Investigating the Epigenetic Regulation of the BDNF, PGC-1α, Myogenin and MHC-IIb Genes

Tara Thompson[1], Andrew T. Ludlow[2], Stephen M. Roth, Ph.D.[2]
[1]Rutgers, The State University of New Jersey, New Brunswick, NJ
[2]Department of Kinesiology, School of Public Health, University of Maryland, College Park, MD

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[3].

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 IIb (MHC-IIb).

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[1], PGC-1 α[2], and myogenin[3]. The effect of cytosine methylation on the myosin heavy chain type IIb 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) IIB [5]. The myosin heavy chain composition of a 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[6].

PURPOSE

The purpose of this investigation was to determine the gene expression profile in myoblasts and myotubes treated with methylating or demethylating agents. This information will set the stage for future analysis of DNA methylation in these genes.

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 (GM), 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 passed 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 100mm 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 cDNA created in the RT PCR were then visualized using agarose gel electrophoresis.

RESULTS

Figure 1. Myoblasts treated with Demethylating and Methylating agents.

Figure 2. Myotubes treated with Demethylating and Methylating agents.

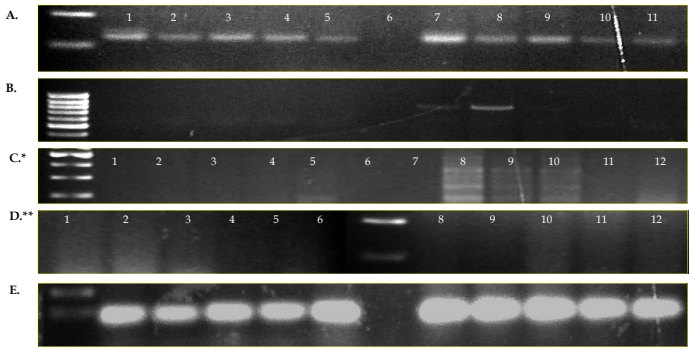

Figure 3. Representative agarose gel images (n=3) show the gene expression profile of C2C12 myoblast and mytoubes treated with methylating and demethylating agents. A) *Bdnf* transcript 1. B) *Myogenin*. C) *Ppargc1* alpha. D). *Myh4* - myosin heavy chain type IIB. E) *Gapdh*. Agarose gel images A, B and E were loaded as follows - 100bp molecular, 1 = MB 48hrs, 2 = MB treated for 48hrs with 10uM 5-Azacytodine, 3 = MB treated for 48hrs with 10uM 5-azacytodine vehicle, 4 = MB treated for 48hrs with 100uM *S*-adenosylmethionine, 5 = MB treated for 48hrs with *S*-adenosylhomocysteine, 6 = No template loading control, 7 = MT after 48hrs, 8 = MT treated for 48hrs with 10uM 5-Azacytodine, 9 = MT treated for 48hrs with 10uM 5-azacytodine vehicle, 10= MT treated for 48hrs with 100uM *S*-adenosylmethionine,11= MT treated for 48hrs with *S*-adenosylhomocysteine. Gel C* loaded as follows 100bp molecular, 1 = MB 48hrs, 2 = MB treated for 48hrs with 10uM 5-Azacytodine, 3 = MB treated for 48hrs with 10uM 5-azacytodine vehicle, 4 = MB treated for 48hrs with 100uM *S*-adenosylmethionine, 5 = MB treated for 48hrs with *S*-adenosylhomocysteine, 6 = No template loading control, 7 = Empty, 8 = MT after 48hrs, 9= MT treated for 48hrs with 10uM 5-Azacytodine, 10 = MT treated for 48hrs with 10uM 5-azacytodine vehicle, 11 = MT treated for 48hrs with 100uM *S*-adenosylmethionine,12 = MT treated for 48hrs with *S*-adenosylhomocysteine. D** loaded as follows 1 = MB 48hrs, 2 = MB treated for 48hrs with 10uM 5-Azacytodine, 3 = MB treated for 48hrs with 10uM 5-azacytodine vehicle, 4 = MB treated for 48hrs with 100uM *S*-adenosylmethionine, 5 = MB treated for 48hrs with *S*-adenosylhomocysteine, 6 = No template loading control, 100bp ladder, 8 = MT after 48hrs, 9= MT treated for 48hrs with 10uM 5-Azacytodine, 10 = MT treated for 48hrs with 10uM 5-azacytodine vehicle, 11 = MT treated for 48hrs with 100uM *S*-adenosylmethionine,12 = MT treated for 48hrs with *S*-adenosylhomocysteine. *Gapdh* was used as a loading control.

DISCUSSION

Bdnf transcript 1 - No differences in gene expression levels were observed in C2C12 skeletal muscle myoblasts or myotubes regardless of methylating/demethylating agents. The *Bdnf -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 5-azacytodine increased expression levels compared to controls, while *S*-adenosylmethionine attenuated myogenin expression, similar to previous work[7].

-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.

Ppargc1 alpha - Further optimization of primers is necessary to confirm expression of the *Ppargc1 alpha* gene in C2C12 myblasts and/or myotubes.

Myh4- Myosin heavy chain type IIb - No 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 *Myh4* 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 *Myh4*. To confirm differentiation we will probe for other markers of differentiation, such as troponin I fast and the other myosin heavy chain isoforms.

REFERENCES

1. Lubin, F.D., Roth, T.L., Sweatt, J.D. (2008). Epigenetic Regulation of BDNF gene transcription in the consolidation of fear memory. The Journal of Neuroscience 28(42): 10576-10586.
2. Ling, C., Del Guerra, S., Lupi, R., Ronn, T., Granhall, C., Luthman, H., Masiello, P., Marchetti, P., Groop, L., Del Prato, S. (2008). Epigenetic regulation of PPARGC1A in human type 2 diabetic islets and effect on insulin secretion. Diabetologia 51:615-622.
3. Lucarelli, M., Fuso, A., Strom, R., Scarpa, S.(2001). The Dynamics of Myogenin Site-specific Demethylation Is Strongly Correlated with Its Expression and with Muscle Differentiation. The Journal of Biological Chemistry.
4. Franko, A., Mayer, S., Theil, G., Mercy, L., Arnould, T., Hornig-Do, H., Wiesner, R.J., Goffart, S. (2008). CREB-1α Is Recruited to and Mediates Upregulation of the Cytochrome c Promoter during Enhanced Mitochondrial Biogenesis Accompanying Skeletal Muscle Differentiation. Molecular and Cellular Biology p2446-2459.
5. Mousavi, K., Jasmin, B.J. (2006). BDNF is expressed in Skeletal Muscle Satellite Cells and Inhibits Myogenic Differentiation. Journal of Neuroscience 26(21)5739-5749.
6. Schiaffino and Reggiani. Myosin isoforms in mammalian skeletal muscle. J Appl Physiol (1994) vol. 77 (2) pp. 493-501
7. Scarpa, S, Lucarelli, M, Palitti, F, Carotti, D, Strom, R. Simultaneous myogenin expression and overall DNA hypomethylation promote in vitro myoblast differentiation. Cell Growth Differ (1996) vol. 7 (8) pp. 1051-8

Funding was provided by the University of Maryland Summer Training and Research (UM STAR) Program (NIH R25 HL092604)