoD, and inhibition of the atrophy-related factors Atrogin1 and MURF1. **(C)** In contrast to miR-29c, miR29-b induces the expression of Atrogin1 and MURF1 leading to muscle atrophy. In physiological conditions, this effect might be impaired by miRNA sequestration/degradation through binding to tissue specific long non-coding RNAs (lncRNA) or endogenous target transcripts or by other unknown mechanisms.