Invstigating the Epigenetic Regulation of the BDNF, PGC-1α, Myogenin and MHC-IId Genes

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INTRODUCTION

Epigenetics refers to a chemical modification of DNA that is heritable and caused by mechanisms other than changes in underlying DNA sequence. Cytosine methylation is a well known epigenetic mechanism that controls the expression of housekeeping and possibly also tissue-specific genes. Despite many investigations concerning DNA methylation, specific information about the mechanism that regulates this process or its exact functional role in the activation of some genes is lacking.

The aim of this study was to begin investigating the role of cytosine methylation in the expression of several genes by examining the potential for methylation to alter their expression in skeletal muscle tissue. The genes in question include: brain-derived neurotropic factor transcript 1 (BDNF), peroxisome proliferator activated receptor gamma coactivator-1 (PGC-1α), myogenin, and myosin heavy chain type IId (MHC-IId).

These genes are of particular interest because they show a definite on/off switch closely connected with strict transcriptional control at the onset of cell differentiation. Research has shown that cytosine methylation is an essential active mechanism in the transcriptional control of BDNF, PGC-1α, and myogenin. The effect of cytosine methylation on the myogenin heavy chain type IId gene, however, is unknown. PGC-1α's role in mitochondrial function makes it relevant to this investigation on skeletal muscle differentiation. BDNF helps to support the survival of existing neurons and encourage the growth and differentiation of new neurons and synapses. It plays an important regulatory function during myogenic differentiation and its expression is associated with developing myofibers, which later express myosin heavy chain (MHC) IId. The myosin heavy chain component of the fiber plays the dominant role in defining muscle fiber type and myogenin is a regulatory factor involved in the coordination of skeletal muscle development and repair.

METHODS

Cell Culture. The experiments were performed on the C2C12 mouse muscle line. Cells were cultured in F-14 medium, supplemented with 1% penstrep and 10% fetal calf serum (CM), which favors cell growth with limited differentiation, for 48hrs. The resulting myoblast cells were then treated with two demethylating agents (5-Aza and SAH) and two methylating agents (5-Aza Veh and SAM). Cells were also passaged and treated with differentiation media, resulting in myotubes that were also treated with the above mentioned drugs.

Gene Expression: RNA Isolation. After treatment, the total RNA was isolated from 10mm plates with trizol. The purity of each RNA sample was checked using a spectrophotometer and the expression levels of all four genes were determined using RT PCR. The gene expression bands from the DNA created in the RT PCR were then visualized using agarose gel electrophoresis.

RESULTS

Rbf1 transcript 1 - No differences in gene expression levels were observed in C2C12 skeletal muscle myoblasts or myotubes regardless of methylating/demethylating agents. The Rbf1-1 transcript expression is not likely to be controlled by CpG methylation.

- Future studies will confirm the presence of this transcript in adult skeletal muscle by clonal analysis.

Myogenin - Myoblasts did not express myogenin in any treatment. In myotubes S-adenosylhomocysteine increased expression levels compared to controls, while S-adenosylmethionine attenuated myogenin expression, similar to previous work.

- Future work will be to determine and confirm the methylation status of CpG sites in the promoter of the myogenin gene known to be associated with myogenin's expression level.

Mpk1- Mpk1 is a mycin heavy chain type IId. Differences in gene expression levels were observed in C2C12 skeletal muscle myoblasts (as expected) or myotubes (unexpected results). Cells may not have been cultured long enough after induction of differentiation to obtain Mpk1 gene expression results.

- Future studies will be to culture C2C12 cells for 72 and 96 hrs post differentiation and test for gene expression differences at Mpk1. To confirm differentiation we will probe for markers of differentiation, such as tropinin-I fast and the other myosin heavy chain isoforms.

REFERENCES


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