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Retinoid acid-induced microRNA-27b-3p impairs C2C12 myoblast proliferation and differentiation by suppressing α-dystrobrevin

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ABSTRACT

We previously reported that excess retinoic acid (RA) resulted in hypoplastic and derangement of myofilaments in embryonic tongue by inhibiting myogenic proliferation and differentiation through CamKIID pathway. Our further studies revealed that the expression of a series of miRNAs was altered by RA administration in embryonic tongue as well as in C2C12 cells. Thus, if excess RA impairs myogenic proliferation and differentiation through miRNAs is taken into account. In present study, miR-27b-3p was found up-regulated in RA-treated C2C12 cells as in embryonic tongue, and predicted to target the 3′UTR of α-dystrobrevin (DTNA). Luciferase reporter assays confirmed the direct interaction between miR-27b-3p and the 3′UTR of DTNA. MiR-27b-3p mimics recapitulated the RA repression on DTNA expression, C2C12 proliferation and differentiation, while the miR-27b-3p inhibitor circumvented these defects resulting from excess RA. As expected, the effects of siDTNA on C2C12 were coincided with those by RA treatment or miR-27b-3p mimics. Therefore, these findings indicated that excess RA inhibited the myoblast proliferation and differentiation by up-regulating miR-27b-3p to target DTNA, which implied a new mechanism in myogenic hypoplasia.

1. Introduction

Retinoic acid (RA) is a metabolic product of vitamin A requiring for organogenesis and growth in chordate animals [1]. Putatively, RA is regarded as a signaling molecule involved in neuronal patterning, neural differentiation, axon out-growth, myogenesis, limb patterning and development of many other organs [2,3]. Disruption of RA signaling has been implicated in degeneration of motor neurons and the deterioration of Alzheimer's disease [4,5]. Conversely, overdoses of RA are highly teratogenic due to the induction of abnormalities in organogenesis [6–8]. Administration of exogenous RA in mouse fetuses was reported to cause tongue deformity, cleft palate, and inhibit osteogenic differentiation of stem cells [7,9–11]. However, there are few studies concerning on the molecular mechanism how excess RA leads to these defects. Although we have identified CamKIID as an important component in the RA-induced tongue deformity, if there are more molecules or signaling pathways involved in RA-induced tongue deformity remains to be elucidated.

MicroRNAs (miRs) are highly conserved short single-stranded RNAs, which are comprised ~22-nucleotides in length. MiR can be imperfectly complementary to the 3′ untranslated regions (3′UTR) of its target mRNA, leading to translational repression or degradation of mRNAs through the RNA-induced silencing complex (RISC) at the post-transcriptional level. It is becoming increasingly evident that a cohort of miRs are involved in multiple physiological and biological processes, including myogenesis and muscle regeneration [12,13]. The miR-27 family consists of two members, miR-27a and miR-27b, which are located on chromosome 8 and 13, respectively [14]. Both miR-27a and miR-27b have been reported to promote myeloblast differentiation and inhibit multiple steps of adipogenesis [15,16]. Additionally, miR-27b also promote the differentiation of mesenchymal stem cells into osteoblasts by activating Wnt/β-catenin signaling and participate in numerous types of cancer, such in breast and ovarian [17,18]. Our preliminary study detected the change of miR-27b expression in RA-induced deformed tongue. However, the involvement of miR-27bin tongue muscle development remains unknown.

Our preliminary study predicted that the 3′UTR of α-dystrobrevin (DTNA) was a potential target of miR-27b-3p. Dystrobrevins have two isoforms: α-dystrobrevin (DTNA) and β-dystrobrevin. DTNA is believed to predominantly exert biological function in muscles, while β-dystrobrevin presents mainly in non-muscle tissues [19]. As a submembrane cytoskeletal protein, dystrobrevins belong to the family of a large macromolecular complex of proteins, named the dystrophin-associated protein complex (DAPC). The conformation of DAPC is

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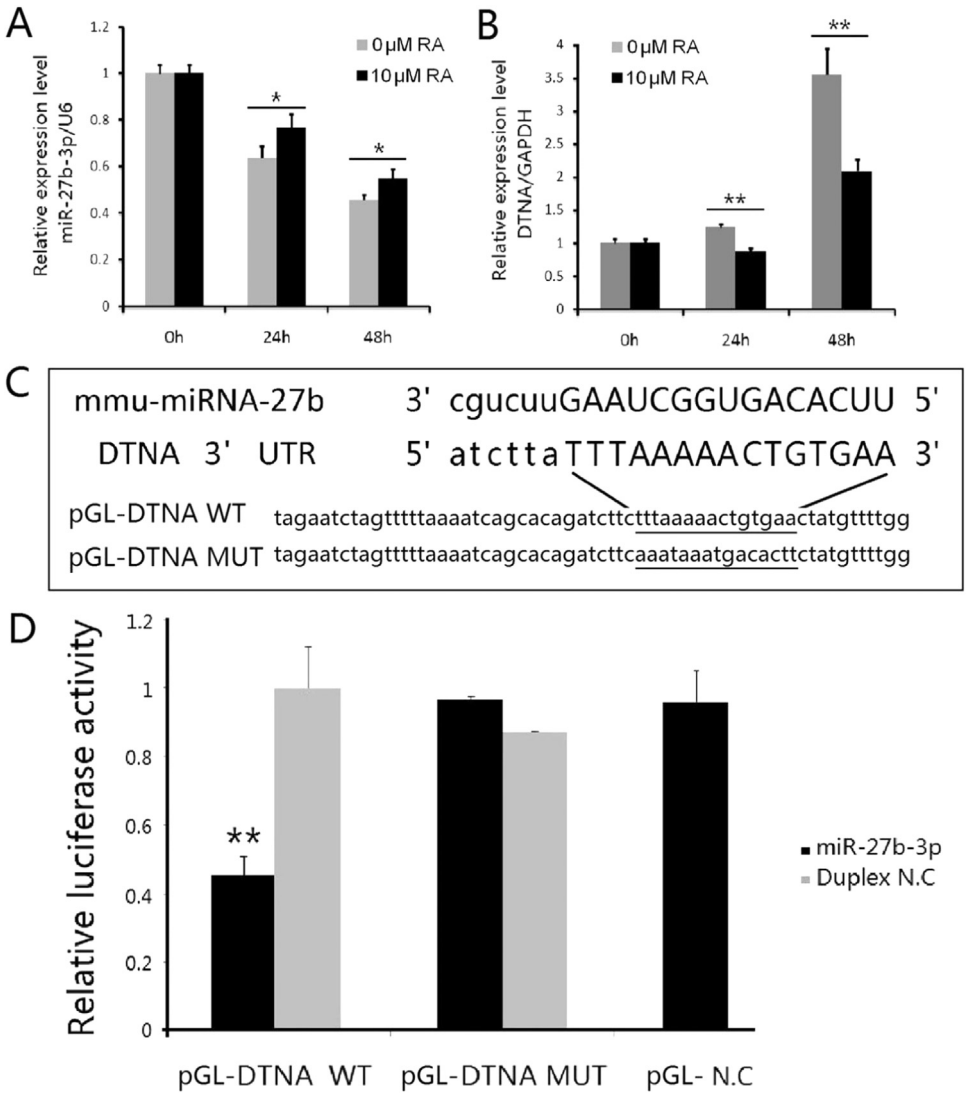

Fig. 1. miR-27b-3p was able to interact with DTNA directly. (A-B) The relative levels of miR-27b-3p and DTNA in the C2C12 myoblasts after addition of 10 μM of RA. In 48 h after RA addition, both the decrease of miR-27b-3p expression (A) and the increase of DTNA expression (B) were slowed down. **(C-D) Direct interaction between miR-27b-3p and DTNA**. The sequences predicted by TargetScan for the imperfect binding between mouse miR-27b (mmu-miRNA-27b) and 3′ UTR of DTNA, and the vector sequences of WT (pGL-DTNA WT) and mutant (pGL-DTNA MUT) miR-27b designed for luciferase activity assay (C). After 24 h of the co-transfection, the luciferase activity of pGL-DTNA WT vector was significantly reduced by miR-27b-3p compared with that by duplex N.C control; in contrast, no noticeable change in the pGL-DTNA MUT vector luciferase activities was detected when co-transfected with miR-27b-3p or duplex N.C (D). (The relative expression levels at difference time points in (A) & (B) were normalized by the GAPDH expression; the pGL-DTNA N.C in (D) was the empty vector working as inner control; ** in (D) means $P < 0.01$).

critical for the mechanical stabilization of skeletal sarcolemma, the scaffold of various signaling and the channel proteins of muscle cells by ensuring the structural integrity and contraction of myofibers [20–22]. The deficiency of DTNA in mice causes a mild muscular dystrophy, characterized by neuromuscular junction abnormalities and myofiber degeneration [23,24]. Since the DTNA deficient mouse exhibited muscular phenotype similar to that found in RA-induced deformed tongue, we hypothesize that excessive RA results in the disordered arrangement of muscles filaments by regulating miR-27b-3p expression which suppresses DTNA expression.

2. Materials and methods

2.1. Microarray

All procedures with mice were carried out in accordance with the strict ethical guidelines of the Ethics Committee of the Dalian Medical University (The ethical guidelines No. L2014034). 10–12 weeks old female C57/BL6J mice were obtained from the Animal Laboratory Center of the Dalian Medical University. The appearance of vaginal plug was designated to embryonic day 0.5 (E 0.5). The pregnant mice were randomly assigned to control and treatment groups on E10.5. The treatment group received RA (100 mg/kg), dissolved in edible oil. And the control groups were received with edible oil only. When the fetus reached E15.5, embryonic mice were dissected, and muscle samples were collected from the genioglossus and tongue body of fetuses. All samples were immediately frozen and stored separately at −80 °C (n=4 from each group). The de-phosphorylated total RNA (100 ng) and the denature, ligation and hybridization were measured according to the manufacturer's instructions with a μParafloTM mouse MicroRNA Microarray Array 19.0 (LC Sciences). Fluorescence images were scanned on a microarray scanner (GenePix 4000B, Molecular Device) and digitized with Array-Pro image analysis software (Media Cybernetics). All the microarray experiments data were submitted to the Gene Expression Omnibus (GEO). The GEO accession number is GSE67553.