

CHARACTERIZATION OF MYOSTATIN IN BROOK TROUT

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EXTENDED ABSTRACT ONLY-DO NOT CITE

Myostatin (MSTN), a member of the Transforming Growth Factor- β (TGF- β) superfamily, has been established as a regulator of development and growth in several vertebrates. MSTN, originally termed growth and differentiation factor - 8 (GDF-8), is a negative regulator of muscle mass and thus has the potential to be an important factor in the production of farmed animals. A dramatic increase in muscle mass has been observed in mutant mice and artificially selected bovine species such as the Belgian Blue (Lee and McPherron, 1999). To understand the role that MSTN could have in the growth and development of fish, the present study characterized MSTN expression in the brook trout.

Methods

Sampling

Male and Female brook trout were sampled in April (1 yr old), July (1.5 yr old), and October (2 yr old). For the analysis of brook trout embryos, eggs were fertilized and raised in Heath incubators for 12 weeks. Samples were taken 10 times throughout the period from fertilization to swim-up.

mRNA and RNA Analysis

The full-length MSTN cDNA clone observed in brain and muscle tissue (b/m MSTN) obtained from cDNA library screening and the brook trout cDNA fragment from ovarian tissue (ov BT MSTN), were used to probe brook trout mRNA tissue blots. Total RNA samples were analyzed quantitatively using Taqman probes designed to hybridize to brook trout b/m MSTN, ov MSTN, and 18S RNA. Quantitative RT-PCR (Thermoscript One-Step System - Invitrogen) was used with the Opticon Continuous Fluorescence Detection System (MJ Research).

Protein Analysis

Recombinant MSTN protein was produced using the region coding for the mature peptide bp 794-1120 (Accession# AF247650). This 326 bp fragment obtained from PCR was directly inserted into the pBAD TOPO/*LacZ* vector and transformed into TOP10 *E. coli* cells (Invitrogen). Recombinant protein was produced, purified using His-Bind Resin chromatography (Novagen), and used to produce a polyclonal antibody in rabbits. Protein (10 ug) from adult muscle tissue or whole embryos was transferred to membranes, hybridized with antibody, and quantified by Western blotting.

Results

On Northern blots, a 2.7 kb transcript was observed in red muscle when probed with the b/m BT MSTN cDNA (Figure 1). A less abundant transcript was present in brain (Figure 1). When the full-length b/m BT MSTN cDNA was used as a probe with ovarian tissue taken at ovulation, no hybridization was observed. On Northern blots of ovarian tissue taken at different reproductive stages and probed with the ov BT MSTN cDNA fragment, increased transcript levels were observed during ovulation in several individuals (Roberts and Goetz, 2001)

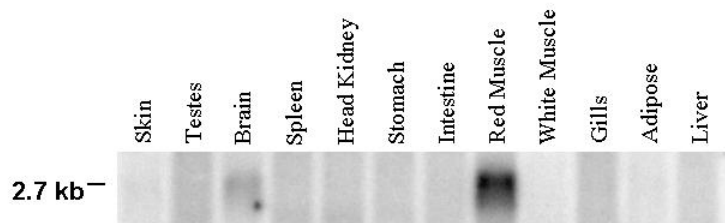


Fig. 1: Northern blot of mRNA (0.5 ug/lane) from brook trout tissues

Quantitative RT-PCR data were similar to the results of Northern analysis with red muscle generally having higher levels of b/m MSTN compared with white muscle (Figure 2). The difference in amounts of b/m MSTN RNA among red and white muscle varied, with the greatest difference observed in the fall. Levels of ov MSTN RNA were observed in all muscle tissue samples taken, though the levels were less than b/m MTSN RNA.

No b/m MSTN was detected in any of the embryos sampled. Levels of ov MSTN were observed in all embryo samples except for unfertilized eggs and only occasionally in eggs 2 days after fertilization. Levels of ov MSTN were comparable to levels observed in adult muscle tissue.

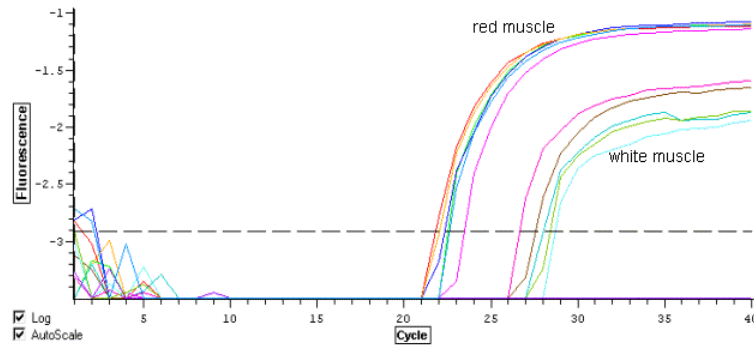


Fig 2. Representative b/m MSTN quantitative real time RT-PCR data from female brook trout red (n=5) and white (n=5) muscle tissue sampled in October.

The MSTN antibody recognized a single immunoreactive band of ~50 kDa. Protein expression was similar to RT-PCR data in that higher levels of an immunoreactive protein were observed in red muscle with the greatest difference seen in the Fall. In general, males had slightly higher levels of protein in both red and white muscle. In brook trout embryos, expression of immunoreactive MSTN protein was evident 45 days after fertilization.

Conclusions

These data are consistent with investigations characterizing MSTN in other teleosts in which multiple isoforms of MSTN were reported. This study demonstrates the presence of MSTN RNA and immunoreactive protein in several life stages of brook trout and the differential expression among muscle type, gender, and season. One explanation for the difference in MSTN expression in adult fish could be related to the reproductive status, as gonad development has been shown to influence somatic growth rates. Quantitative RT-

PCR analysis also demonstrated that both MSTN isoforms are found in adult muscle tissue. Therefore, the previously named “ov MSTN” is actually expressed in multiple tissues. The presence of ov MSTN in brook trout embryos could be the result of a maternal transfer of MSTN from the ovary to the developing larvae. The absence of b/m MSTN RNA in larval fish could indicate a functional role for this isoform as a negative regulator of muscle mass since muscle increases during this stage of development.

References

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