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Differential expression of *HDAC* and *HAT* genes in atrophying skeletal muscle

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Abstract

Introduction—Histone deacetylase (HDAC) proteins, which counter the activity of histone acetyltransferases (HATs), are necessary for normal muscle atrophy in response to several pathophysiological conditions. Despite this, it remains unknown whether a common or unique transcriptional profile of HDAC and HAT genes exist during the progression of muscle atrophy.

Methods—Muscles were harvested from cast immobilized, denervated, or nutrient deprived animals for qRT-PCR analysis of HDAC and HAT gene expression.

Results—The mRNA levels of *Hdac2, Hdac4, Hdac6, Sirt1, p300, Cbp and Pcaf* increased, and *Hdac7* decreased, in skeletal muscle in each experimental model of muscle atrophy. *Hdac1* and *Hdac3* were increased only in cast immobilized and denervated muscles.

Conclusion—While specific HDACs and HATs are increased in multiple models of muscle atrophy, increased expression of class I HDACs was unique to muscle disuse, reinforcing that specific HDAC inhibitors may be more effective than pan-HDAC inhibitors at countering muscle atrophy.

Keywords

muscle wasting; histone deacetylase; histone acetyltransferase; muscle disuse; denervation

Introduction

Histone deacetylase (HDAC) proteins remove acetyl groups from target proteins, and recently a critical role of specific HDAC proteins has emerged in the regulation of skeletal muscle atrophy.[1–5] Indeed, of the 11 HDAC and 7 Sirtuin proteins, data supports the direct involvement of HDACs 1, 4, 5, 6 and SIRT1 in the regulation of muscle atrophy during various catabolic conditions.[1–3,6] Despite these findings, the results following treatment with HDAC inhibitors to counter muscle atrophy have not yielded similar results.[7,8] These contrasting findings may be because the HDAC inhibitor used in these studies, trichostatin A (TSA), is a pan HDAC inhibitor and too non-specific. In this regard, increasing evidence supports the notion each HDAC has unique protein targets with non-redundant functions[9].

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Conflict of Interests

No conflict of interests

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Thus, in future studies it will be important to move towards the use of more specific HDAC inhibitors. However, before this can be considered, a comprehensive analysis of how individual HDACs, and their counterpart histone acetyltransferase (HATs), change during various atrophy conditions is necessary. Therefore, the purpose of the current study was to compare the expression changes of various HDACs and HATs in skeletal muscle during multiple atrophying conditions to determine if a common or unique transcriptional profile exists.

Materials and Methods

C57BL/6 mice (nutrient deprivation and cast immobilization) weighing ~20g, or Sprague Dawley rats (denervation) weighing ~175g were purchased from Charles River Laboratories (Wilmington, Massachusetts) and used for all animal experiments which were approved by the University of Florida Institutional Animal Care and Use Committee.

Mice were cast immobilized as described previously,[10] and muscles were removed 4 or 10 days later. Food was withheld from nutrient deprived mice for 3 days and then muscles were harvested. Denervated rats underwent bilateral sciatic nerve transection, as described by others,[11] and muscles were harvested after 3 and 7 days. Muscles (n=6 per group) were removed and processed for RNA isolation using a TRIzol-based method as previously described.[10] cDNA was generated from 1 μg of RNA and used as a template for quantitative RT-PCR using various primers for *p300*, *Cbp*, *Pcaf*, *Gcn5*, *Moz*, *Hdacs1-11*, and *Sirt1*.

Results

Since the atrophy and ubiquitin proteasome pathway biomarkers, *atrogin-1* and *MuRF1* showed a typical increase in gene transcription (Figure 1A–B), we next measured the mRNA level of the HATs, *p300*, *Cbp*, *Pcaf*, *Gcn5* and *Moz*, and *Hdacs1-11* and *Sirt1* (Figure 1C–S). *p300*, *Cbp* and *Pcaf* were significantly increased in all models of muscle atrophy, at all time-points measured except for *p300* following 10-days of immobilization (Figures 1C–E). *Gcn5* was significantly increased following 4-days of immobilization, and 3 and 7-days of denervation but not during nutrient deprivation (Figure 1F). *Moz* was significantly increased following 4-days of immobilization, 7-days of denervation and nutrient deprivation (Figure 1G). *Hdac2*, *Hdac4*, *Hdac6* and *Sirt1* significantly increased in all conditions, and *Hdac1* and *Hdac3* increased in response to cast immobilization and denervation, but not nutrient deprivation (Figure 1H–J, 1L, 1P and 1R). *Hdac7* was significantly decreased in all conditions and *Hdac9* was significantly decreased in response to nutrient deprivation and 7-days of denervation (Figures 1N and 1O).

Discussion

In the current study we demonstrate that *p300*, *Cbp*, *Pcaf*, *Hdac2*, *Hdac4*, *Hdac6* and *Sirt1* mRNA levels all increase, and *Hdac7* mRNA decreases, in skeletal muscle in response to three different models of muscle atrophy. The common increase in the mRNA levels of *Hdac4* and *Hdac6* is interesting given their direct implication in the regulation of skeletal muscle mass. Indeed, overexpression of HDAC4 is sufficient to cause muscle fiber atrophy[6]

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and skeletal muscle specific knockout/knockdown of HDAC4 attenuates denervation-induced muscle atrophy.[1,6] However, knockdown of HDAC4 does not attenuate fasting-induced muscle atrophy[6] demonstrating in the absence of HDAC4 other mechanisms still drive muscle wasting during this condition. In addition, recent data also shows HDAC6 is causative in muscle atrophy associated with both denervation and chronic angiotensin II signaling.[4,12].

The current work also demonstrates *Hdac1, Hdac2 and Hdac3* are commonly increased in response to cast immobilization and denervation, suggesting increased expression of these class I HDACs is associated with conditions of muscle disuse. In support of this, recent work from our lab showed inhibition of class I HDACs, via treatment of mice with MS-275 (Entinostat), inhibits cast immobilized-induced muscle atrophy and weakness.[3]

The most well characterized role of HDACs/HATs is through the regulation of gene transcription via deacetylation-acetylation of histone proteins.[13] In this regard deacetylation of histones causes a decrease in gene transcription whereas acetylation of histones increases gene transcription.[14–16] Thus, HDACs could cause muscle atrophy, in part, through decreased transcription of genes required for the maintenance of muscle mass. Alternatively HDACs/HATs could regulate muscle mass via the regulation of atrophy-related transcription factors by either acting as co-factors within multimolecular transcriptional complexes[17] and/or by directly acetylating/deacetylating such transcription factors. In this latter regard, the nuclear-cytosolic localization and/or DNA-binding activities of the transcription factors Forkhead boxO, nuclear factor-kappaB and CCAAT/enhancer-binding protein beta are all regulated via acetylation-deacetylation[3,5,12,18–20] and each is required for muscle atrophy during various conditions.[21–24] Another potential mechanism is via the regulation of protein stability. Indeed deacetylation-acetylation can either promote or block the protein degradation[25] or change protein-protein interactions since acetylation of lysine residues can create docking sites for other proteins.[26]

Conclusion

The current study is the first to comprehensively examine the gene expression changes of 12 HDACs and 5 HATs in skeletal muscle during three independent models of muscle wasting. Our findings have established that *Hdac2*, *Hdac4*, *Hdac6* and *Sirt1* are commonly increased in all 3 models of muscle atrophy, while Class I HDACs are commonly increased in models of disuse atrophy. We are well aware that changes in protein expression, localization and, most importantly, substrate interactions, dictate the downstream consequences of HDACs, not changes in mRNA. However, the biological consequences of the identified common mRNA transcriptional profile may be inferred from the published work demonstrating that *Hdac1*, *Hdac2*, *Hdac4* and *Hdac6* are required for normal muscle atrophy in response to various conditions. Therefore selective chemical probes for these specific HDACs may provide the greatest chance of success in inhibiting muscle atrophy.

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Abbreviations

CBP	CREB (cAMP-responsive element binding protein)-binding protein
GCN5	General control of amino-acid synthesis
HAT	Histone Acetyltransferase
HDAC	Histone Deacetylase
p300	E1A binding protein p300
PCAF	P300/CBP-associated factor
MOZ	Monocytic leukemia zinc-finger protein
TA	Tibialis anterior
TSA	Trichostatin A

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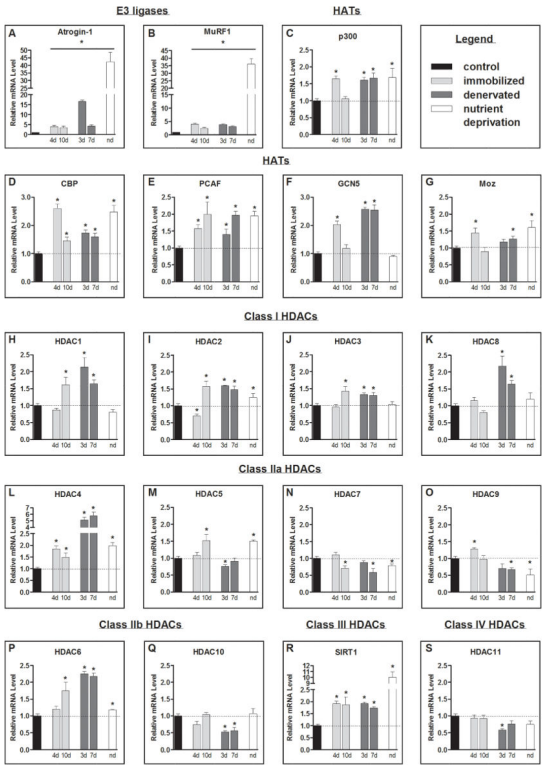

Figure 1.

Atrophy gene expression profile. Relative mRNA levels of; the E3 ligases (A) *atrogin-1* (Mm_026346.3/Rn_133521.1) and (B) *MuRF1* (Mm_001039048.2/Rn_080903.1), the HATs: (C) *p300* (*E1A binding protein p300;* Mm_177821.6/Rn_576312.4), (D) *Cbp* (*CREB* (c*AMP-responsive* e*lement* b*inding protein*)-*binding protein;* Mm_001025432.1/ Rn_133381.3), (E) *Pcaf* (*P300/CBP-associated factor;* Mm_001177798.1/ Rn_001107442.1), (F) *Gcn5* (*general control of amino-acid synthesis;* Mm_029090.3/ Rn_001107050.1), and (G) *Moz* (*Monocytic leukemia zinc-finger* protein; Mm_001081149.1/Rn_001100570.1); the class I *Hdacs*: (H–K) *Hdac1* (Mm_008228.2/ Rn_001025409.1), *Hdac2* (Mm_008229.2/Rn_053447.1), *Hdac3* (Mm_010411.2/ Rn_053448.1), and *Hdac8* (Mm_027382.3/Rn_001126373.2), class IIa *Hdacs*: (L–O) Hdac*4* (Mm_207225.1/Rn_343629.4), *Hdac5* (Mm_001077696/Rn_053450.1), *Hdac7* (Mm_001204275.1/Rn_345868.4), *Hdac9* (Mm_024124.3/Rn_001200045.1), class IIb *Hdacs*: (P& Q) Hdac*6* (Mm_001130416.1/Rn_228753.5) and Hdac*10* (Mm_199198.2/ Rn_001035000.1), the class III *Hdac*: (R) *Sirt1* (Mm_001159589.1/Rn_001080493.1) and the class IV *Hdac*: (S) *Hdac11* (Mm_144919.2/Rn_001106610.2) from the soleus muscle of control and 4 day (4d) and 10 day (10d) immobilized mice, tibialis anterior (TA) muscle of control and 3 day (3d) and 7 day (7d) denervated rats, and nutrient deprived (nd) mice. All values were normalized to *18s*. Bars represent means ± SE for 6 muscles/group. *P<0.05 vs control.