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Differences in gene expression in myostatin-mutant Belgian White-Blue satellite cell cultures during differentiation

Julianna Kobolák[1], Botond Görhöny[1], István Király[2], Károly Kiss[2], Károly Bölcskey3, Elen Gócza[1], Zsuzsanna Bősze[1]

1 Institute of Animal Sciences, Agricultural Biotechnology Center, H-2100 Gödöllő, Hungary; 2 Petőfi Mgtsz H-9512 Ostffyasszonyfa, Hungary; 3 Research Institute for Animal Breeding and Nutrition, H-2053 Herceghalom, Hungary

Abstract

The myostatin protein is a regulator factor in normal muscle that determines the amount of muscle mass. If the myostatin gene is mutant, its negative regulating function does not work resulting in muscle hypertrophy and hyperplasia. In the view of quality meat production, this is an outstanding trait. This phenomenon occurs in Belgian White-Blue breed, where 'double-muscled' phenotype is common due to successful selection. Crossing with Belgian White-Blue shows that, although, the gene is recessive and monofactorial, its effect is apparent even in heterozygotes due to its partial dominance: the meat:bone ratio and meat yield is better than in the other parent breed.

Muscle stem cell (satellite cell) cultures were isolated from biopsy of hindlimb muscle (*m.gluteus medius*) of one-year-old Belgian White-Blue bulls. For controls, muscle biopsies and cell cultures obtained from Holstein-Friesian bulls of the same age were used. Expression of 13 genes playing role in muscle differentiation was examined. In mutant cells, a significant increase was observed in the level of MRFs, Igf-1, Cdk2, and a decrease was observed in p21. The difference detected in early stages of differentiation supposes that myostatin mutation may affect muscle differentiation causing hypertrophy.

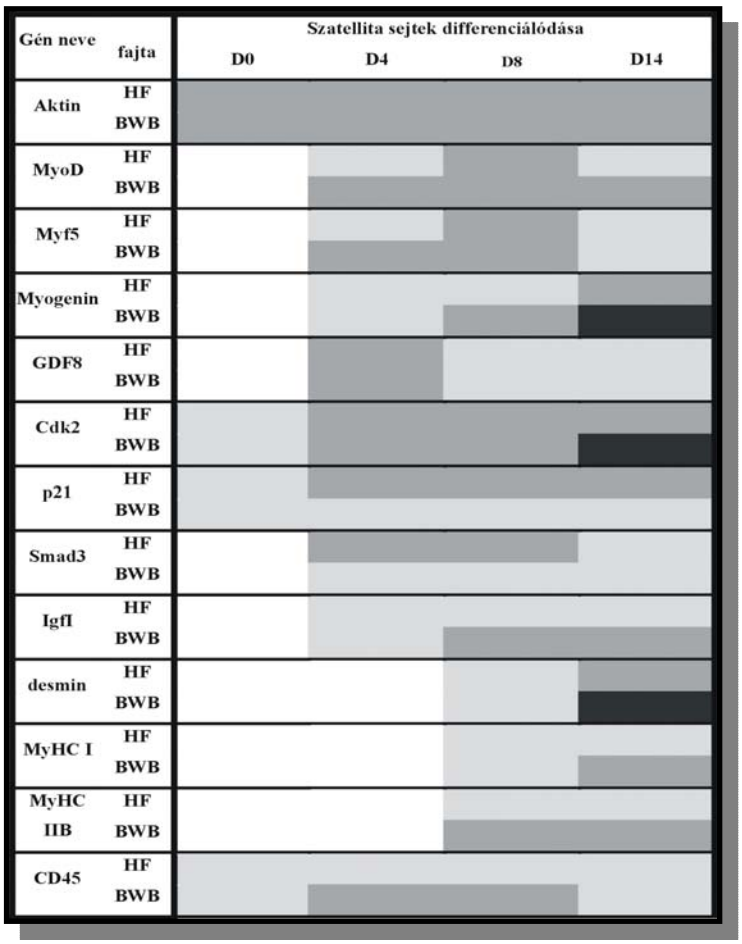
Myostatin mutation

In cattle:

- **Belgian White Blue *(culard)***
- **autosomal, recessive, non-complete penetrance (BTA2q12-22 region) [nt821(del11)]**
- **Muscle hypertrophy and hyperplasia**

Results

- The expression patterns of thirteen genes linked to myostatin cascade have been compared during differentiation in vitro.
- A significant increase was measured in the expression of the MRF factors, the Igf-1 and the Cdk2 genes in Belgian White-Blue satellite cell cultures, while the level of p21 decreased significantly.
- The study of MyHC isotype of muscle fibers differentiating in vitro revealed an excess of MyHC IIB mRNA to the detriment of type IIa, a phenomenon typically observed in adults in vivo. The differences measured in gene expressions during differentiation also support the role of myostatin played in muscle differentiation.
- The results showed a correlation with our earlier results of the study on muscle differentiation in myostatin mutant mouse strain (Cmpt).
- It can be concluded that, for the factors studied, there is no difference between the two species based on the comparison of the myostatin-regulated muscle differentiation cascades. Therefore, the results of our study on the myostatin-mutant mouse strain (Kobolak et al., 2004) can be regarded as a model of the changes occurring in the domestic animal, the Belgian White-Blue.



Abbreviations:

BWB: Belgian White-Blue cattle

HF: Holstein-Friesian

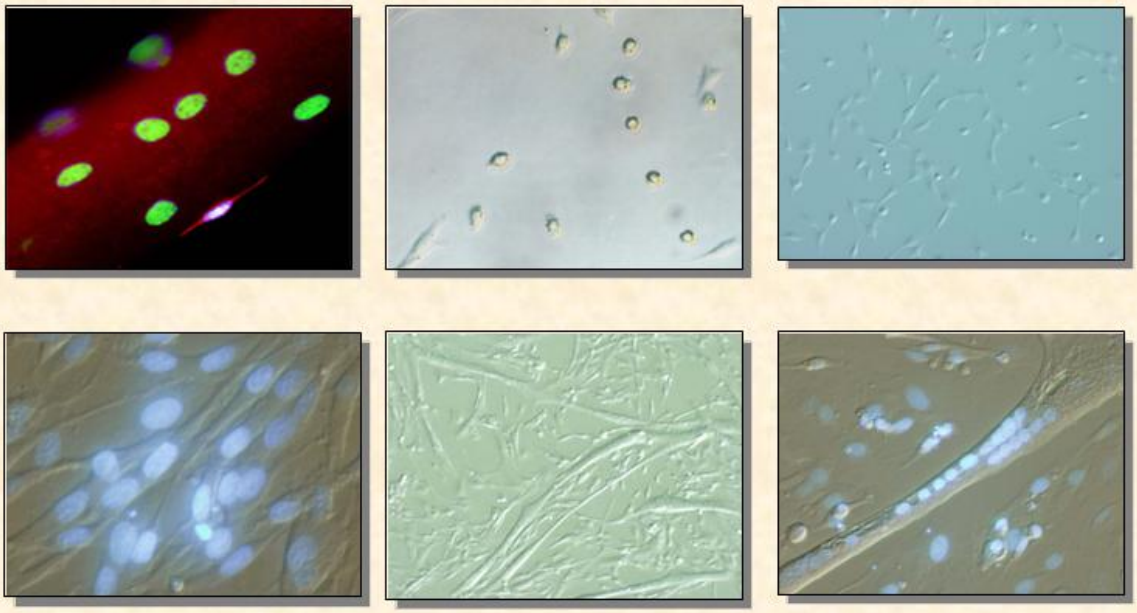
D0: satellite cells on Day 0

D4: myoblast appears on Day 4

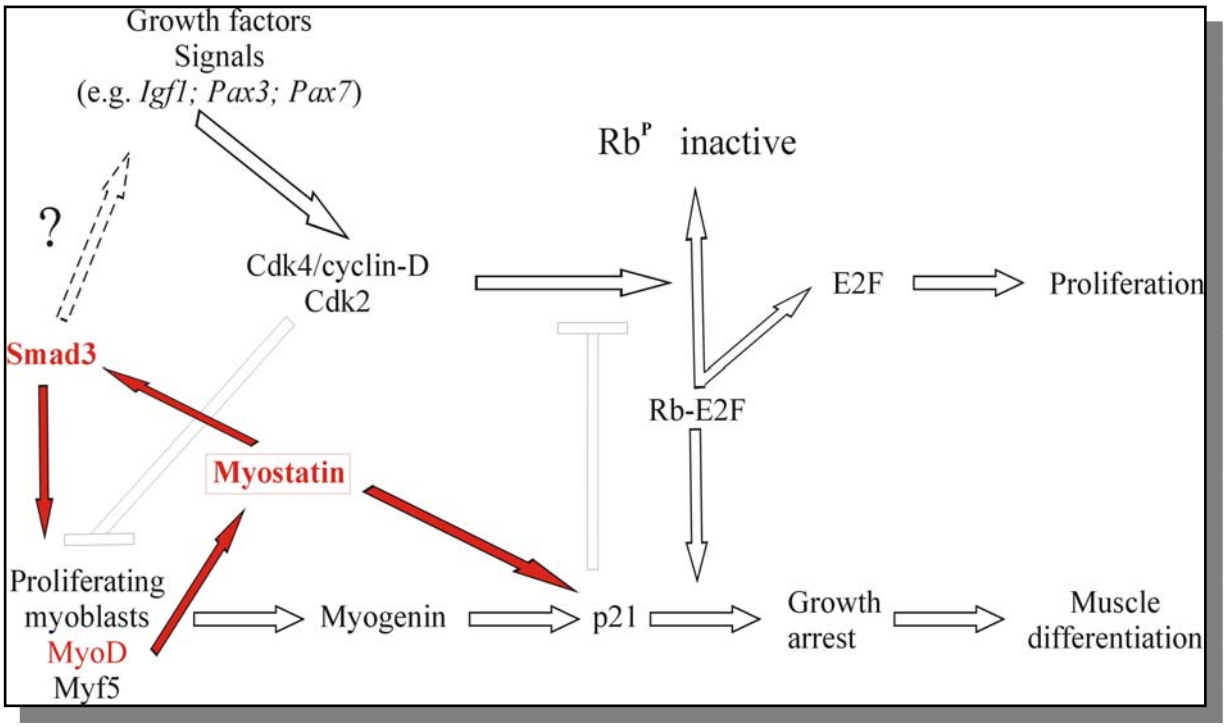
D8: cell fusion occurs on Day 8

D14: multinuclear muscle cells formed

Satellite cell culture and differentiation in vitro



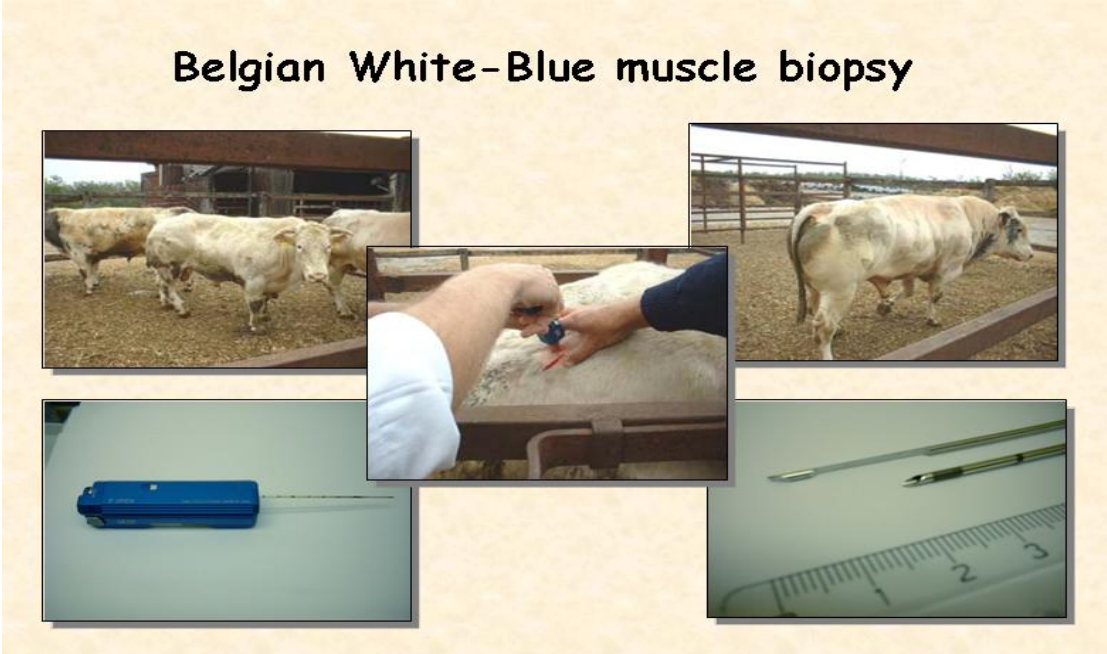
Role of myoblast proliferation and myostatin in differentiation



Methods

Primer muscle culture

Muscle was isolated using open biopsy from the hindlimb muscle (*m. gluteus medius)* of the Belgian White-Blue. Samples from animals of Holstein-Friesian served as control. Primer muscle cells were cultured according to Freshney (2000): following a digestion with collagenase and dispase, the biopsies were filtered with a 70 µm screen and were cultivated in culture medium in Petri-dishes pretreated with collagen. In order to remove debris and fibroblasts, cells were transferred onto fresh collagen after 2 hrs. Unlike to the method of Freshney, the cultivation medium was changed to F10 medium (Sigma), 5 nM bFGF (Sigma), 0.5% chick embryo extract (SLI, UK), 20% FBS (EuroClone) and antibiotics. The isolated cell cultures were characterized and differentiated *in vitro*. Following a 3-5 days of cultivation, the medium was replaced with differentiation medium [DMEM (low glucose; Sigma), 2% HS (Gibco) and antibiotics] to induce fusion of the cells.



Isolation of satellite cells

The method of Yablonka-Reuveni and Nameroff (1987) modified by the Goodell laboratory was used. In short: the satellite cells can be separated from the myoblasts at the 40 and 70% border of Percoll-gradient following a centrifugation as described at the primer cultures. After washing the Percoll out, the medium F10 (described at the primary cultures) was used. The Percoll separation was combined with the preplating technique in order to obtain higher efficiency. After the 4th and the 5th preplating, a satellite cell population of 98% purity was obtained which was cultivated further in a collagen-treated Petri-dish.

RT-PCR reaction

RNA was extracted from tissue cultures using TRI-reagent (Sigma) according to the manufacturer's instruction. For RT-PCR reactions, reagents of 'Titan One Tube RT-PCR Kit' (Roche) was used, according to the manufacturer's instruction. 1 μg of the RNA template was used. The reaction in the 50 μg reaction mix was run in a Perkin Elmer 9600 thermal cycler according to the following program: the reverse transcription (30 min, 42°C) was followed by the inactivation of the enzyme (10 min, 95°C) and a 40 cycles of amplification:

30 sec 95°C (denaturation),
60 sec 60°C (primer annealing),
90 sec 72°C (elongation).

The reaction was terminated with a final elongation (10 min, 72 °C). The samples were analysed by gelelectrophoresis (3% agarose, 1xTAE gel).

Table 1: The sequences of specific primers used to identify gene products

Jelölés	Szekvencia (5'→3')	Fragment mérete	Jelölés	Szekvencia (5'→3')	Fragment mérete
B-MyoD_F	GACGGCTCTCTCTGCAACTT	270	B-Smad3_F	GACGCCAGTTCTACCTCCTG	242
B-MyoD_R	TAGTCGTCTTGCGTTTGCAC		B-Smad3_R	TCTGGAATATTGCTCTGGGG	
B-Myf5_F	ACCAACCCTAACCAGAGGCT	279	B-Igf-1_F	CATCCTCCTCGCATCTCTTC	198
B-Myf5_R	GGGCTGTTACATTCAGGCAT		B-Igf-1_R	CCTCCTCAGATCACAGCTCC	
B-Myogenin_F	TGGGCGTGTAAGGTGTGTAA	184	B-Desmin_F	GGGACATCCGTGCTCAGTAT	321
B-Myogenin_R	TGGGCGTGTAAGGTGTGTAA		B-Desmin_R	GTGGCGGTACTCCATCATCT	
B-CD45_F	ACCTGGACACCACCTCAAAG	243	B-Actin_F	GGAGCTCATCTATGAGAAGGC	202
B-CD45_R	AAACCATTGACCTTGCTTGG		B-Actin_R	AAGACGAAGGAGCTGCAGAAC	
B-GDF8_F	TGAGGCCTGTCAAGACTCCT	200	B-MyHC IIb_F	TAGGGTGAGGGAGCTTGAAA	203
B-GDF8_R	GCCTGGGTTCATGTCAAGTT		B-MyHC IIb_R	CCTCCTCAGCCTGTCTCTTG	
B-Cdk2_F	CGACTTTCAGATCCCGTTGT	203	B-MyHC I_F	GGCCCAGAAACAAGTGAAGA	202
B-Cdk2_R	AGATTCTTTCGGTACCCGCT		B-MyHC I_R	GTCTTGCTCTGCCAGTTTCC	
B-p21_F	GGCAGTGATGCCCAACTTAT	211			
B-p21_R	TCCTCTGCCTGTTCTGGAGT				

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