Molecular Pathogenesis of Genetic and Inherited Diseases

Myotubularin-Deficient Myoblasts Display Increased Apoptosis, Delayed Proliferation, and Poor Cell Engraftment

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X-linked myotubular myopathy is a severe congenital myopathy caused by deficiency of the lipid phosphatase, myotubularin. Recent studies of human tissue and animal models have discovered structural and physiological abnormalities in myotubularin-deficient muscle, but the impact of myotubularin deficiency on myogenic stem cells within muscles is unclear. In the present study, we evaluated the viability, proliferative capacity, and in vivo engraftment of myogenic cells obtained from severely symptomatic (Mtm1/H92544) myotubularin-deficient mice. Mtm1/H92544 muscle contains fewer myogenic cells than wild-type (WT) littermates, and the number of myogenic cells decreases with age. The behavior of Mtm1/H92544 myoblasts is also abnormal, because they engraft poorly into C57BL/6/Rag1null/mdx5cv mice and display decreased proliferation and increased apoptosis compared with WT myoblasts. Evaluation of Mtm1/H92544 animals at 21 and 42 days of life detected fewer satellite cells in Mtm1/H92544 muscle compared with WT littermates, and the decrease in satellite cells correlated with progression of disease. In addition, analysis of WT and Mtm1/H92544 regeneration after injury detected similar abnormalities of satellite cell function, with fewer satellite cells, fewer dividing cells, and increased apoptotic cells in Mtm1/H92544 muscle. These studies demonstrate specific abnormalities in myogenic cell number and behavior that may relate to the progression of disease in myotubularin deficiency, and may also be used to develop in vitro assays by which novel treatment strategies can be assessed. (Am J Pathol 2012, 181:961–968; http://dx.doi.org/10.1016/j.ajpath.2012.05.016)

X-linked myotubular myopathy (XLMTM) is a severe form of congenital myopathy with an estimated incidence of 1:50,000 male births that most often presents with severe perinatal weakness and respiratory failure.1–3 XLMTM is caused by mutations in the MTM1 gene that encodes a phosphoinositide phosphatase called myotubularin. Myotubularin plays a role in multiple cellular processes, including endosomal trafficking,4 excitation contraction coupling,5–7 intermediate filament organization,8 and apoptosis.9 Muscle biopsy specimens from patients with XLMTM display excessively small fibers with increased numbers of central nuclei and aggregation of organelles within the central regions of many cells.3 A murine model of myotubularin deficiency, the Mtm1/H92544 mouse [also referred to as Mtm1 knock-

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CD31 antibody (Sca1, clone E13-161.7; BD Biosciences, San Jose, CA) and propidium iodide (Sigma Aldrich, St Louis, MO) for 45 minutes. Cells were then analyzed and sorted using a BD FACS Aria cell sorter to isolate the SCA1^-CD45^-PDGFRα^-PI^- (quadruple-negative) population.

CD31^-CD45^-VCAM^+ Myogenic Cells

Primary muscle cells were dissociated from Mtm1^4^ and WT mice at 45 to 49 DOL, as previously described. The antibodies used for FACS analysis included fluorescein isothiocyanate–conjugated anti-CD31 antibody (clone 390; BD Biosciences), phosphatidylethanolamine-conjugated anti-CD45 (clone 30-F11; BD Biosciences), and APC-conjugated CD106 (clone 429; BioLegend).

Proliferation Assays

Quadruple-negative cells from 25- to 30-day-old WT or Mtm1^4^ mice were plated onto 96-well plates at a density of either 1000, 5000, or 10,000 cells per well in Human Skeletal Muscle Growth Media (Promocell, Heidelberg, Germany). Proliferation was measured as the absorbance at 490 nm using a Synergy 2 plate reader (Biotek Instruments, Winooski, VT). The average of three wells for each condition was recorded for each time point, data were standardized by comparing the values from WT with Mtm1^4^ cells from each individual animal pair, and the entire experiment was repeated three times. Statistical differences between corrected values were compared using a two-way analysis of variance.

Caspase Assays

After FACS, 250,000 quadruple-negative cells from 25- to 30-day-old WT or Mtm1^4^ mice were plated onto a 10-cm collagen-coated culture dish in Human Skeletal Muscle Growth Media (Promocell). Three aliquots of 100,000 quadruple-negative cells were also frozen as a time = 0 condition. After 2 or 4 days in culture, three aliquots of 80,000 cells were frozen at −80°C. Apoptosis was evaluated using a Caspase-Glo 3/7 Assay kit (Promega), following the manufacturer’s instructions, and read on a single-tube luminometer (Berthold Instruments, Oak Ridge, TN). Statistical differences between corrected values were compared using a two-way analysis of variance. Depending on cell yields available from each animal, between 1 and 3 replicates at each time point were performed on each animal cell isolate, using five different animal isolates for each genotype.

Fusion Assays

Primary myogenic cells from 25- to 30-day-old WT or Mtm1^4^ mice were enriched using the preplating technique. Myogenic cells were cultured on Matrigel-coated plates in Dulbecco’s modified Eagle’s medium (DMEM) high glucose supplemented with 30% fetal bo-
vine serum (FBS) and 3 μg/mL basic fibroblast growth factor. For fusion assays, 10,000 cells per well were plated in eight-well permanox slides coated with Matrigel. Cells adhered overnight in high serum medium, then switched to differentiation medium (DMEM low glucose supplemented with 4% FBS). Differentiation medium was changed daily, and fusion was assessed at days 0, 2, and 5 after the induction of differentiation. Cells were fixed and stained with a rabbit monoclonal anti-desmin antibody (Epitomics) diluted 1:100. The percentage of fused cells was calculated using 10 randomly photographed fields for each animal. Data were collected from three animals per genotype. Alternatively, quadruple-negative cells from 25- to 30-day-old WT or Mtm1/H9254 mice were plated onto gelatin in a 48-well culture plate at 50,000 cells per well in DMEM High Glucose plus 20% FBS and 3 μg/mL basic fibroblast growth factor (Invitrogen, Carlsbad, CA). Media were changed every 2 days until the cultures reached 70% confluence, and then media were changed to DMEM Low Glucose (Invitrogen) plus 2% FBS to promote differentiation. Media were changed daily for 3 days. After methanol fixation, immunohistochemical (IHC) staining was performed using standard techniques, with anti-human desmin primary antibodies (1:100, M0760; Dako, Carpentrya, CA) and AlexaFluor 568–conjugated goat anti-mouse IgG secondary antibodies (Invitrogen). Slides were coverslipped in Vectashield Hard Set mounting media containing DAPI (Vector Laboratories, Burlingame, CA) and evaluated using a Nikon TE-2000S microscope (Nikon, Melville, NY). The fusion index in control and myotubularin-deficient cultures was calculated as the ratio of fused nuclei within myotubes/number of total nuclei. Fusion indices were compared in control and mutant cultures using the Student’s t-test. These studies were performed using myoblasts isolated from two different mice of each genotype and two to three replicates performed per mouse, depending on cell yields.

**FDB Preparations**

Flexor digitorum brevis (FDB) muscles from 25- to 30-day-old WT or Mtm1/H9254 mice were extracted using standard techniques and plated on laminin-coated chamber slides. After incubating for 6 hours in DMEM with 10% FBS, cultured fibers were fixed in paraformaldehyde and stained for Pax7 (Developmental Studies Hybridoma Bank, Iowa City, IA) and DAPI using standard IHC techniques. Data were quantified as the number of fibers containing satellite cells per 100 fibers evaluated. The number of nuclei per fiber was nearly identical when comparing WT with KO cultures within the images quantified.

**Western Blot Analysis**

Quadruple-negative cells were pelleted and then lysed using MPER buffer (Thermo Scientific, Waltham, MA) containing protease and phosphatase inhibitors at a concentration of 1 μL per 10,000 cells isolated. Western blot procedures were performed as previously described. Transferred proteins were probed with antibodies against Pax7 (Developmental Studies Hybridoma Bank) and glyceraldehyde-3-phosphate dehydrogenase (6C5; Abcam, Cambridge, MA) and visualized using enhanced chemiluminescence. Quantification of protein levels normalized to glyceraldehyde-3-phosphate dehydrogenase was performed with the program Quantity One version 4.2.1 (Bio-Rad Laboratories), on an Image Station 440 (Kodak DS).

**Pax7 mRNA Expression**

Total RNA was purified from frozen muscles using TRIzol reagent (Invitrogen), according to manufacturer’s instructions, treated with DNase, and reverse transcribed using Super Script II RNase H Reverse Transcriptase (Invitrogen) in the presence of Random Primers (Promega). Real-time PCR was performed using an ABI Prism 7900 apparatus (Applied Biosystems, Foster City, CA) at 60°C as the melting temperature, with the following primers: ribosomal protein large P0, 5′-CTCCAAGCAGATGCAGCAG-CAGA-3′ and 5′-ATAGCCTTGCCCATCTATGGG-3′; and Pax7 (paired-box gene 7), 5′-GGAAAACAGTGTGC-CATCT-3′ and 5′-CCTGTCTTTTGGCACCATT-3′.

**Cell Transplantation**

Quadruple-negative cells from WT and Mtm1/H9254 donor mice cells were pelleted and resuspended in 1.2% barium chloride (Sigma Aldrich). The tibialis anterior (TA) muscles of isoflurane-anesthetized, 5- to 6-month-old C57BL/6 Rag1null mice were injected with a 10- to 15-μL suspension containing 50,000 cells. Mice were sacrificed 1 month after injection, and the TA muscles were frozen in isopentane for histological analysis. Cross sections (8-μm thick) were cut and stained with dystrophin (ab15277; Abcam) and DAPI at 80-μm intervals to evaluate dystrophin expression related to engraftment. Sections with the maximum number of positive fibers for a given muscle were photographed and counted. The maximum number of positive fibers in a given section was quantified for TA muscles injected with WT (n = 4) and KO (n = 8) cells, and compared using the Student’s t-test. Additional cryosections (8-μm thick) were stained with H&E or Gomori trichrome to histologically evaluate the engraftment sites, as described later.

**Cardiotoxin Injection**

The right TA muscle of three isoflurane-anesthetized WT or Mtm1/H9254 mice at 28 DOL were injected with 15- to 20-μL cardiotoxin (Sigma Aldrich) diluted at a final concentration of 0.5 μg/μL in sterile PBS. At 5 to 6 days after injection, the animals were euthanized and the TA muscles were extracted and snap frozen in isopentane.

**Tissue Histological Data**

Cross sections (8 μm thick) of isopentane–frozen TA or quadriceps muscle were taken midway down the length...
of the muscle and stained with H&E and Gomori trichrome using standard techniques. IHC for Ki-67, a marker of cellular proliferation, was performed on TA muscles of cardiotoxin-injected animals by standard IHC techniques using Ki-67 primary antibody (ab16667; Abcam) and biotinylated horse anti-rabbit IgG (Vector Laboratories). TUNEL staining was performed on these muscles using an In Situ Cell Death Detection Kit POD (Roche, Indianapolis, IN), per the manufacturer’s instructions. IHC for Pax7 was performed on TA muscles from cardiotoxin-injected animals and on quadriceps muscles from three uninjured WT and three Mtm1 knockout (KO) at 21 and 42 DOL after fixation of slides in 4% paraformaldehyde, blocking with both H2O2 and unconjugated Donkey Anti-Mouse IgG Fab Fragment (Jackson ImmunoResearch, West Grove, PA) in 1% bovine serum albumin, and exposure to Pax7 primary antibody (Developmental Studies Hybridoma Bank) and biotin-SP-conjugated donkey anti-mouse IgG Fab fragment (Jackson ImmunoResearch) secondary antibodies. Light microscopic images were captured using an Olympus DP72 camera and cellSens Standard software (Olympus, Center Valley, PA). Images were quantified by counting the number of positive cells within a field and dividing by the total number of fibers (for Pax7 and TUNEL) or nuclei (for Ki-67) within that field.

**Results**

To enrich for myogenic cells using FACS, our primary approach was to purify myogenic cells that were quadruple negative for SCA1, CD45, PDGFRA, and propidium iodide. SCA1 labeled most nonmyogenic cells in skeletal muscle,19–21 although rare SCA1-expressing myogenic cells were also reported.22–24 CD45 labeled immune cells, PDGFRA was a fibroadipogenic cell marker,25,26 and propidium iodide was a marker of dead cells. Pooled limb muscles isolated from 26- to 30-day-old Mtm1/4 mice and their WT male littermates consistently showed more quadruple-negative cells in Mtm1/4 tissue (P < 0.01, n = 5), despite much higher tissue yields from the WT littermates (Figure 1A). Fusion assays revealed many desmin-expressing myoblasts and myotubes in both WT and Mtm1/4 cultures, which confirmed that our sorting strategy enriched cultures for myogenic cells (Figure 1B). When areas of equivalent cellularity were quantified in WT and Mtm1/4 myogenic cell cultures, the fusion index did not significantly vary between WT and Mtm1/4 cultures (Figure 1B).

We further investigated the myogenic cell populations in Mtm1/4 mice by evaluating the expression of Pax7, a marker of satellite cells, at the quiescent or early activated stage, in the quadruple-negative populations, and in isolated myofibers. When evaluating Pax7 expression per 200,000 cells, quadruple-negative Mtm1/4 cells showed significantly decreased levels of Pax7 (36% ± 11% of WT values) expression (Figure 1C). Similarly, immunostaining for Pax7+ satellite cells in cultured FDB fibers revealed that the number of Pax7+ satellite cells in Mtm1/4 FDBs (47.76 ± 10.0 fibers containing Pax7+ cells per 100 fibers evaluated) was significantly lower than in WT FDBs (78.62 ± 8.16 fibers containing Pax7+ cells per 100 fibers evaluated) isolated from 26- to 30-day-old animals (Figure 2A). These findings suggested that the number of satellite cells in Mtm1/4 animals was decreased compared with WT littermates, despite more quadruple-negative cells in Mtm1/4 mice.

We further evaluated the number of prospective satellite cells in WT and Mtm1/4 mice by using a different FACS strategy to isolate CD31+ CD45+ CD106+ (Vcam-1) cells.27 This strategy revealed that Mtm1/4 animals at 45 to 49 DOL had significantly fewer Vcam-1+ satellite cells compared with age-matched littermates (Figure 2B), further suggesting an age-related decrease in myogenic cells in Mtm1/4 mice.

To evaluate the relationship between satellite cell number and progression of disease, Pax7 immunostaining was performed on quadriceps muscles from Mtm1/4 animals at 21 and 42 DOL (Figure 3A). These studies detected significantly fewer satellite cells when comparing Mtm1/4 muscle with age-matched WT animals. In addition, there was a significant decrease in the number of satellite cells found when comparing Mtm1/4 animals at 21 and 42 DOL, confirming a marked decrease in the satellite cell population with the progression of disease. This finding was also confirmed through the evaluation of Pax7 mRNA levels at 14, 21, and 30 DOL in the Mtm1/4 TA muscle (Figure 3B). Pax7 mRNA expression was de-
creased compared with WT values at 21 and 30 DOL. In addition, there was a significant decrease in Pax7 mRNA expression when comparing Mtm1Δ4 animals at 14 and 30 DOL, consistent with the progression-related decrease in satellite cells observed in our IHC studies. Although a decline in Pax7 expression was evident in Mtm1Δ4 animals, quadruple-negative cells displayed myogenic activity in vitro and equal fusion capacity to WT cells. To determine whether myotubularin-deficient myogenic progenitors had the ability to function in a myotubularin-competent environment in vivo, we transplanted quadruple-negative cells from either Mtm1Δ4 or WT mice at 25 to 30 DOL into the TA muscles of dystrophin-deficient C57BL/6 Rag1null mdx5cv mice and evaluated dystrophin expression 1 month after transplantation. Mdx5cv mice were used in this assay because they displayed approximately 10-fold less revertant fibers compared with mdx mice.28 H&E and Gomori trichrome staining of implanted muscles showed no differences between muscles injected with WT or Mtm1Δ4 cells. On IHC evaluation for dystrophin-positive fibers, which are an indicator of the successful engraftment of transplanted myogenic cells, Mtm1Δ4 cells showed a dramatically decreased amount of engraftment (9.0 ± 3.8 dystrophin-positive fibers) compared with WT cells (38.5 ± 8.1 dystrophin-positive fibers), despite being transplanted at equivalent cell numbers (Figure 4A). Although this technique was unable to distinguish between engrafted and revertant fibers, there were fewer than nine dystrophin-positive fibers in six of the eight Mdx5cv muscles injected with Mtm1Δ4 cells. These results suggested that the revertant fibers compose a minority of the dystrophin-positive fibers observed after the injection of Mdx5cv muscles with WT cells.

These engraftment findings suggested that Mtm1Δ4 myogenic cells may be impaired with respect to survival and/or function. To address this, cell proliferation and apoptosis assays were performed. Quadruple-negative cells from 25- to 30-day-old Mtm1Δ4 mice displayed decreased proliferation (51% to 70% of WT values, P < 0.05) immediately after sorting, but the difference in proliferation in WT and Mtm1Δ4 animals was less pronounced (KO = 36% to 87% of WT values, P = ns) by 4 days after isolation (Figure 4B). Apoptosis after isolation was also measured using caspase-3 and caspase-7 activity, because abnormalities of the caspase apoptotic pathway and increased apoptosis were reported in myotubularin knockdown studies using HeLa cells.9 Caspase-3 and caspase-7 activity was increased in Mtm1Δ4 cells at 0, 2, and 4 days after isolation (178%, 151%, and 266% of WT levels, respectively; P < 0.05), consistent with persistently increased apoptosis in Mtm1Δ4 myogenic cells (Figure 4C). Collectively, our find-

Figure 2. Decreased expression of Pax7 and number of prospective satellite cells are found in myotubularin-deficient muscle. A: Pax7 immunostaining of FDB fibers reveals fewer fibers containing Pax7+ nuclei in myofibers isolated from Mtm1Δ4 (KO) animals. *P < 0.05. B: FACS of 45- to 49-day-old WT and Mtm1Δ4 (KO) mouse muscle for CD31+CD45+CD106+ (Vcam1+ myogenic cells) reveals a depletion of this population in Mtm1Δ4 muscle. P < 0.05. SSC, side scatter.

Figure 3. Depletion of satellite cells with progression of disease in Mtm1Δ4 muscle. A: Immunostaining results for Pax7 in the quadriceps muscles of WT and Mtm1Δ4 (KO) mice at 21 and 42 DOL are quantified to evaluate the satellite cell populations in vivo. Arrows indicate Pax7-positive nuclei in WT and KO animals. B: mRNA levels of 14-, 21-, and 30-day-old mice (n = 9) are quantified by quantitative RT-PCR, normalized to the ribosomal protein large P0 mRNA level, and compared with WT. Scale bar = 20 μm. *P < 0.05.
ings indicated that the poor engraftment seen after transplantation of myoblasts from Mtm1/H92544 mice was likely because of impairment of myogenic cell survival and proliferation, rather than abnormalities related to cell fusion.

The in vivo relevance of our findings was addressed through evaluation of the healing process and the baseline Pax7/H11001 satellite cell levels in Mtm1/H92544 and WT mice. Five days after injection of cardiotoxin, which produces a focal muscle injury and stimulates satellite cell activation, proliferation, and differentiation, Mtm1/H92544 and WT muscle showed similar degrees of fiber regeneration and response to injury (Figure 5). Uninjured TA muscles showed pathological characteristics typical of myotubularin-deficient muscle, including marked fiber size variation and myofiber smallness in the absence of endomysial fibrosis, myonecrosis, or inflammation. Centrally nucleated fibers, which were less commonly seen in Mtm1/H924 mice, compared with other murine29 and canine30 models of myotubularin deficiency, were not significantly increased in the uninjured TA muscles at this time point (Figure 5). Regenerating areas in injured TA muscles were readily identifiable by the presence of numerous centrally nucleated fibers, surrounded by a mixed population of mononuclear cells composed of satellite cells, macrophages, and lymphocytes. Although mild fibrosis, interstitial edema, and an increase in the number of small blood vessels were noted on H&E and Gomori trichrome stains, these findings were present to an equivalent extent in regenerating WT and Mtm1/H92544 muscles. On immunostaining for Pax7, Ki-67, and apoptotic cells (using a TUNEL assay), however, differences in the response to injury in WT and Mtm1/H92544 mice became apparent. After injury, Mtm1/H92544 muscle contained fewer satellite cells, fewer proliferative cells, and more apoptotic cells than WT muscle (Figure 5). Although not all of the proliferating and apoptotic cells might correspond to satellite cells, these data provided in vivo evidence for abnormal satellite cell behavior in Mtm1/H92544 animals, involving abnormal levels of both proliferation and apoptosis.

Discussion

Several studies have been conducted on myotubularin-deficient cells in the past. Studies performed on myotubes from human patients reported normal cytoskeletal development, fusion, and behavior,31,32 but proliferation, apoptosis, and engraftment were not evaluated. More recent work on Mtm1/H9244 cells, cells with myotubularin knockdown, HeLa, and primary human cells, however, has revealed that myotubularin-deficient cells show abnormalities of cytoskeletal

![Figure 4. Poor survival of myotubularin-deficient cells in vitro. A: One month after transplantation of freshly sorted quadruple-negative cells into the TA muscles of C57BL/6 Rag1null mdx5cv mice, staining for dystrophin (Dys; red, bottom panels) and DAPI (blue) reveals significantly fewer successfully engrafted fibers after transplantation of Mtm1/H924 (KO) cells (n = 8) compared with WT cells (n = 4). *P < 0.01. Quantification of dystrophin+ fibers is performed by counting the highest number of dystrophin-expressing fibers within a single section of the entire TA muscle after surveying sections every 80 μm through the entire TA muscle. Top panels: H&E and trichrome stains of the injection site showing little or no fibrosis from the injected cells. B: Incorporation of MTS/PMS, which correlates with proliferation, is markedly higher in freshly isolated WT cells compared with cells from Mtm1/H924 (KO) littermates. There is no significant difference in proliferation after 4 days in culture. MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; PMS, phenazine methosulfate. C: A caspase-3/caspase-7 assay, which measures apoptosis through the activation of caspase-3/caspase-7, reveals increased caspase activation in Mtm1/H924 (KO) cells after FACS and at 2 and 4 days in culture. *P < 0.05. Avg. RLU, relative light unit average.]
Although we were unable to detect abnormalities in desmin expression or cytoskeletal organization in cultured myoblasts and myotubes while staining for desmin in fusion assays, the techniques we used were not nearly as sensitive as those described by Hnia et al. We were, however, able to demonstrate the presence of increased apoptosis in primary myotubularin-deficient myoblasts through increased activation of caspase-3 and caspase-7, consistent with the results reported in myotubularin-deficient cells.

Abnormalities in the number and behavior of myogenic cells were also noted in Mtm1/H9254 mice. Although there were more quadruple-negative cells in Mtm1/H164 animals compared with WT littermates, further investigation by FACS, IHC, gene expression, and Western blot analysis revealed that Pax7 expression is consistently decreased when comparing Mtm1/H164 with WT cell fractions and muscle tissue. In addition, data from these studies indicate that the number of myogenic cells markedly decreases with the progression of disease in Mtm1/H9254 mice. Our findings suggest that such depletion may occur through both slow proliferation and increased apoptosis of these myogenic cells. Depletion of satellite cells has been described in mice and humans deficient for dystrophin and selenoprotein N and in models of aging and denervation. Although this finding is nonspecific, it provides evidence for an additional degenerative component of the muscle dysfunction seen in myotubularin deficiency.

As an enzyme involved in sarcotubular and myofibrillar organization, endosomal trafficking, and excitation contraction coupling, myotubularin may affect cell survival pathways through several mechanisms. Pathways involved in the induction of apoptosis include the activation of death receptors, the release of apoptogenic factors from mitochondria, and the unfolded protein response produced as the result of the accumulation of misfolded proteins in the endoplasmic reticulum. Abnormalities in intracellular calcium produced in myotubularin deficiency may cause mitochondrial stress and activation of the mitochondrial apoptotic pathway. Alternatively, abnormalities of sarcotubular organization may lead to the accumulation of proteins and induce the unfolded protein response. Further investigation is required to definitively identify the mechanism(s) by which myotubularin deficiency impairs satellite cell survival.

Because of the deficiency of a single, ubiquitously expressed enzyme that has been modeled in several animal systems, XLMTM is a disease with excellent potential for the development of successful treatment strategies. Significant limitations to the development or identification of potentially useful agents include the lack of a high-throughput in vitro assay on which new treatments could be tested and the difficulty in generating sufficient animals for preclinical trials on a rapid time scale. Although the relationship between the increased apoptosis and slowed proliferation in Mtm1/H164 cells and the progression of disease in Mtm1/H164 animals remains unclear, these cellular phenotypes represent reproducible phenotypes that can easily be tested in a high-throughput manner. The usefulness of myoblast cultures in evaluating novel treatment strategies will be studied as our laboratory continues to test novel agents in Mtm1/H9254 mice.

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