Characterization of Gene Expression in Double-Muscled and Normal-Muscled Bovine Embryos

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Summary and Implications

Using suppressive subtractive hybridization, 19 genes were confirmed to be differentially expressed between double-muscled and normal bovine embryos. The identified genes play roles in several cellular processes, including transcription, cell proliferation, protein synthesis and degradation, and metabolism. These genes can provide insight into molecules with which myostatin may interact. This information could potentially aid in the development of strategies to improve lean tissue deposition in livestock species.

Introduction

Myostatin has been shown to function as an inhibitor of skeletal muscle growth. Inactivation of the myostatin gene in certain cattle breeds results in "double muscled" animals with substantially increased muscle mass and decreased fat. Mice engineered to lack active myostatin exhibit a 200-300% increase in muscle mass, as compared to wild-type mice. Although myostatin has been shown to inhibit both proliferation and differentiation of skeletal muscle cells, the precise mechanism by which it acts has not yet been determined.

Materials and Methods

Suppressive subtractive hybridization was used to identify differential gene expression between normal and double-muscled bovine embryos.

Embryo collection

Embryos were collected from two different groups of dams. The first were Piedmontese x Angus crosses with one functional copy of the myostatin gene, and one containing the mutation that causes double muscling in the Piedmontese breed. The cows were bred through artificial insemination with semen from a single Piedmontese x Angus bull, who was also heterozygous for the mutation in the myostatin gene. The other group of dams consisted of Belgian Blue x Hereford crosses possessing one mutated copy of the myostatin gene. They were artificially inseminated with semen from a heterozygous Belgian Blue x MARC III bull. Shortly after the time at which myostatin is first expressed in the embryo, the dams were slaughtered, and the embryos were collected and genotyped.

RNA processing

Total RNA was isolated from normal and double-muscled embryos. The samples were treated with Proteinase K and DNaseI to remove protein and DNA contamination, respectively. Following extraction, polyA mRNA was purified for use in suppressive subtractive hybridization.

Suppressive subtractive hybridization and DNA isolation

Differentially expressed mRNAs were selectively amplified using Clontech's PCR-Select cDNA Subtraction Kit. PCR products were subcloned into a vector and isolated in preparation for sequencing.

Sequencing and analysis

Clones of interest were sequenced, and known genes with high levels of homology were identified through comparison with sequences available in the NCBI BLAST database.

cDNA macroarray

A cDNA macroarray was generated in order to confirm the differential expression of clones identified by subtractive hybridization. cDNA clones were amplified by PCR and spotted onto a nylon membrane. Radiolabelled target, consisting of cDNA from the sequenced clones, was hybridized to the membrane, and signal intensity was quantified.

COMPASS and BLAST analysis

Clones confirmed to be differentially expressed on the macroarray were further analyzed. Using the COMPASS comparative mapping tool, predicted chromosomal locations were determined.

Radiation hybrid panel

Radiation hybrid and somatic cell hybrid panels were used to determine more precise map locations for genes thought to be positioned near a myostatin-interacting QTL on chromosome 5.

Results and Discussion

Suppressive subtractive hybridization identified 30 clones that were possibly differentially expressed between normal and double-muscled bovine embryos. Of these, 19 were verified via macroarray analysis to have differences in expression levels of two-fold or greater (Table 1). The known functions of several of the identified genes suggest that they may act directly in the control of skeletal muscle growth by myostatin.

Table 1. Genes identified by suppressive subtractive hybridization.

Symbol [a]	Myostatin response[b]	Chromosomal location	Cellular role
DDX17	0.67	5	Transcription
SALL1	2.50*	18	Transcription
SET	1.01	8 or11	Transcription
RAB2	1.36	14	Intracellular signaling
KIAA0697	22.80*	6	Unknown
ACTB	1.87	3	Cellular structure
RPL18	14.17*	7	Protein synthesis & degradation
TF	0.64	1	Metabolism
Unique	2.43*	--	Unknown
SOD1	2.03*	3	Metabolism
HBE1	3.52*	15 or 25	Metabolism
MTND5	1.64	13 or 18	Metabolism
TMSB10	2.44*	2	Cell proliferation
MLL2	0.16*	5	Cell proliferation
ATP5H	0.24*	5	Altered metabolism
HMGA2	0.24*	5	Transcription factor
RAF1	0.19*	22	Intracellular signaling
RPL3	0.29*	5	Protein synthesis & degradation
EEF1A1	1.03	6	Protein synthesis & degradation
RPS5	0.52	9	Protein synthesis & degradation
AFP	0.91	6 or 17	Metabolism
Unique	0.24*	--	Unknown
RPL11	0.23*	15 or 1	Protein synthesis & degradation
TUBB	0.19*	18	Cellular structure
RPS9	1.46	7 or 18	Protein synthesis & degradation
HBZ	0.26*	29 or 5	Metabolism
TUBGCP6	0.21*	22 or 17	Metabolism
Unique	0.41*	--	Unknown
PTMA	1.10	7	Cellular structure
RPS3	0.35*	6	Protein synthesis & degradation

* At least a 2-fold difference in expression levels.

[a] Gene name was determined by the closet human homologue. Identity had to be greater than 80% over a distance of >60 bp.

[b] Response is reported as wild-type expression divided by double-muscled. Those genes with a greater than two-fold difference in expression are denoted with an asterisk.