Atrophy-related ubiquitin ligases atrogin-1 and MuRF-1 are associated with uterine smooth muscle involution in the postpartum period

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Atrophy-related ubiquitin ligases atrogin-1 and MuRF-1 are associated with uterine smooth muscle involution in the postpartum period. Am J Physiol Regul Integr Comp Physiol 292: R971–R976, 2007. First published September 28, 2006; doi:10.1152/ajpregu.00617.2006.—The regulation of cell size depends on a delicate balance between protein synthesis and breakdown. Skeletal and cardiac muscle adapt to hormonal and neuronal stimuli and can rapidly hypertrophy and atrophy; however, the extent to which these processes occur in smooth muscle is less clear. Atrophy in striated muscle results from enhanced protein breakdown and is associated with a common transcriptional profile and activation of the ubiquitin-proteasome pathway, including induction of the muscle-specific ubiquitin protein ligases atrogin-1 and muscle ring-finger protein 1 (MuRF-1). Here we show that atrogin-1 is also expressed in smooth muscle, and that both atrogin-1 and MuRF-1 are upregulated in the uterus following delivery, as rapid involution occurs. While these two genes are similarly induced in all types of muscle during rapid loss of cell mass, other striated muscle atrophy-specific transcriptional changes are not observed during uterine involution, suggesting different underlying molecular mechanisms. These results raise the possibility that activation of atrogin-1 and MuRF-1 may be a common general adaptation in cells undergoing a rapid reduction in size.

atrophy; atrogen; ubiquitin

CELL MASS IS DETERMINED BY THE COMPETING PROCESSES OF PROTEIN SYNTHESIS AND PROTEIN BREAKDOWN. IN SKELETAL MUSCLE, RAPID LOSS OF MUSCLE MASS OCCURS IN RESPONSE TO SYSTEMIC PERTURBATIONS SUCH AS AFTER FOO DEPRIVATION AND IN MANY MAJOR DISEASE STATES (E.G., CANCER CACHEXIA, DIABETES, UREMIA, CARDIAC FAILURE, SEPSIS) AND ALSO WITH DISUSE OR DENERVATION (17). DURING THESE CATABOLIC CONDITIONS, MUSCLE PROTEIN IS RAPIDLY MOBILIZED AS A SOURCE OF AMINO ACIDS FOR GLUCONEOGENESIS OR SYNTHESIS OF STRESS-RELATED PROTEINS. IT HAS BEEN PROPOSED THAT THESE VARIOUS TYPES OF SKELETAL MUSCLE ATROPHY OCCUR THROUGH COMMON CELLULAR MECHANISMS INVOLVING SIMILAR BIOCHEMICAL AND TRANSCRIPTIONAL ADAPTATIONS, INCLUDING ACTIVATION OF THE UBQUITIN-PROTEASOME PATHWAY (UPP) (16, 18, 26).

EVIDENCE FOR A SPECIFIC TRANSCRIPTIONAL PROGRAM UNDERLYING ATROPHY IN SKELETAL MUSCLE HAS COME FROM THE USE OF cDNA MICROARRAYS. THESE ANALYSES IDENTIFIED A COMMON SET OF CHANGES IN THE mRNA CONTENT OF SKELETAL MUSCLES FROM FASTED MICE AND FROM RATS WITH CANCER CACHEXIA, STREPTOZOTOCIN-INDUCED DIABETES MELLITUS, AND UREMIA AND FOLLOWING DENERVATION (16, 18). THESE TRANSCRIPTIONAL CHANGES WERE FOUND UNDER CONDITIONS WHERE THE MUSCLES WERE UNDERGOING RAPID ATROPHY AND HAD HIGH RATES OF PROTEIN DEGRADATION. APPROXIMATELY 120 GENES WERE SHOWN TO BE COORDINATELY INDUCED OR SUPPRESSED IN THE MUSCLES IN THESE DIFFERENT CATABOLIC STATES AND WERE TERMED “ATROGENES” (16, 18). AMONG THE MOST STRONGLY INDUCED ATROGENES WERE COMPONENTS OF THE UPP. THIS PATHWAY LINKS CHAINS OF UBQUITIN TO CELLULAR PROTEINS TARGETED FOR DEGRADATION, THROUGH THE CONCERTED ACTION OF THREE ENZYMES, E1, E2, AND E3. PROTEINS TAGGED WITH UBQUITIN ARE THEN TARGETED TO THE PROTEASOME, WHERE THEY ARE DEGRADED TO SMALL PEPTIDES WITHIN ITS CENTRAL CHAMBER. UPP COMPONENTS IDENTIFIED AS ATROGENES INCLUDE GENES FOR POLYUBQUITIN AND UBQUITIN FUSION PROTEINS, MULTIPLE SUBUNITS OF THE 20S PROTEASOME, AND ESPECIALLY THE UBQUITIN LIGASES, OR E3S, ATROGIN-1/MAFBX AND MUrf-1 (2, 9). BOTH ATROGIN-1 AND MUrf-1 mRNA ARE INDUCED EARLY DURING THE ATROPHY PROCESS, AND, ON FASTING, THE RISE IN ATROGIN-1 EXPRESSION PRECEDES THE LOSS OF MUSCLE WEIGHT (9). ANIMALS LACKING ATROGIN-1 OR MUrf-1 ARE RESISTANT TO MUSCLE ATROPHY FOLLOWING DENERVATION (2). ALTHOUGH A NUMBER OF POTENTIAL TARGETS OF UBQUITIN CONJUGATION BY ATROGIN-1 HAVE BEEN IDENTIFIED, THERE IS NO CLEAR CONSENSUS ON THE CELLULAR FUNCTION OF ATROGIN-1 IN THE DEVELOPMENT OF ATROPHY.

CARDIAC MUSCLE ALSO HYPERTROPHIES AND ATROPHIES IN RESPONSE TO PHYSIOLOGICAL CONDITIONS. CARDIAC HYPERTROPHY OCCURS DURING NORMAL PHYSIOLOGICAL GROWTH OF THE ORGANISM AND AS AN ADAPTIVE RESPONSE TO PRESSURE OR VOLUME STRESS, MUTATIONS IN CARDIAC PROTEINS, OR METABOLIC PERTURBATIONS (13). ON THE OTHER HAND, THE HEART UNDERGOES A REDUCTION IN SIZE IN RESPONSE TO DECREASED NUTRITIONAL INPUT AND DECREASED LOAD. FOR EXAMPLE, PATIENTS WITH ANOREXIA NERVOSA HAVE MARKEDLY REDUCED HEART SIZE (6), AND WEIGHT REDUCTION IN OBSESE PATIENTS IS ASSOCIATED WITH REDUCED HEART SIZE IN THE ABSENCE OF CHANGES IN BLOOD PRESSURE OR OTHER HEMODYNAMIC PARAMETERS (12). HEART SIZE ALSO DECREASES AFTER LEFT VENTRICULAR ASSIST DEVICE SUPPORT (34) AND BY REDUCTIONS IN VOLUME AND PRESSURE OVERLOAD (25, 31). SIMILAR CHANGES IN MANY OF THE GENES IDENTIFIED IN ATROPHYING SKELETAL MUSCLE HAVE RECENTLY BEEN FOUND IN CARDIAC MUSCLE UNDER ATROPHIC CONDITIONS (29), SUGGESTING THAT SIMILAR PATHWAYS ARE ACTIVATED IN SKELETAL AND CARDIAC MUSCLE TO INDUCE RAPID LOSS IN PROTEIN CONTENT/CELL MASS.

MUCH LESS IS KNOWN ABOUT THE REGULATION OF CELL SIZE IN SMOOTH MUSCLE. SMOOTH MUSCLE IS THE PREDOMINANT CELL TYPE IN THE MEDIA OF BLOOD VESSELS AS WELL AS IN THE MUSCLE LAYERS OF THE UTERUS AND URINARY BLADDER. IN THE UTERUS, THE MYOMETRIUM HYPERTROPHIES SIGNIFICANTLY DURING PREGNANCY AND THEN RAPIDLY INVOLUTES FOLLOWING DELIVERY. THIS INCREASE IN ORGAN SIZE HAS MAINLY BEEN EXPLAINED BY HYPERTROPHY RATHER THAN HYPERPLASIA OF THE SMOOTH MUSCLE CELLS, AND BY AN INCREASE IN COLLAGEN...
content (11). Following parturition, the rat uterus regains its nonpregnant weight within little more than a week through a largely undefined process of enhanced protein breakdown at least in part mediated by autophagic vacuoles (11). It is not known to what extent the transcriptional changes found in atrophying skeletal and cardiac muscle are shared by smooth muscle as it responds to atrophic stimuli.

In this study, we show that the involuting uterus expresses high levels of atrogin-1, the muscle-specific ubiquitin protein ligase associated with atrophying skeletal and cardiac muscle. Furthermore, another muscle-specific E3, MuRF-1, is also highly expressed in uterus following parturition. On the other hand, other transcriptional markers of atrophying skeletal muscle do not change in this tissue, suggesting that, at the transcriptional level, involution of the uterus and skeletal and cardiac muscle atrophy are functionally unique but share important molecular features.

MATERIALS AND METHODS

Experimental animals. Cardiac, skeletal, and smooth muscle tissues were dissected from C57B6 mice (Charles River), immediately placed in TRIzol (Life Technologies), and stored at −80°C until further use. Female Sprague-Dawley rats (company) were killed by CO2 asphyxiation during the first trimester (day 6, n = 3) and third trimester (day 18, n = 6) and on postpartum day 1 (n = 5), postpartum day 4 (n = 3), and postpartum day 7 (n = 2). All animals were housed in the Beth Israel animal facility under a 12:12-h day-night diurnal cycle and fed standard laboratory chow. Following death, the uterus was rapidly dissected, and uterine tissue was immediately stripped of placenta and connective tissue, minced, placed in RNAlater (Ambion), and frozen at −80°C until further use. Heart and skeletal muscle (gastrocnemius) were also harvested and stored in RNAlater. Total RNA was extracted using TRIzol according to the manufacturer’s instructions.

Real-time PCR. Atrogin-1 mRNA levels were determined by real-time PCR using the Applied Biosystems 7500 real-time PCR analyzer according to the method recently described by others (24, 33). Multiplexed amplification reactions were performed using 18S rRNA as an endogenous control (18S rRNA primers/VIC-labeled probe; Applied Biosystems, no. 4310893E), using the TaqMan One-Step PCR Master Mix Reagents Kit (no. 4309169, Applied Biosystems). The following settings were used: Stage 1 (reverse transcription), 48°C for 30 min; Stage 2 (denaturation), 95°C for 10 min; and Stage 3 (PCR), 95°C for 15 s and 60°C for 60 s for 40 cycles. The sequences of the forward, reverse, and double-labeled oligonucleotides were as follows: atrogin-1, forward 5′-CTT ACA GAC TGG ACT TCT CGA -3′, reverse 5′-CAC TTC CAA CAG CCT TAC TAC GT-3′; TaqMan probe, sequence 5′-FAM-TGC CAT CCT GGA TTC CAG AGG ATT CAA C-TAMRA-3′; MuRF-1, primers (Applied Biosystems ID no. Rn00590197_m1). Fluorescence data were analyzed by SDS1.7 software (Applied Biosystems). The Ct (threshold cycle) values for each reaction were transferred to a Microsoft Excel spreadsheet, and calculation of relative gene expression was performed from this data according to published algorithms (TaqMan Cytokine Gene Expression Plate 1 protocol, Applied Biosystems). All RNA samples were analyzed in triplicate, with the mean value used in subsequent analyses.

Mean expression ± SE at each trimester or postpartum time point was determined by combining the average expression values for each animal from the time point.

Northern blot analysis. For Northern blot analysis, total RNA (15 μg) was electrophoresed on 1% formaldehyde-agarose gels, transferred to Zeta-probe membranes (Bio-Rad, Hercules, CA), and UV cross-linked as described (9). MuRF-1 and FoxO1 probes were prepared as previously described (26). To generate full-length cathepsin L and metallothionein-1 probes, a plasmid-based mouse skeletal muscle cDNA library was used as the template in PCR amplification, as previously described (9). Full-length polyubiquitin probe was as described (22). PCR products were labeled by random priming (PrimeIt Kit, Stratagene). Hybridization was performed by the method of Church and Gilbert (5) at 65°C overnight. Hybridized membranes were analyzed by use of a Fuji Phosphorimager with QuantityOne software (Bio-Rad). Blots were stripped and rehybridized with a mouse GAPDH probe (Ambion) to ensure equivalent gel loading. mRNA levels for each gene in each sample were determined by dividing the autoradiographic density for that gene by the GAPDH density. Relative expression for each gene was determined by dividing the mean postpartum day 1 expression by the mean third trimester expression.

Production of an anti-atrogin-1 antibody and Western analysis. pCS2-atrogin-1 was cut with EcoRI and StuI to liberate 650 bp of atrogin-1. This fragment was inserted into pET28b (Novagen) previously digested with NotI and blunt-ended with Klenow fragment followed by digestion with EcoRI. The resulting plasmid contained a 110-amino acid NH2-terminal fragment of the atrogin-1 gene behind a His6 tag and an isopropylthiogalactoside-inducible promoter. This fragment was purified from Escherichia coli BL21(DE3) under denaturing conditions using the His6 affinity matrix Ni-NTA Agarose (Qiagen) according to the manufacturer’s instructions. On dialysis against PBS, ~30% of the eluted protein remained soluble. This protein antigen was used to generate a polyclonal antiserum (Zymed), which was affinity purified using the same antigen by the manufacturer. IgG was prepared from the crude rabbit serum using established protocols (10). Anti-atrogin-1 IgG was affinity purified from the IgG according to the procedures of Harlow and Lane (10), using an Affigel-10 matrix (Bio-Rad Laboratories) onto which the purified atrogin-1 fragment was bound. Western blots were preformed as described (19), using 20 μg of protein from representative uterine samples solubilized in RIPA buffer. The same blot was probed with rabbit anti-atrogin-1 antibody and rabbit anti-dyenin antibody as a loading control.

Statistics. The experiments were repeated four or five times to ascertain reproducibility of results.

Results were expressed as means ± SE. Comparison of groups was made by Student’s t-test, with significance denoted at P < 0.05.

RESULTS

The ubiquitin protein ligase MAFbx/atrogin-1 is highly expressed in atrophying skeletal and cardiac muscle and plays a critical role in the development of atrophy in both organs (9, 29). We measured by real-time PCR the expression of atrogin-1 in skeletal and cardiac muscle as well as in uterus, esophagus, bladder, and aorta to see whether the gene was also
expressed to a significant degree in smooth muscle of normal wildtype mice (Fig. 1). While atrogin-1 was expressed highly in both fast- and slow-twitch skeletal muscle (tibialis anterior and soleus, respectively), and especially in heart, in agreement with prior data (9), low levels of expression were also apparent in the tissues containing smooth muscle. Because atrogin-1 is dramatically induced as skeletal and cardiac muscle undergoes atrophy, and is required for the process (2), we tested whether its expression was similarly regulated in uterine smooth muscle, which undergoes hypertrophy during pregnancy and then rapid involution following delivery. Uterus tissue was harvested from four to five Sprague-Dawley rats during the first and third trimesters of pregnancy as well as at 1, 4, and 7 days postpartum. Mean animal weights at each stage were similar, although animals in the third trimester were significantly heavier (Fig. 2A). Uterus weights increased greatly during pregnancy but, interestingly, were significantly lower by only 1 day after delivery (Fig. 2B). By 4 days postpartum, uterus weight had fallen to first trimester levels. We reasoned that this dramatic loss of uterine mass might share mechanistic similarities with the rapid atrophy that occurs in skeletal muscle following denervation or disuse and many systemic catabolic states.

To begin to address this possibility, we measured levels of atrogin-1 mRNA in the uterus samples before and after delivery. Atrogin-1 message was 4-fold higher 1 day postpartum when compared with levels in the third trimester and 14-fold higher that in the tissue during the first trimester (Fig. 3). By 4 and 7 days after delivery, atrogin-1 mRNA had fallen to the lower levels seen in the third trimester. This induction of atrogin-1 was specific to the uterus, since no significant difference in amounts of atrogin-1 mRNA was found in the skeletal or cardiac muscle from these same animals (Fig. 4). To measure protein levels of atrogin-1 in these uterine samples, we generated a polyclonal antibody to the NH2-terminal half of the atrogin-1 protein. This antiserum detected a single 40-kDa protein in extracts from atrophying skeletal muscle (data not shown). Similarly, the antibody detected significant atrogin-1 protein in extracts from uterine tissue only the day following delivery, when atrogin-1 mRNA was most highly expressed (Fig. 5). These data suggest that atrogin-1 induction in the uterus correlates with the period of rapid weight loss following delivery. In these same samples, we also analyzed expression

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Fig. 1. Atrogin-1/MAfbx is expressed in all muscle types. Real-time PCR for atrogin-1 in total RNA from various mouse tissues.

Fig. 2. Rat and uterus weight during pregnancy and following delivery. A: total body weight. B: uterus wet weight. T1, first trimester; T3, third trimester; PP1, postpartum day 1; PP4, postpartum day 4; PP7, postpartum day 7.

Fig. 3. mRNA levels for atrogin-1 and MuRF-1 rise in the immediate postpartum period. Real-time PCR of atrogin-1 (black bars) and MuRF-1 (gray bars) in total RNA from uterus at different stages of parturition. *P < 0.05, PP1 compared with T1, T3, PP4, or PP7.

Fig. 4. mRNA levels for atrogin-1 do not change in heart and skeletal muscle in the postpartum period. Real-time PCR of atrogin-1 in total RNA from the gastrocnemius muscle (black bars) and heart (gray bars) at different stages of parturition.
of MuRF-1, another ubiquitin protein ligase markedly upregulated in atrophying skeletal and cardiac muscle. Like atrogin-1, MuRF-1 was induced over twofold the day following delivery compared with the third trimester samples (Fig. 3).

Previous work has defined a set of common transcriptional changes in skeletal muscle genes in all forms of atrophy (18, 26). These atrogenes define a transcriptional program in atrophying skeletal muscle. It is not known whether similar changes occur in other tissues during changes in mass or whether smooth and skeletal muscles share the same pathways of tissue atrophy. Using Northern blot analysis, we measured the mRNA content of four atrogenes in addition to atrogin-1 and MuRF-1 in the uterus during the third trimester as well as 1 day postpartum, to examine whether similar transcriptional changes occur in uterus as it undergoes involution (Fig. 6). In addition to atrogin-1 and MuRF-1, polyubiquitin is another gene upregulated in atrophying skeletal and cardiac muscles. Polyubiquitin is posttranslationally cleaved to ubiquitin monomers, and its induction generally represents activation of the UPP (15). One day following delivery, polyubiquitin mRNA was upregulated 1.7-fold compared with the third trimester samples. On the other hand, cathepsin L, a lysosomal protease upregulated generally in atrophying skeletal muscle, was not significantly induced in the involuting uterus. In addition, the atrogene FoxO1 was also measured in the third trimester and the 1-day-postpartum uterus. This gene is a member of the forkhead family of transcription factors and is also generally upregulated in atrophying skeletal muscle. Furthermore, these factors are activated by dephosphorylation and nuclear translocation in atrophying muscle and themselves can produce atrophy in skeletal muscle fibers (27, 30). In the uterus samples, however, no increase in FoxO1 mRNA was seen the postpartum samples. Finally, metallothionein-1 (Mt1), a gene involved in response to cellular and oxidative stress that is strongly induced in atrophying skeletal muscle, was likewise unchanged in the involuting uterus. Taken together, these data suggest that the transcriptional profile of involuting uterus is different from that of atrophying skeletal muscle, although both share important common features.

DISCUSSION

Maintenance of cell size reflects a balance between processes of cell growth and atrophy and depends on relative amounts of protein synthesis and protein degradation. Large fluctuations in mass are seen in all types of muscle tissue in varied physiological and pathophysiological processes. For instance, striated skeletal muscle hypertrophies in response to exercise and undergoes marked atrophy with disuse or in catabolic states. Likewise, cardiac muscle hypertrophies and can rapidly atrophy. Smooth muscle has been less studied, but the uterus is well known to hypertrophy during pregnancy and return to baseline size rapidly following delivery. The molecular mechanisms that underlie atrophy in skeletal and cardiac muscle are beginning to be elucidated. A program of transcriptional changes, including activation of the ubiquitin-proteasome pathway and suppression of energy metabolism and growth-promoting genes, has been identified in atrophying skeletal muscle regardless of cause (18, 26) and is largely conserved in the atrophying heart (29). Here we show that involuting uterine smooth muscle following delivery of the fetus shares some important transcriptional features with atrophy in striated muscle. In both situations, there is marked induction of polyubiquitin and atrogin-1 and MuRF-1, ubiquitin ligases implicated in the rapid atrophy of striated muscle. On the other hand, many other transcriptional hallmarks of atrophying muscle, such as activation of metallothionein and cathepsin L, are not observed in the involuting uterus immediately following delivery. Although expression of these genes was only measured 1 day following delivery, and activation might occur later in the involution process, these genes are activated in atrophying skeletal muscle early, during the period of most rapid weight loss.

Girotti and Zingg (8) recently performed transcriptional profiling of the uterus at different stages of parturition and found each to be accompanied by a specific pattern of gene expression. Their analyses did not identify atrogin-1 or MuRF-1 as differentially regulated. These studies did show induction of IGF-binding protein-5 (IGFBP5) following delivery. IGFBP5 was also shown to increase in uterus following exposure to IGF-I and estrogen (14). On the other hand, in atrophying skeletal muscle, IGFBP5 is dramatically suppressed (18). These results demonstrate that uterine involution and skeletal muscle atrophy are fundamentally different processes at the transcriptional level.
Earlier studies have shown that uterine involution is associated with marked increases in many proteases, many of them extracellular [e.g., matrix metalloproteinase-7 (MMP-7), collagenase-2 (MMP-8), and plasminogen activator (1, 23, 28, 32)]. Additionally, the process of autophagy, in which bulk cytoplasm and organelles are sequestered into autophagosomes that fuse with lysosomes, where they are degraded, has been implicated in the process (11). While the lysosomal protease cathepsin L is not induced in the involuting uterus, we found one component of the autophagic vacuole, LC3, to be activated by covalent lipidation in the uterus following delivery, lending support for activation of this proteolytic pathway (S. H. Lecker, unpublished data). Recent experiments have also demonstrated activation of autophagy in atrophying skeletal muscle; however, there the forkhead transcription factors seem to play an important role in its activation (A. Goldberg and M. Sandri, personal communication), whereas in the involuting uterus, no evidence for forkhead activation has been found. Activation of the ubiquitin-proteasome pathway in the involuting uterus has not been reported but is strongly suggested by the activation of polyubiquitin, atrogin-1, and MuRF-1. Our experiments did not measure expression of proteasomal subunits, many of which are induced in atrophying skeletal muscle.

Suppression of IGF-I signaling pathways leading to dephosphorylation and activation of FoxO transcription factors are critical regulatory steps in the activation of atrophy and in the induction of the ubiquitin ligases atrogin-1 and MuRF-1 in striated muscle. FoxO1 is not expressed at increased levels in the uterus following delivery as it is in atrophying muscle and heart, suggesting that suppression of the IGF-I signaling pathway may not be critical for the involution process in the uterus. Furthermore, atrogin-1 is not induced in heart or skeletal muscle in the postpartum period. Taken together, these data suggest that the signaling events triggering atrogin-1 expression are different in uterus smooth muscle and in striated muscle. In the future, it will be important to elucidate the pathways that lead to activation of atrogin-1 and MuRF-1 in the involuting uterus. In skeletal muscle, glucocorticoids are potent inducers of these genes, at least partially through activation of FoxO factors (27). It the uterus, other steroid hormones such as estrogen and progesterone control uterine growth, and studies are underway to define their effects on atrogin-1 and MuRF-1 expression.

These studies have shown that atrogin-1 and MuRF-1 are found in tissues where smooth muscle predominates, in addition to the previously demonstrated importance of these genes in striated muscle tissues. Atrogin-1 has also been found in regressing gastrointestinal stromal tumors following therapy with Gleevec (imatinib) (7). A trogin-1 expression in these cell types shows that atrogin-1 induction is not strictly specific to striated muscle and suggests instead that atrogin-1 expression may define states of rapid protein turnover. Some F-box proteins are themselves unstable proteins with short half-lives due to autoubiquitination (3). The rapid appearance and disappearance of the atrogin-1 protein in the postpartum period may suggest that it is regulated in a similar manner. Understanding the molecular function of atrogin-1 in these processes will require elucidation of the proteins with which it interacts, and the proteins it targets for proteasomal degradation.

As genes associated with uterine muscle involution, atrogin-1 and MuRF-1 may have roles in other physiological and pathophysiological uterine conditions. Although there have been several attempts to characterize the transcriptional profile of common smooth muscle uterine tumors, leiomyomas (20, 21), none has described levels of atrogin-1 or MuRF-1 expression or the amount of suppression in these tumors. It is also unknown whether activation of these genes might correlate with states of uterine hyperactivity such as preterm labor, where uterine involution might be triggered (4). In conclusion, we have identified the ubiquitin ligases atrogin-1 and MuRF-1 as genes activated in uterine smooth muscle as it undergoes reduction in size following delivery. These findings broaden the relevance of these genes beyond states of skeletal muscle atrophy.

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