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Short comunication

Quantitative Expression Analysis of *Myostatin* Gene in Nile Tilapia (*Oreochromis niloticus*) Tissues in Adult Stage

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ABSTRACT

In this study the differential expression of *Myostatin* gene was investigated in adult stage in Nile tilapia (*Oreochromis niloticus*) by realtime PCR. *Myostatin* gene was found to be expressed in various body tissues including: liver, intestine, gills, skin, spleen, kidneys, muscle, eyes and brain tissues. However, the highest expression level of *Myostatin* was found in brain tissue while the lowest expression level was in the spleen and eyes. These results support the early hypothesis suggested that *Myostatin* in fish have retained a different partition of the expression patterns compared with mammals.

Key words: myostatin, Nile tilapia, *Oreochromis niloticus*, GDF-8, gene expression

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1. INTRODUCTION

Myostatin (also known as GDF8) which is a member of the transforming growth factor-ß superfamily. Myostatin is expressed in developing and adult muscle tissue and functions to negatively regulate muscle growth in mammals (McPherron *et al.*, 1997). Muscle growth is known to result from the proliferation of myoblasts and their subsequent differentiation into muscle fibers. This process is regulated in vivo through mechanisms that involve cell-to-cell interactions, cell-to-matrix interactions, and extracellular secreted factors including myostatin (Lee 2004).

A myostatin-null phenotype in domestic mammals is characterized by extreme gains in muscle mass, commonly referred to as 'double muscling' (Kambadur *et al.*, 1997, McPherron & Lee 1997). In addition, a 50 splice site mutation in the first intron of the human *Myostatin* gene has been reported in a child with extraordinary musculature (Schuelke *et al.*, 2004). Myostatin null-phenotype in mice generated by gene silencing show a widespread and dramatic increase in skeletal muscle mass known as double-muscling due to muscle fiber hyperplasia and hypertrophy, suggesting a possible inhibitory role of Myostatin during muscle growth and development (Radaelli *et al.*, 2003 and Garikipati *et al.*, 2006). These findings together suggest that the biological functions of Myostatin are conserved in all mammals. *MSTN* has also been cloned from representatives of various vertebrate groups (Biga *et al.*, 2005).Increased muscle growth in all these models results from both muscle cell hyperplasia and hypertrophy as *MSTN* influences myosatellite cells directly (Thomas *et al.*, 2000, Rios *et al.*, 2001, 2002, Langley *et al.*, 2002, 2004, McCroskery *et al.*, 2003). These results together suggest that the biological functions of *MSTN* are conserved in all mammals, although they are yet to be described in other vertebrates.

In Teleosts, *MSTN* gene characterization and expression has been examined in several fish species such as zebrafish (McPherron and Lee 1997), tilapia and sea bass (Rodgers *et al.*, 2001), striped bass and white perch (Rodgers and Weber 2001), brook trout, yellow perch, mahi-mahi, little tunny and king mackerel (Roberts and Goetz 2001), Atlantic salmon (Ostbye *et al.*, 2001), rainbow trout (Rescan *et al.*, 2001), sea bream and shi drum (Radaelli *et al.*, 2003, Maccatrozzo *et al.*, 2001a, 2002), and channel catfish (Kocabas *et al.*, 2002). However, two distinct myostatin genes have been demonstrated in Atlantic salmon (Ostbye *et al.*, 2001), rainbow trout (Rescan *et al.*, 2001) and sea bream (Maccatrozzo *et al.*, 2001b).

Unlike mammals, in fish, myostatin is expressed in several tissues in addition to its expression in muscle (Maccatrozzo *et al.*, 2001a, 2001b, 2002, Ostbye *et al.*, 2001, Rescan *et al.*,

2001, Roberts and Goetz 2001, Rodgers *et al.,* 2001, Kocabas *et al.,* 2002) where myostatin mRNA was found to be expressed in tissues including muscle tissue, gill filaments, eyes, spleen, ovaries, gut, brain, and testes (Maccatrozzo *et al.,* 2001a,b, Rodgers *et al.,* 2001). Although little is known about the exact function of *Myostatin* in other body tissues in fish species. However, the expression pattern of *MSTN in Nile tilapia is unknown.*

The aim of this study is to study the expression pattern of *MSTN* gene in different organs in adult *Nile tilapia* fish.

2. MATERIALS AND METHODS

2.1. Fish sampling and RNA extraction:

Adult *Oreochromis niloticus* fish (one year old) were collected from a fish farm in Kafr Elsheikh governorate and different tissue samples were collected from each fish in a 2 ml microfuge tubes, preserved in liquid nitrogen and transferred to the Biotechnology laboratory in Faculty of Veterinary Medicine, Kafr Elsheikh University for RNA extraction.

The total RNA was extracted from liver, intestine, gills, skin, spleen, kidney, muscle, eye and brain tissue samples of the five different adult fish using Tri-sure reagent (Bioline, United Kingdom) according to the manufacturer's protocol. The quality and concentration of the extracted RNA was confirmed with Nanodeop. The integrity and quantity of all extracted RNA were checked by electrophoresis in 1.5% ethidium bromide-stained denaturing gel (Sigma, Germany) at 100 V in 1x Tris-acetate acid-EDTA (TAE) buffer, pH 8.0. The gel image was visualized using UV trans-illuminator (SybGene).

2.2. cDNA Synthesis and Quantitative real-time PCR.

Real-time reverse transcription polymerase chain reaction (RT-PCR) analysis of mRNA expression *for O.niloticus MSTN gene* and β-actin (as a reference gene) was performed using the following primers for Myostatin gene exon1 (Elkatatny *et al.*, 2016) forward primer 5'GCATCTGTCTCAGATCGTGCT3' and reverse primer 5'TGCCATCATTACAATTGTCTCCG3' and *β-actin* (Pang *et al.,* 2013) forward primer 5'CAGCAAGCAGGAGTACGATGAG 3' and reverse primer TGTGTGGTGTGTGGTTGTTTTG. 2µg of total RNA was reverse transcribed to first-strand cDNA kits (Bioline, United Kingdom) according to the manufacturer's instructions. The cDNAs were used as the template for RT-PCR. SensiFastTM SYBR Lo-Rox kit (Bioline, United Kingdom) was used in Mx3005P Real-time PCR system (Agilent Technologies, Santa Clara, CA, USA). The relative differences in gene expression were calculated using threshold cycle (CT) values that were first normalized to those of the *Oreochromis niloticus* β- actin house-keeping gene and using ΔCT value of kidney as a calibrator using the $2^{-\Delta\Delta CT}$ method "as previously described by Livak and Schmittgen (2001). All samples were performed and analyzed in triplicate.

3. RESULTS AND DISCUSSION

The amplification blot of *MSTN* gene in different organs are shown in Fig. (1). Expression level of Nile Tilapia *MSTN* gene in different examined tissues in this work showed highest expression level in the brain tissue (13.62) and skin (9.78), while the lowest expression level was observed in spleen (0.14) and eye tissues (0.18) of the adult fish as shown in figures 1 and 2.

The tissue-specific expression pattern of *MSTN* (*tMSTN*) was determined by RT-PCR. In most mammalian species, *tMSTN* expression is believed to occur primarily in skeletal muscle and to a much lesser degree in bovine cardiomyocytes and Purkinje fibers (Rodgers *et al.,* 2001). However, *tMSTN* expression in the recent study was detected in a variety of tissues, including the gill filaments, intestine, skin, kidney, and brain however, a minimal amount of *Myostatin* was detected in eyes and spleen (Rodgers *et al.,* 2001). *MSTN* gene was also expressed in different organs of rainbow trout including brain, gills, skin, muscle, heart and gonads (Garikipati *et al.*, 2007) in the mouth epithelium and pharynx of adult Zebrafish and in seabream stomach tissue (Radaelli *et al.,* 2003). In adult Barramundi species *MSTN* was found in almost all tissues in different expression levels (De Santis *et al.,* 2008).

The expression of *MSTN* in tissues other than skeletal muscle suggests that the biological actions of *MSTN* in tilapia, and possibly in other fishes, may not be limited to skeletal muscle growth inhibition, but may also contribute to the homeostatic growth control of other tissues. The diversity of tissues expressing *tMSTN* suggests that its actions may be equally diverse and may not necessarily be limited to growth control.

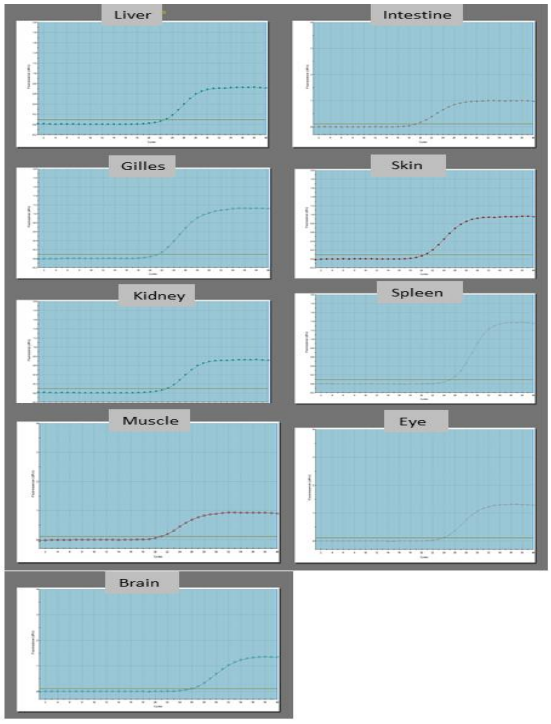

Figure (1): The amplification blot of *MSTN* gene in different organs of Nile tilapia (*Oreochromis niloticus*).

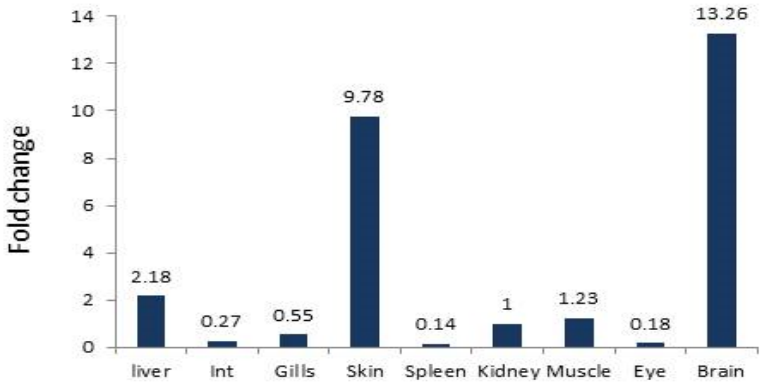

Fig.2. Expression level of Nile Tilapia *MSTN*gene in different organs.

tMSTN could potentially regulate some species-specific physiological processes that are unrelated to skeletal muscle growth altogether. For example, *tMSTN* expression in the gill filaments of this euryhaline teleost suggests that it may participate either directly or indirectly in osmoregulation (Radaelli *et al.*, 2003). Its expression in the brain may suggest that *tMSTN* helps to coordinate neuronal growth and development, which, unlike in mammals, continues throughout the fish lifecycle. *Myostatin* mRNA was found both in kidneys and gills of several fish species (Ostbye *et al.*, 2001, Rodgers *et al.*, 2001, *Kocabas et al.*, 2002, Maccatrozzo *et al.*, 2001a,

2002), although in other cases a lack of *Myostatin* expression in the kidney has been reported (Roberts and Goetz 2001, Rodgers *et al.*, 2001).

We conclude that *MSTN* gene may have other functions rather than growth control in fish. However, further studies are needed in order to clarify whether *Myostatin* gene in fish is a growth-inhibitory factor as it is in mammals or it has a different function.

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