The Journal of Experimental Biology 216, 1265-1269

doi:10.1242/jeb.079590

RESEARCH ARTICLE

miR-206 regulates the growth of the teleost tilapia (*Oreochromis niloticus*) through the modulation of IGF-1 gene expression

Biao Yan, Chang-Dong Zhu, Jin-Tao Guo, Li-Hui Zhao and Jin-Liang Zhao*

Key Laboratory of Freshwater Aquatic Genetic Resources, Shanghai Ocean University, Ministry of Agriculture, Shanghai Ocean University, Shanghai 201306, China

*Author for correspondence (jlzhao@shou.edu.cn)

SUMMARY

MicroRNAs (miRNAs) are ~22-nucleotide noncoding RNAs that play a crucial role in regulating muscle development. Our previous study shows that miR-206 is specifically expressed in tilapia skeletal muscle, and exhibits a dynamic expression pattern at different developmental stages. Here, we reveal that miR-206 emerges as a crucial regulator of tilapia growth. miR-206 loss of function leads to the acceleration of tilapia growth. IGF-1 is identified as the target gene of miR-206. miR-206 directly changes IGF-1 expression by targeting its 3′ UTR, and inhibition of miR-206 substantially increases the IGF-1 mRNA level *in vivo*. Thus, miR-206 could be developed as a molecular marker to assist fish breeding.

Key words: Nile tilapia, microRNA, growth performance, target gene.

Received 26 October 2012; Accepted 26 November 2012

INTRODUCTION

Nile tilapia, *Oreochromis niloticus* (Linnaeus 1758), is one of the most commonly farmed species in freshwater aquaculture. It has become a major source of protein around the world because of its superior growth performance and its outstanding adaptability to a wide range of environments (Davis et al., 2010; Ponzoni et al., 2010). Thus, better understanding of the regulatory mechanism of tilapia growth will provide important information for both developmental biologists and fish breeding experts.

MicroRNAs (miRNAs) are endogenous ~22-nucleotide noncoding RNAs that act as post-transcriptional regulators in animals and plants (Winter et al., 2009). They result in mRNA cleavage or translational repression by forming imperfect base pairs with the 3′ untranslated region (UTR) of the target gene (Bartel, 2004; Winter et al., 2009). miRNAs have been implicated in regulating numerous physiological processes such as embryogenesis, organ development, cellular differentiation and cellular apoptosis. miRNA deregulation can influence normal cell growth and development, resulting in numerous disorders (Fiore et al., 2008; Zhao and Srivastava, 2007). Furthermore, miRNAs are usually expressed in a tissue-specific manner (Kim et al., 2006; Makeyev and Maniatis, 2008; Sweetman et al., 2008). Our previous study has revealed that miR-206 is specially expressed in tilapia skeletal muscle, and its expression level is tightly associated with the developmental stage of tilapia (Yan et al., 2012a). Thus, we speculated that miR-206 may be involved in the regulation of growth in tilapia. However, the precise role of miR-206 in tilapia is still unclear.

Antagomirs are cholesterol-conjugated single-stranded RNA molecules, which are 21–23 nucleotides in length and complementary to the mature target miRNA (Krützfeldt et al., 2005). They are perfectly complementary to the specific miRNA target with either mispairing at the cleavage site of Ago2 or some sort of base modification to inhibit Ago2 cleavage (Krützfeldt et al., 2005). Antagomirs can block endogenous miRNA expression in mouse, zebrafish and cell lines (Ma et al., 2010; Morton et al., 2008; Selvamani et al., 2012). Here, we employed this method to inhibit endogenous miR-206 expression, and then examine the regulatory role of miR-206 in tilapia growth. We found that the administration of miR-206 antagomir results in a significant reduction in endogenous miR-206 expression, and a marked growth acceleration in tilapia through upregulation of insulin-like growth factor 1 (IGF-1) expression.

MATERIALS AND METHODS

Fish and growth experiment

Nile tilapia [*Oreochromis niloticus* (Linnaeus 1758)] were obtained from the fishery farm of Shanghai Ocean University. They were temporarily raised in a water circulation system in 100-liter tanks, and water temperature was kept at 28±3°C under a 12 h:12 h light:dark photoperiod. Each fish was anesthetized in sodium bicarbonate-buffered MS-222 (200 mg l^{-1}, Sigma, St Louis, MO, USA) prior to collection of required tissue sample. The study was performed according to the Guide for the Care and Use of Laboratory Animals of China.

Tilapia weighing approximately 1 g were injected with miR-206 antagomir, mismatched antagomir, or left untreated. Thirty fish for each treatment were cultured in 501 fiberglass tanks, and four replicates were set for this experiment. During the growth experiment, fish were fed with commercial feed – floating pellets containing 30% protein. Approximately 20% of the total volume of water in the tanks was changed daily. Dissolved oxygen, pH, total ammonia and temperature were measured every day. Water quality parameters were not analyzed in depth, but tests were conducted to determine whether there were significant differences in the key parameters (pH, dissolved oxygen, total ammonia) among different treatments. At the termination of the experiment, all the fish in each treatment were weighed collectively, and the average final mass was recorded. The following indices were calculated to interpret

growth performance (El-Sayed and Kawanna, 2008; Yan and Wang, 2010; Chen et al., 2011): (1) initial mass (g), wet mass of fish at the beginning of culture; (2) final mass (g), wet mass of fish at the final of culture; (3) daily mass gain (DMG), calculated as (final mass–initial mass)/culture days; (4) coefficient of variation (CV) for final mass, calculated as mean standard deviation of the final mass/mean final mass; (5) feed conversion ratio, calculated as feed consumption/mass gain; and (6) feed intake, calculated as 100×feed consumption/[days×(final mass+initial mass)/2)].

Quantitative PCR

Total RNAs were extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA), and miRNAs were extracted using the miRNeasy kit (Qiagen, Gaithersburg, MD, USA). Real-time PCR for IGF-1 was performed using SYBR Green PCR mixture (Takara Bio, Otsu, Shiga, Japan) in a MyiQ5 Real-time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Gene expression was normalized relative to the housekeeping gene (β-actin). The primer sequences are as follows: IGF-1, 5′-GTCTGTGGAGAGCGAGGCTTT-3′ (forward), 5′-CACGTGAC-CGCCTTGCA-3′ (reverse); β-actin, 5′-GTCCCTGTACGC-CTCTGGTCG-3′ (forward), 5′-GCCGGACTCATCGTACTCCTG-3′ (reverse). The relative amount of miRNA was detected using stem-loop PCR, and U6 RNA was detected as the internal control. Relative gene or miRNA expression was determined using the comparative C_t method, which is also referred to the $2^{-\Delta\Delta C_t}$ method (Chen et al., 2005; Schmittgen and Livak, 2008).

Silencing of miR-206 *in vivo* using the antagomir method

The antagomirs used in the study are single-stranded RNAs, which consist of 21–23 nucleotides modified as follows: antagomir-133a, CsAsGCUGGUUGAAGGGGACCsAs As As-Chol-3′; antagomir-206, CsCsACACACACUUCCUUACAUUs CsC sAs-Chol-3′; and mismatch antagomir, CsAsCGGUUCCAGGCACUGUsG sUs As-Chol-3′. All nucleotides are 2′-OMe-modified; the 's' represents a phosphorothioate linkage; 'Chol' represents cholesterol linked through a hydroxyprolinol linkage. They were deprotected, desalted and purified by high-performance liquid chromatography. Antagomir was dissolved in phosphate-buffered saline before injection. Tilapia weighing approximately 1 g received a 0.02 ml tail-vein injection of saline or antagomir at a dose of 60 mg kg^{-1} body mass twice a week. Tissues were harvested, snap-frozen and stored at –80°C.

Cell culture and 3′-UTR luciferase reporter assay

HEK 293T cells were obtained from the American Type Culture Collection (Rockville, MD, USA). They were maintained in Dulbecco's modified Eagle's medium with 10% fetal bovine serum, 100 U ml^{-1} penicillin, 100 μg ml^{-1} streptomycin and 250 ng ml^{-1} amphotericin B, and maintained at 37°C in a humidified 5% CO_2 incubator.

To generate the 3′ UTR luciferase reporter construct, the full length of the 3′ UTR from IGF-1 was cloned into the downstream of the firefly luciferase gene in pGL3-control vector (Promega, Madison, WI, USA). The QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA) was used to introduce one or two point mutations into the seed region of pGL3-IGF-1, giving pGL3-IGF-1 m1 and pGL3-IGF-1 m2. For luciferase reporter assays, HEK 293T cells were plated at 2×10^5 cells per well in 12-well dishes. Cells were transfected with either wild-type or mutant IGF-1 3′ UTR constructs, with and without miR-206 mimic or negative control mimic using Lipofectamine 2000 (Invitrogen). Luciferase activity was measured on a scintillation counter using a dual luciferase reporter system.

Statistical analysis

Values were expressed as means ± s.e.m. unless otherwise indicated. Significant differences across treatments were evaluated using one-way ANOVA with the Bonferroni *post hoc* test. The significance level was chosen at $P<0.05$.

RESULTS

Antagomir treatment represses endogenous miR-206 expression in tilapia

In our previous study, we successfully used the antagomir method to suppress endogenous miR-30c expression in tilapia (Yan et al., 2012b). Here, we determined whether the antagomir method is able to efficiently repress miR-206 expression *in vivo*. As shown in fig. 1 in Yan et al. (Yan et al., 2012b), miR-206 expression could be efficiently blocked by its corresponding antagomir (miR-206 antagomir) but not the mismatch miRNA (miR-133 antagomir). Furthermore, the silencing effect of antagomir could be detected at different time points (Fig. 1). Thus, the antagomir method is suitable for miRNA loss-of-function experiments in tilapia.

Effect of miR-206 silencing on the growth performance of tilapia

To determine the role of miR-206 in regulating tilapia growth, we employed the antagomir technique to achieve efficient miR-206 loss-

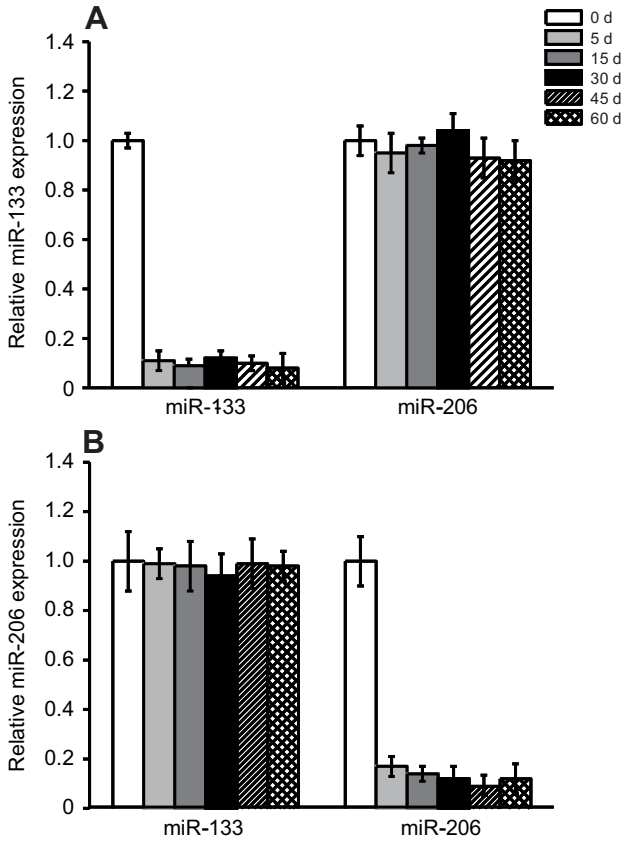

Fig. 1. Effect of antagomir treatment on endogenous miRNA expression in Nile tilapia, *Oreochromis niloticus*. Tilapia were injected with (A) miR-133 antagomir or (B) miR-206 antagomir. The expression of miR-133 or miR-206 was detected using stem-loop PCR. The relative amount of miR-133 or miR-206 was normalized to the endogenous control, U6 snRNA expression. Results are expressed as means ± s.e.m. of three independent experiments. Each sample was analyzed in triplicate. The results are expressed as the change in treated groups relative to the untreated group.

Table 1. Effects of miR-206 intervention on the growth performance of tilapia

	Untreated	Mismatched antagomir	miR-206 antagomir
Initial mass (g)	1.0±0.09[a]	0.95±0.04[a]	0.98±0.11[a]
Final mass (g)	25±2.4[a]	23±4.0[a]	36±5.2[b]
Survival rate (%)	98[a]	96[a]	95[a]
CV	0.10[a]	0.17[a]	0.14[a]
Daily mass gain	0.53[a]	0.49[a]	0.78[b]
Feed conversion ratio	3.3±0.26[a]	3.6±0.75[a]	2.4±0.39[b]
Feed intake (%)	3.2±0.23[a]	3.0±0.49[a]	2.94±0.31[a]

Values are expressed as means ± s.e.m. of four replicates. Means in the same row with different superscripts are significantly different from each other (one-way ANOVA with Bonferroni *post hoc* test, $P<0.05$).

of-function *in vivo*, and then compared the growth performance between different treatments. During the 45-day culture period, water temperature ranged from 22 to 28°C. Aeration was provided continuously, and dissolved oxygen was maintained at levels greater than 5 mg l^{-1}. pH was maintained between 7.4 and 8.4. Total ammonia (NH_3/NH_4) ranged from 1.2 to 2.5 mg l^{-1}. The survival rate of all fish was above 95%.

As shown in Table 1, no significant difference was observed for the initial mass and feed intake values across different groups. The growth rate (DMG) and feed conversion ratio were significantly affected by miR-206 intervention ($P<0.05$). The growth rate of miR-206 antagomir injection group was approximately 30% greater than that in the group injected with mismatch antagomir or the untreated group. Moreover, higher feed conversion ratios were observed in the miR-206 antagomir injection group than in the other two groups. These results suggest that miR-206 silencing results in growth acceleration in tilapia.

Identification of the target gene of miR-206 in tilapia

To understand the mechanism of miR-206 regulation of tilapia growth, we sought to identify miR-206 target genes that are responsible for regulating tilapia growth. In general, miRNA function involves uninterrupted base pairing between nucleotides 2 and 7 of the miRNA and a complementary sequence in the 3′ UTR of the target mRNA (Ulitsky et al., 2010). Based on this evidence, IGF-1 was predicted to be a potential target gene of miR-206. We first evaluated the correlation between the expression of miR-206 and IGF-1 in HEK 293T cells. Compared with the control group, transfection with miR-206 inhibitor but not the negative control miRNA inhibitor caused a significant decrease in miR-206 expression. Meanwhile, IGF-1 expression was markedly upregulated upon miR-206 inhibition (Fig. 2A,B). Moreover, we found that miR-206 inhibition by antagomir treatment resulted in a significant reduction in miR-206 expression, but a significant increase in IGF-1 expression in tilapia (Fig. 2C,D).

Next, we tested whether IGF-1 expression is post-transcriptionally regulated by miR-206. The 3′ UTR of IGF-1 was cloned into the downstream of the luciferase gene, and a luciferase reporter assay was conducted in HEK 293T cells. Cotransfection of miR-206 mimic with the luciferase reporter gene linked to the wild-type 3′ UTR of IGF-1 resulted in a significant decrease in luciferase activity. In contrast, cotransfection of a control miRNA mimic (not complementary to the 3′ UTRs of IGF-1) with the wild-type 3′ UTR constructs did not result in a decrease in luciferase activity. Furthermore, cotransfection of miR-206 mimic with the constructs containing mutated IGF-1 3′ UTR sequences did not result in a marked decrease in luciferase activity (Fig. 2E). Taken together, these results suggest that IGF-1 is a target gene of miR-206.

DISCUSSION

Growth in fish involves the recruitment and hypertrophy of muscle fibres. Fish muscle growth is not linear and occurs through a combination of hyperplasia and hypertrophy in post-juvenile stages. miRNAs are a class of non-coding RNAs that have emerged as crucial regulators in the expression and function of eukaryotic genomes. They can regulate skeletal muscle development through targeting of the crucial genes controlling myogenesis (Eisenberg et al., 2009; Williams et al., 2009). Given that fish growth is tightly associated with skeletal muscle development, the expression of muscle-specific miRNAs may be involved in regulating fish growth (Johnston et al., 2011; Rescan, 2008). Thus, the elucidation of miRNA function in muscle development will enhance our understanding of skeletal muscle biology and may provide information relevant to marker-assisted fish breeding. Previous studies reveal that cardiac and/or skeletal muscle is highly enriched in many miRNAs, including miR-1, miR-133, miR-206, miR-208, miR-486 and miR-499 (Güller and Russell, 2010; Ge and Chen, 2011). Here, we show that miR-206 participates in a regulatory circuit that allows rapid gene program transition during the regulation of tilapia growth.

miR-206 is a muscle-specific miRNA, and its role in muscle development has been verified in some animal models, including mouse, rat and zebrafish (Anderson et al., 2006; Kim et al., 2006; Mishima et al., 2009; Shan et al., 2009). Our previous study reveals that miR-206 is specifically expressed in tilapia skeletal muscle, and shows a dynamic expression pattern among different developmental stages (Yan et al., 2012a). These results lead us to consider whether its expression level can affect the growth performance of tilapia. As expected, miR-206 loss-of-function *in vivo* significantly improves tilapia growth performance. It is well known that the miRNA-guided regulatory network induces mRNA cleavage or translational repression by forming imperfect base pairs with the 3′ UTR region of the target gene. We further reveal that IGF-1 is a direct target gene of miR-206.

IGF-1 is one of the important members in IGF signaling, and is involved in the regulation of growth and development of skeletal muscle in many vertebrates (Duan et al., 2010). For example, the birth mass of IGF-1 or IGF-2 knockout mice is approximately 60% of that of their wild-type littermates (Liu et al., 1993). Overexpression of IGF-1 in mice increases the body mass by 30% (Mathews et al., 1988). In many fish species, IGF-1 blood levels or tissue IGF-1 mRNA levels positively correlate with dietary ration, dietary protein content and body growth rate (Carnevali et al., 2006; Beckman et al., 2004). Treatment of fish with IGF-1 implants contributes to accelerating growth (McCormick et al., 1992). In this study, miR-206 inhibition results in a significant increase in IGF-1 expression. Thus, we speculate that miR-206

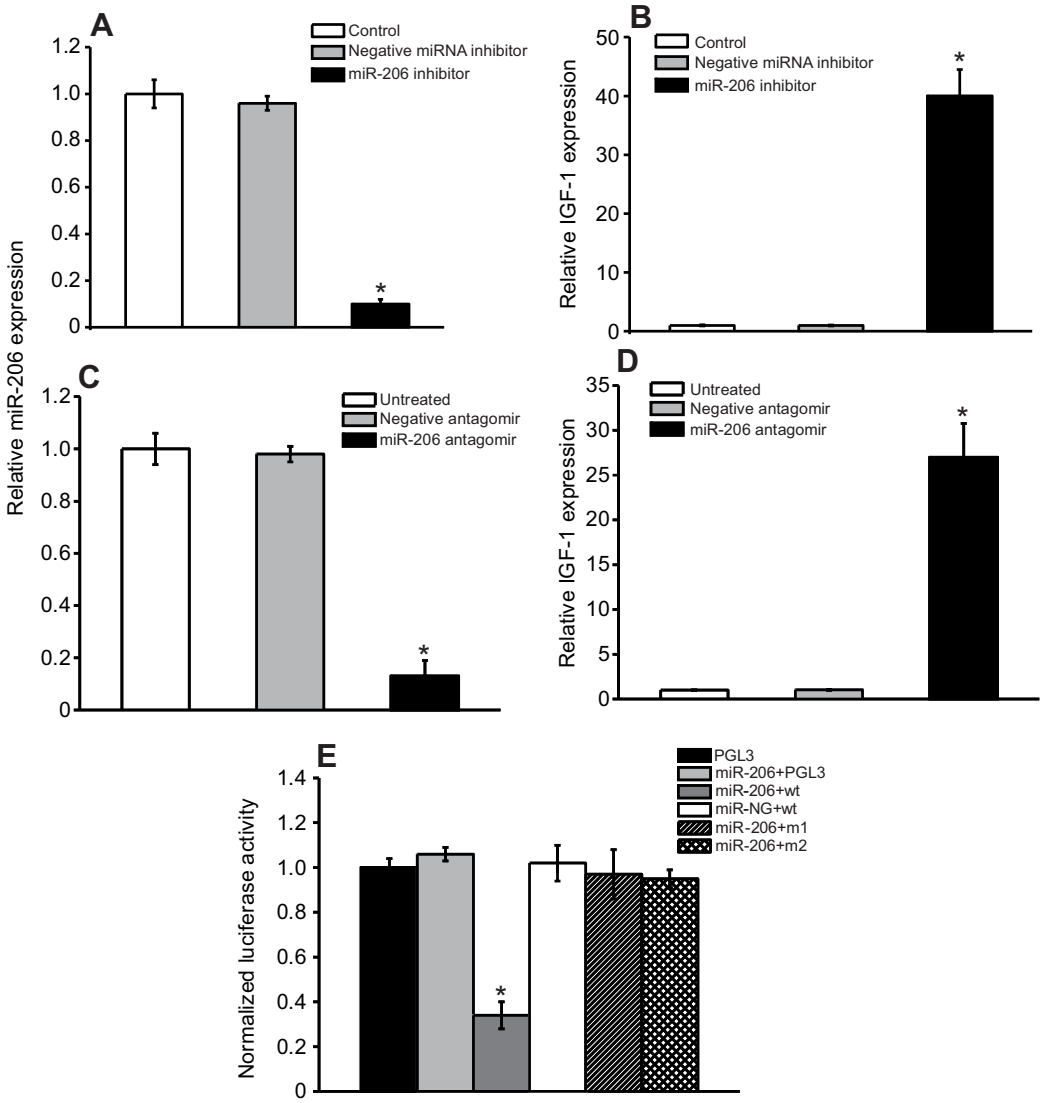

Fig. 2. Identification of IGF-1 as the target gene of miR-206 in Nile tilapia. (A) HEK 293T cells were transfected with negative control miRNA inhibitor, miR-206 inhibitor or left untreated for 48 h. The level of miR-206 expression was determined using stem-loop PCR. The relative amount of miR-206 was normalized to endogenous control U6 snRNA expression. (B) HEK 293T cells were treated as in A. The level of IGF-1 expression was detected using real-time PCR. The relative amount of IGF-1was normalized to β-actin expression. (C,D) Tilapia were injected with miR-206 antagomir, mismatched antagomir or left untreated. The expression of miR-206 or IGF-1 was detected as described in the Materials and methods. Results are expressed as means ± s.e.m. of three independent experiments. Asterisks indicate a significant difference compared with the control group. (E) Luciferase assays were carried out to address whether IGF-1 was directly targeted by miR-206. Luciferase activities were normalized to the group transfected with PGL3. HEK 293T cells were transfected as shown, and luciferase activity was determined 48 h after transfection. The cells tranfected with PGL3 plus miR-206 were used as the control group. Data represent the means ± s.e.m. from three independent experiments (*P<0.05). wt, PGL3-IGF-3′-UTR; m1, PGL3-IGF-3’-UTR mutant 1; m2, PGL3-IGF-3′-UTR mutant 2; miR-NG, miRNA that is not complementary to the 3′ UTRs of IGF-1.

regulation of tilapia growth is mainly mediated by the upregulation of IGF-1 expression. IGF-1 mRNA is detected in all life stages of fish, ranging from the unfertilized egg to the adult. The temporal and spatial expression patterns of fish IGF-1 seem to be similar to those in mammals. In addition, nutrition condition or hormone treatment changes IGF-1 levels in fish as they do in mammals. These features suggest that the IGF system is highly conserved between teleost fish and mammals (Duan, 1998; Bower et al., 2008; Johnston et al., 2011). Thus, understanding of tilapia IGF-1 action would contribute to knowledge of the basic growth physiology of vertebrates and provide important information about muscle hypertrophy, muscle atrophy and muscle regeneration of other vertebrates.

In summary, we found that miRNA antagomir is able to repress endogenous miRNA expression in tilapia. Treatment with miR-206 antagomir results in a significant reduction in endogenous miR-206 levels, and an obvious acceleration of tilapia growth through an increase in IGF-1 expression. Given the role of miR-206 in tilapia growth, it can be developed as a promising molecular marker to assist the selection of faster-growing fish strains.

FUNDING

This work was supported by China Agriculture Research System (grant no.CARS-49-4B to J.-L.Z.), National Key Technology Research and Development Program of the Ministry of Science and Technology of China (grant no. 2012BAD26B03-01 to B.Y.), and the Specialized Research Fund for the Doctoral Program of Higher Education of China (grant no. 20123104120005 to B.Y.).

REFERENCES

Anderson, C., Catoe, H. and Werner, R. (2006). MIR-206 regulates connexin43 expression during skeletal muscle development. *Nucleic Acids Res.* **34**, 5863-5871.

Bartel, D. P. (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* **116**, 281-297.

Beckman, B. R., Shimizu, M., Gadberry, B. A. and Cooper, K. A. (2004). Response of the somatotropic axis of juvenile coho salmon to alterations in plane of nutrition with an analysis of the relationships among growth rate and circulating IGF-I and 41 kDa IGFBP. *Gen. Comp. Endocrinol.* **135**, 334-344.

Bower, N. I., Li, X., Taylor, R. and Johnston, I. A. (2008). Switching to fast growth: the insulin-like growth factor (IGF) system in skeletal muscle of Atlantic salmon. *J. Exp. Biol.* **211**, 3859-3870.

Carnevali, O., de Vivo, L., Sulpizio, R., Gioacchini, G., Olivotto, I., Silvi, S. and Cresci, A. (2006). Growth improvement by probiotic in European sea bass juveniles (*Dicentrarchus labrax*, L.), with particular attention to IGF-1, myostatin and cortisol gene expression. *Aquaculture* **258**, 430-438.

Chen, C., Ridzon, D. A., Broomer, A. J., Zhou, Z., Lee, D. H., Nguyen, J. T., Barbisin, M., Xu, N. L., Mahuvakar, V. R., Andersen, M. R. et al. (2005). Real-time quantification of microRNAs by stem-loop RT-PCR. *Nucleic Acids Res.* **33**, e179.

Chen, W., Ai, Q., Mai, K., Xu, W., Liufu, Z., Zhang, W. and Cai, Y. (2011). Effects of dietary soybean saponins on feed intake, growth performance, digestibility and intestinal structure in juvenile Japanese flounder (*Paralichthys olivaceus*). *Aquaculture* **318**, 95-100.

Davis, L. K., Fox, B. K., Lim, C., Lerner, D. T., Hirano, T. and Grau, E. G. (2010). Effects of 11-ketotestosterone and fishmeal in the feed on growth of juvenile tilapia (*Oreochromis mossambicus*). *Aquaculture* **305**, 143-149.

Duan, C. (1998). Nutritional and developmental regulation of insulin-like growth factors in fish. *J. Nutr.* **128 Suppl.**, 306S-314S.

Duan, C., Ren, H. and Gao, S. (2010). Insulin-like growth factors (IGFs), IGF receptors, and IGF-binding proteins: roles in skeletal muscle growth and differentiation. *Gen. Comp. Endocrinol.* **167**, 344-351.

Eisenberg, I., Alexander, M. S. and Kunkel, L. M. (2009). miRNAS in normal and diseased skeletal muscle. *J. Cell. Mol. Med.* **13**, 2-11.

El-Sayed, A. F. M. and Kawanna, M. (2008). Optimum water temperature boosts the growth performance of Nile tilapia (*Oreochromis niloticus*) fry reared in a recycling system. *Aquac. Res.* **39**, 670-672.

Fiore, R., Siegel, G. and Schratt, G. (2008). MicroRNA function in neuronal development, plasticity and disease. *Biochim. Biophys. Acta* **1779**, 471-478.

Ge, Y. and Chen, J. (2011). MicroRNAs in skeletal myogenesis. *Cell Cycle* **10**, 441-448.

Güller, I. and Russell, A. P. (2010). MicroRNAs in skeletal muscle: their role and regulation in development, disease and function. *J. Physiol.* **588**, 4075-4087.

Johnston, I. A., Bower, N. I. and Macqueen, D. J. (2011). Growth and the regulation of myotomal muscle mass in teleost fish. *J. Exp. Biol.* **214**, 1617-1628.

Kim, H. K., Lee, Y. S., Sivaprasad, U., Malhotra, A. and Dutta, A. (2006). Muscle-specific microRNA miR-206 promotes muscle differentiation. *J. Cell Biol.* **174**, 677-687.

Krützfeldt, J., Rajewsky, N., Braich, R., Rajeev, K. G., Tuschl, T., Manoharan, M. and Stoffel, M. (2005). Silencing of microRNAs *in vivo* with 'antagomirs'. *Nature* **438**, 685-689.

Liu, J. P., Baker, J., Perkins, A. S., Robertson, E. J. and Efstratiadis, A. (1993). Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). *Cell* **75**, 59-72.

Ma, L., Reinhardt, F., Pan, E., Soutschek, J., Bhat, B., Marcusson, E. G., Teruya-Feldstein, J., Bell, G. W. and Weinberg, R. A. (2010). Therapeutic silencing of miR-10b inhibits metastasis in a mouse mammary tumor model. *Nat. Biotechnol.* **28**, 341-347.

Makeyev, E. V. and Maniatis, T. (2008). Multilevel regulation of gene expression by microRNAs. *Science* **319**, 1789-1790.

Mathews, L. S., Hammer, R. E., Behringer, R. R., D'Ercole, A. J., Bell, G. I., Brinster, R. L. and Palmiter, R. D. (1988). Growth enhancement of transgenic mice expressing human insulin-like growth factor I. *Endocrinology* **123**, 2827-2833.

McCormick, S. D., Kelley, K. M., Young, G., Nishioka, R. S. and Bern, H. A. (1992). Stimulation of coho salmon growth by insulin-like growth factor I. *Gen. Comp. Endocrinol.* **86**, 398-406.

Mishima, Y., Abreu-Goodger, C., Staton, A. A., Stahlhut, C., Shou, C., Cheng, C., Gerstein, M., Enright, A. J. and Giraldez, A. J. (2009). Zebrafish miR-1 and miR-133 shape muscle gene expression and regulate sarcomeric actin organization. *Genes Dev.* **23**, 619-632.

Morton, S. U., Scherz, P. J., Cordes, K. R., Ivey, K. N., Stainier, D. Y. R. and Srivastava, D. (2008). microRNA-138 modulates cardiac patterning during embryonic development. *Proc. Natl. Acad. Sci. USA* **105**, 17830-17835.

Ponzoni, R. W., Khaw, H. L., Nguyen, N. H. and Hamzah, A. (2010). Inbreeding and effective population size in the Malaysian nucleus of the GIFT strain of Nile tilapia (Oreochromis niloticus). *Aquaculture* **302**, 42-48.

Rescan, P. Y. (2008). New insights into skeletal muscle development and growth in teleost fishes. *J. Exp. Zool.* **310B**, 541-548.

Schmittgen, T. D. and Livak, K. J. (2008). Analyzing real-time PCR data by the comparative C_T method. *Nat. Protoc.* **3**, 1101-1108.

Selvamani, A., Sathyan, P., Miranda, R. C. and Sohrabji, F. (2012). An antagomir to microRNA Let7f promotes neuroprotection in an ischemic stroke model. *PLoS ONE* **7**, e32662.

Shan, Z. X., Lin, Q. X., Fu, Y. H., Deng, C. Y., Zhou, Z. L., Zhu, J. N., Liu, X. Y., Zhang, Y. Y., Li, Y., Lin, S. G. et al. (2009). Upregulated expression of miR-1/miR-206 in a rat model of myocardial infarction. *Biochem. Biophys. Res. Commun.* **381**, 597-601.

Sweetman, D., Goljanek, K., Rathjen, T., Oustanina, S., Braun, T., Dalmay, T. and Münsterberg, A. (2008). Specific requirements of MRFs for the expression of muscle specific microRNAs, miR-1, miR-206 and miR-133. *Dev. Biol.* **321**, 491-499.

Ulitsky, I., Laurent, L. C. and Shamir, R. (2010). Towards computational prediction of microRNA function and activity. *Nucleic Acids Res.* **38**, e160.

Williams, A. H., Liu, N., van Rooij, E. and Olson, E. N. (2009). MicroRNA control of muscle development and disease. *Curr. Opin. Cell Biol.* **21**, 461-469.

Winter, J., Jung, S., Keller, S., Gregory, R. I. and Diederichs, S. (2009). Many roads to maturity: microRNA biogenesis pathways and their regulation. *Nat. Cell Biol.* **11**, 228-234.

Yan, B. and Wang, Z. (2010). Growth, salinity tolerance and microsatellite analysis of the F_2 reciprocal hybrids of *Oreochromis niloticus*× *Sarotherodon galilaeus* at different salinities. *Aquac. Res.* **41**, e336-e344.

Yan, B., Guo, J. T., Zhao, L. H. and Zhao, J. L. (2012a). microRNA expression signature in skeletal muscle of Nile tilapia. *Aquaculture* **364-365**, 240-246.

Yan, B., Guo, J. T., Zhao, L. H. and Zhao, J. L. (2012b). MiR-30c: a novel regulator of salt tolerance in tilapia. *Biochem. Biophys. Res. Commun.* **425**, 315-320.

Zhao, Y. and Srivastava, D. (2007). A developmental view of microRNA function. *Trends Biochem. Sci.* **32**, 189-197.