Absence of p21<sup>CIP</sup> Rescues Myogenic Progenitor Cell Proliferative and Regenerative Capacity in Foxk1 Null Mice*

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Foxk1 is a forkhead/winged helix transcription factor that is restricted to myogenic progenitor cells in adult skeletal muscle. Mice lacking Foxk1 (Foxk1<sup>−/−</sup>) display growth retardation and a severe impairment in skeletal muscle regeneration following injury. Here we show that myogenic progenitor cells from Foxk1<sup>−/−</sup> mice are reduced in number and have perturbed cell cycle progression (G<sub>G1</sub>/G<sub>S</sub> arrest). Molecular analysis of Foxk1<sup>−/−</sup> myogenic progenitor cells revealed increased expression of the cyclin-dependent kinase inhibitor, p21<sup>CIP</sup>, independent of changes in other cell cycle inhibitors, including p53. Combinatorial mating of Foxk1<sup>−/−</sup> mice with p21<sup>CIP</sup><sup>−/−</sup> mice, to generate double mutant progeny, resulted in a complete restoration of the growth deficit, skeletal muscle regeneration, myogenic progenitor cell number, and cell cycle progression that characterizes the Foxk1<sup>−/−</sup> mice. We conclude that Foxk1 is essential for regulating cell cycle progression in the myogenic progenitor cell and that the cyclin-dependent kinase inhibitor, p21<sup>CIP</sup>, may be a downstream target of Foxk1.

The capacity of skeletal muscle for self-repair is due to a rare population of myogenic progenitor cells (previously termed muscle satellite cells) that reside at the periphery of adult muscle fibers (1, 2). Previous work has demonstrated the plasticity or multipotential capacity of these adult progenitor cells (3), but the molecular events that regulate their quiescence, proliferation, and differentiation during cellular growth and regeneration remain poorly defined.

The forkhead/winged helix (Fox) transcription factor family is characterized by a 100-amino acid winged helix DNA binding domain and has been shown to play essential roles during embryogenesis and in the control of cellular proliferation/determination in adult progenitor cell populations (4–13). We have identified a novel member of the forkhead/winged helix transcription factor family, termed Foxk1 (previously referred to as myocyte nuclear factor or MNF) (14, 15). Foxk1 is expressed early, selectively, and transiently in the developing neural tube, heart, and somites of the mouse embryo. However, during the latter stages of embryogenesis and in the adult, Foxk1 is restricted to a subpopulation of mononuclear cells within the skeletal muscle, which were identified as myogenic satellite (progenitor) cells (14). Importantly, Foxk1 was the first selective molecular marker of the quiescent myogenic progenitor cell population. Furthermore, we have demonstrated that mice lacking Foxk1 (Foxk1<sup>−/−</sup>) exhibit a growth deficiency and a severe impairment in skeletal muscle regeneration following injury.

The mechanistic defect associated with the impaired skeletal muscle regeneration in Foxk1 mutant mice is the focus of the current work. Overall, it was hypothesized that dysregulation, or loss, of Foxk1 attenuates skeletal muscle growth and regeneration due to an impairment of the cell cycle regulatory genes within the myogenic progenitor cell population. The data presented here establish Foxk1 as an important regulator of the myogenic progenitor cell population and provide evidence supporting the hypothesis that the cyclin-dependent kinase inhibitor, p21<sup>CIP</sup>, is a downstream target of Foxk1.

**EXPERIMENTAL PROCEDURES**

Mice—Foxk1<sup>−/−</sup> (15), C57BL/6 wild type (WT),<sup>1</sup> and p21<sup>CIP</sup><sup>−/−</sup> mice (Jackson Laboratory, Bar Harbor, ME) were used in these studies. Combinatorial mating of Foxk1<sup>−/−</sup> and p21<sup>CIP</sup><sup>−/−</sup> mice was undertaken to generate mice lacking both Foxk1 and p21<sup>CIP</sup>. Genotyping was performed by PCR analysis of genomic DNA using the following primer pairs: Foxk1 wild type allele, For 5′-GCAAGTCTCTTGCTCCAG-GAGG-3 and Rev 5′-CCGAGGAGGAGCCACTCTCTG-3′; Foxk1 mutant allele, For 5′-CCCTCGGACGACAGAGATGCG-3′ and Rev 5′-GCTCTGAGGAGGAGACTGCTAG-3′; p21<sup>CIP</sup> wild type allele, For 5′-GGCGTAACTCAACAGCCCT-3′ and Rev 5′-AGACACCG-GCACACTTGGCTC-3′; p21<sup>CIP</sup> mutant allele, For 5′-GGCTGAACT-CAACCCACCT-3′ and Rev 5′-GCTATCGAGATCCGTGGGC-3′. Thermocycler conditions for p21<sup>CIP</sup> are: step 1, 94 °C for 4 min; step 2, 94 °C for 1 min; step 3, 62 °C for 1 min; step 4, 72 °C for 1.5 min; step 5, 72 °C for 3 min. Steps 2–4 are repeated for 40 cycles. Thermocycler conditions for Foxk1 are: step 1) 94 °C for 2 min; step 2, 94 °C for 15 s; step 3, 62 °C for 15 s; step 4, 72 °C for 15 s; step 5, 72 °C for 3 min. Steps 2–4 are repeated for 30 cycles.

Primary Myogenic Progenitor Cell Culture—Asynchronously dividing primary myogenic progenitor cell cultures were harvested from hindlimb skeletal muscle of neonatal (2-day-old) mice (16). Cells were plated and grown on collagen coated plates in F-10 growth medium (20% fetal bovine serum, 0.5% penicillin/streptomycin, 25 ng/ml basic fibroblast growth factor). In selected experiments, IGF-I (50 ng/ml) or WT conditioned medium was added to the growth medium of WT and Foxk1<sup>−/−</sup> cells and further cultured for 4 days.

Single Skeletal Muscle Fiber Isolation—Single skeletal muscle fibers were harvested from the fast twitch extensor digitorum longus muscles of 2–4-month-old mice (17, 18). After 72 h in culture, the number of myogenic progenitor cells that had migrated from the fiber were quantified.

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‡ The abbreviations used are: WT, wild type; IGF, insulin growth factor; RT, reverse transcriptase; FACS, fluorescence-activated cell sorting; BrdUrd, 5′-bromo-2′-deoxyuridine.
Regulatory Mechanisms of Foxk1—Myogenic Progenitor Cells

Cardiotin Injury of Mouse Skeletal Muscle—Gastrocnemius muscles from WT, Foxk1−/−, and Foxk1−/−; p21CIP−/− mice (2-month old) were injected with cardiotin (150 μl/mouse of 10 μl cardiotin (Naja nigricollis; Calbiochem)) (15). Muscles were harvested 10 days post-injection and fixed overnight with 4% paraformaldehyde. Paraaffin-embedded sections were stained with hematoxylin/eosin to examine the resident myogenic progenitor cell population in Foxk1−/− mice. Ultrastructural analyses revealed that although Foxk1−/− skeletal muscle possesses a resident myogenic progenitor cell (satellite cell) population (Fig. 1a), the quantity of myogenic progenitor cells is significantly decreased by 71% compared with age/sex-matched WT mice (2.86 ± 0.05% in WT versus 0.84 ± 0.05% in Foxk1−/−; n = 3 for each group; p < 0.05; Fig. 1c). Single fiber myogenic progenitor cell quantification also demonstrated a 73% reduction in the number of myogenic progenitor cells migrating from Foxk1−/− fibers compared with gender and size-matched WT fibers following 72 h in culture (104 ± 10 in WT (n = 19) versus 29 ± 6 in Foxk1−/− (n = 29); p < 0.05; Fig. 1, b and c). These results support the hypothesis that Foxk1 is an important regulatory factor of the myogenic progenitor cell population.

Cell Cycle Progression in the Foxk1−/− Myogenic Progenitor Cell Is Impaired—To examine the mechanism associated with the reduced myogenic progenitor cell number in the absence of Foxk1, we compared the cell cycle profile of WT and Foxk1−/− myogenic progenitor cells using FACS analysis. Loss of Foxk1 disrupts cell cycle progression, causing an increase in S and G2/M cells as indicated by the DNA content of the cells (Fig. 1d). Furthermore, the proliferative capacity of the Foxk1−/− myogenic progenitor cell population, as determined by BrdU Incorporation, was decreased by ~50% compared with the WT myogenic progenitor cell cultures (43 ± 0.5% in the WT, n = 6 versus 22 ± 0.5% in the Foxk1−/−, n = 8; p < 0.05). These results establish that Foxk1 is involved in the cell cycle process, since myogenic progenitor cells lacking Foxk1 show perturbed cell cycle progression and decreased proliferative capacity.

The G1/G0 Arrest Observed in Foxk1−/− Myogenic Progenitor Cells Is a Cell Autonomous Defect—To ascertain whether the impaired cell cycle progression of the Foxk1−/− myogenic progenitor cells is the result of intrinsic or extrinsic factors, Foxk1−/− myogenic progenitor cells were exposed to WT conditioned medium or IGF-I. IGF-I was chosen as it has been shown to modulate the transcriptional activity and cell cycle control of other forkhead/winged helix transcription factors and influence the G1/S cell cycle progression (19). We hypothesized that if the cell cycle defect in the absence of Foxk1 was the result of impaired extrinsic cues, then exposure of Foxk1−/− myogenic progenitor cells to WT conditioned medium or IGF-I should rescue the cell cycle perturbation (i.e. G0/G1 arrest). Neither WT conditioned medium (n = 3) nor IGF-I (n = 5) was capable of rescuing the G0/G1 arrest observed in the Foxk1−/− myogenic progenitor cells (Fig. 1d), supporting the hypothesis that the cell cycle perturbation in Foxk1−/− myogenic progenitor cells is the result of intrinsic rather than extrinsic defects or cues.

The G1/G0 Arrest Observed in Foxk1−/− Myogenic Progenitor Cells Is a Cell Autonomous Defect—As these data supported a cell autonomous perturbation of cell cycle progression in the Foxk1−/− myogenic progenitor cell population, we examined the transcriptional profile of cell cycle regulatory genes to identify putative candidates responsible for the G0/G1 arrest. Real-time RT-PCR, semiquantitative RT-PCR, and Western analyses revealed a significant up-regulation of the cyclin-dependent kinase inhibitor, p21CIP, independent of changes in other cyclin-dependent kinase inhibitors (p53, p27, or p57 (Fig. 2, a–c)). A significant decrease in cdk1 in the absence of Foxk1 was also observed. The decrease in cdk1 expression is consistent with the hypothesis that Foxk1 is myogenic progenitor cells are arrested at the G0/G1 phase. Collectively, these data support the hypothesis that Foxk1 regulates cell cycle progression and the cyclin-dependent kinase inhibitor, p21CIP, in the myogenic progenitor cells.

Abolition of p21CIP Rescues the Impaired Myogenic Progenitor Cell Number and Cell Cycle Perturbation of the Foxk1−/− Myogenic Progenitor Cells—We hypothesized that the up-regulation of p21CIP expression was responsible for the observed
G$_2$/G$_1$ arrest in the Foxk1$^{-/-}$ mice would ultimately result in the growth retardation of Foxk1$^{-/-}$ mice, since myogenic progenitor cells are required to maintain muscle mass, even in the absence of overt muscle injury. We therefore undertook a combinatorial mating of the Foxk1$^{-/-}$ mice with p21CIP-deficient mice to generate progeny lacking both Foxk1 and p21CIP (Foxk1$^{-/-}$; p21CIP$^{-/-}$) and determine whether we could rescue the defects observed in the Foxk1$^{-/-}$ myogenic progenitor cells.

Quantitation of myogenic progenitor cell number using electron microscopy revealed that mice lacking both Foxk1 and p21CIP had a normal compliment of myogenic progenitor cells, restoring the decreased number of myogenic progenitor cells in the Foxk1$^{-/-}$ skeletal muscle (2.76 ± 0.9% in Foxk1$^{-/-}$; p21CIP$^{-/-}$ versus 0.84 ± 0.5% in Foxk1$^{-/-}$). The rescued myogenic progenitor cell number in the Foxk1$^{-/-}$; p21CIP$^{-/-}$ skeletal muscle was further corroborated using single fiber myogenic progenitor cell quantitation (Fig. 3a).

FACS analysis of the Foxk1$^{-/-}$; p21CIP$^{-/-}$ myogenic progenitor cells demonstrated that the lack of p21CIP expression in Foxk1$^{-/-}$ myogenic progenitor cells prevents the G$_2$/G$_1$ arrest observed in the Foxk1$^{-/-}$ myogenic progenitor cell alone (Fig. 3b). Cell cycle analysis of the p21CIP$^{-/-}$ myogenic progenitor cells did not reveal a significantly enhanced cell cycle progression, which is in agreement with other published reports (20). An increase in cellular proliferation of the double mutant myogenic progenitor cell was also observed using BrdUrd incorporation (Fig. 3, c and d). The percentage of BrdUrd-positive nuclei in the Foxk1$^{-/-}$; p21CIP$^{-/-}$ was significantly greater than that observed in the Foxk1$^{-/-}$ myogenic progenitor cells (Fig. 3d), reaching values comparable with that observed in WT myogenic progenitor cells. We conclude that myogenic progenitor cells lacking Foxk1 have an increase in p21CIP resulting in an impaired cell cycle progression.

The Growth Retardation and the Impaired Skeletal Muscle Regeneration Observed in the Foxk1 Null Mice Are Rescued in Mice Lacking Both Foxk1 and p21CIP. Ten days following cardiotoxin-induced skeletal muscle injury in WT mice, skeletal muscle architecture is largely restored. In contrast, Foxk1$^{-/-}$ mice manifest a myonecrotic response that persists more than 3 weeks following the injury (15). The impairment in Foxk1$^{-/-}$ skeletal muscle re-
Mice (Fig. 4 within 10 days of injury comparable with that observed in WT (expression of the cyclin-dependent kinase inhibitor, p21CIP. Increase in p21 CIP gene expression in Foxk1 Foxk1 inhibitors, including p53. Expression of cdk1 is down-regulated in the cells that is independent of changes in other cyclin-dependent kinase inhibitors, including p53. Expression of cd1 is down-regulated in the Foxk1–/− myogenic progenitor cells consistent with the G1/G0 arrest in Foxk1–/− myogenic progenitor cells. The asterisk denotes significance (p < 0.05) compared with WT. c, Western analysis reveals increased p21CIP protein expression in the Foxk1 mutant myogenic progenitor cells compared with the wild type control. Note that no changes in p53 protein expression are observed in the Foxk1 and WT myogenic progenitor cells.

Generation is completely rescued in mice lacking both Foxk1 and p21CIP with restoration of the skeletal muscle architecture within 10 days of injury comparable with that observed in WT mice (Fig. 4b).

Discussion

Elegant studies have challenged previously established paradigms by demonstrating that adult stem cells have a broad capacity or plasticity and are capable of contributing to multiple lineages when placed in a permissive environment (3, 21, 22). In recent years, cellular augmentation therapy has been pursued using alternative adult stem cell populations, including myogenic stem (progenitor) cells, to repopulate the failing heart and myopathic skeletal muscle (23, 24). The studies utilizing the transfer of myogenic progenitor cells for the treatment of debilitating myopathies and congestive heart failure have unfortunately yielded disappointing results. Successful utilization of these adult stem cell/progenitor cell populations for therapeutic applications requires an understanding of the molecular regulation and cell cycle control of these rare and unique cell populations.

Foxk1 Is Expressed Selectively in Myogenic Progenitor Cells—Myogenic progenitor cells are arrested at an early stage of the myogenic program, such that they do not express any of the myogenic basic helix-loop-helix proteins of the MyoD family (25–28). Although a number of studies have examined the physiological responses of these adult muscle progenitor cells to various stimuli, the molecular signals governing their proliferative and differentiation capacity remain poorly defined (4, 25, 28–30).

We have previously established that Foxk1 is restricted to the myogenic progenitor cell population in adult skeletal muscle and is the first molecular marker for this quiescent cell population (14). In the present study, we establish that Foxk1 is necessary for the normal complement of quiescent myogenic progenitor cells that reside in adult skeletal muscle. In the absence of Foxk1 only a small subpopulation of myogenic progenitor cells are established, and they have perturbed cell cycle progression. This defect in myogenic progenitor cell number and cell cycle progression results in the muscle regeneration deficit observed in Foxk1-deficient mice. These results demonstrate that Foxk1 plays a critical role during muscle development and during muscle regeneration in the adult mouse.

Foxk1 Is Important in the Cell Cycle Progression of the Myogenic Progenitor Cell—The exquisitely ordered, functional regulation of the G1-S phase transition of the cell cycle is determined by a balance between positive and negative regulatory pathways. The principle control of cell cycle progression is mediated through the regulation of the activity of cyclin-dependent kinases by both cyclins and the cyclin-dependent kinase inhibitory proteins. p21CIP is an important cyclin-dependent kinase inhibitor that functions, in part, to regulate the G1-S phase transition of the cell cycle. Expression of the p21CIP gene is induced by a wide range of cell growth regulatory signals, including p53-dependent (e.g. DNA damage) and by p53-independent mechanisms during normal tissue development or cellular differentiation (31, 32). Transgenic overexpression of p21CIP in the adult murine hepatocyte was shown to result in a perturbed cell cycle progression during hepatocyte regeneration, a runted liver, and a significant growth deficit of the animal (33). Notably, this is a similar phenotype to that observed in the Foxk1–/− mouse model. Here we present evidence supporting the hypothesis that in the absence of Foxk1, p21CIP expression is up-regulated, resulting in a G0/G1 arrest in the myogenic progenitor cell population under growth promoting conditions. The conclusion that Foxk1 regulates the myogenic progenitor cell population and modulates cell cycle progression is further based on the rescue of the growth deficit, decreased myogenic progenitor cell number, and cell cycle perturbation in mice that are deficient in both Foxk1 and p21CIP.

Mice that are genetically deficient for p21CIP are viable but are radiation-sensitive, and their cells display impaired p53-dependent cell cycle arrest in response to DNA damage (34). Furthermore, cultures of p21CIP null embryonic fibroblasts reveal a mild decrease in G1 length with less than a 5% decrease in G1 cells (20). While no skeletal muscle defects have been previously reported in the absence of a single cyclin dependent kinase inhibitor, mice lacking both p21CIP and p57 have severe defects in skeletal myogenesis characterized by the failure to form differentiated myotubes (32). These results support the essential role of coordinated cell cycle regulation for myoblast proliferation and differentiation during myogenesis (32). In the present study, we hypothesized that the growth retardation in Foxk1–/− mice reflected a defect in the function of the myogenic progenitor cell population, since myogenic progenitor cells are required to maintain muscle mass, even in the absence of overt muscle injury. In the present study, we provide data to support this hypothesis as mice lacking both Foxk1 and p21CIP resulted in a rescue of the growth deficit observed in Foxk1 mutant mice. In addition, doubly mutant mice have normalized both the myogenic progenitor cell number and cell cycle progression. The conclusion that Foxk1 is an important regulator of the myogenic progenitor cell population is further based on the rescue of chemical induced muscle injury in Foxk1–/−: p21CIP–/− mice. Cardiotoxin-induced injury results in an extensive myonecrotic response (>80% of the muscle is destroyed) followed by a predictable, reproducible, and rapid repair process within 10 days of the injury (15). The regenerative response of both WT and Foxk1–/−:p21CIP–/− muscle is
FIG. 3. Myogenic progenitor cell number and cell cycle progression are rescued in Foxk1−/−; p21CIP−/− mice. a, single fiber myogenic progenitor cell quantitation shows a 73% reduction in myogenic progenitor cell number in the absence of Foxk1 compared with WT values. Fibers lacking both Foxk1 and p21CIP show restoration of myogenic progenitor cell number (WT: 104 ± 10, n = 19; Foxk1−/−: 29 ± 6, n = 29; Foxk1−−; p21CIP−−: 19 ± 16, n = 14 myogenic progenitor cells per fiber). The asterisk denotes significant changes (p < 0.05) compared with the wild type controls. Quantitation of the myogenic progenitor cells utilizing electron microscopy further demonstrates a restoration of myogenic progenitor cells in skeletal muscle lacking both Foxk1 and p21CIP (WT: 2.86 ± 0.05%, n = 3; Foxk1−/−: 0.84 ± 0.05%, n = 3; Foxk1−−; p21CIP−−: 2.76 ± 0.99%, n = 4). b, FACS analysis of asynchronously dividing WT, Foxk1−−, p21CIP−−, and Foxk1−−; p21CIP−− myogenic progenitor cells demonstrates that the G0/G1 arrest observed in Foxk1−− myogenic progenitor cells is restored to WT levels in myogenic progenitor cells lacking Foxk1 and p21CIP. Loss of p21CIP alone has no significant effect on cell cycle progression, consistent with previous studies (20). These results support the hypothesis that p21CIP is a downstream target for Foxk1. The asterisk denotes significant changes (p < 0.05) compared with the wild type controls.

c, BrdUrd incorporation assays to assess proliferative capacity confirms that the impaired cellular proliferation observed in the absence of Foxk1 is rescued in the double mutant (Foxk1−−; p21CIP−−) myogenic progenitor cells. Note BrdUrd-positive nuclei are green, and all nuclei are stained with propidium iodide (PI). d, quantitative analysis of the BrdUrd incorporation assays comparing the number of BrdUrd-positive nuclei (proliferating myogenic progenitor cells) as a percent of propidium iodide-positive nuclei (total myogenic progenitor cells). The percent of BrdUrd-positive myogenic progenitor cells in the Foxk1−−; p21CIP−− (40.6 ± 3.9%, n = 3) is not significantly different from WT (43.4 ± 0.5%, n = 6), while both have a significantly greater percentage of BrdUrd-positive nuclei than the Foxk1−− myogenic progenitor cells (22.4 ± 0.5%, n = 8). The asterisk denotes significant changes (p < 0.05) compared with the wild type controls.

FIG. 4. Growth and regenerative Foxk1 defects are rescued in Foxk1−−; p21CIP−− mice. a, Foxk1−−; p21CIP−− mice are comparable in size and indistinguishable from WT mice, while Foxk1−− mice remain significantly growth retarded. b, ten days post-cardiotoxin injection, WT skeletal muscle architecture is completely restored with many newly regenerated myofibers visible (denoted by arrows). Mice lacking Foxk1 still exhibit a hypercellular response with little evidence of regenerated myofibers. In contrast, Foxk1−−; p21CIP−− skeletal muscle has a complete restoration of the skeletal muscle architecture, which is indistinguishable when compared with WT regenerating muscle. Note the presence of numerous regenerated myofibers (arrows) in WT and double mutant skeletal muscle.
indistinguishable, as normal skeletal muscle architecture was observed within 10 days of injury. In contrast, the Foxk1 mutant mice have a severe impairment in muscle regeneration with ineffectual repair even 3 weeks following injury due to the defect in the myogenic progenitor cell population. These results establish Foxk1 as an important cell cycle regulator of the myogenic progenitor cell and supports the hypothesis that p21\(^{CIP}\) is a downstream target of Foxk1. Furthermore, preliminary studies undertaken in our laboratory have identified a Foxk1 binding motif within one of three evolutionarily conserved regions of the p21\(^{CIP}\) promoter (the other two regions are p53 binding sites). Additional studies are under way that will further examine the transcriptional regulation of the p21\(^{CIP}\) gene by Foxk1.

forkhead/winged helix Factors and Stem Cell Biology—Members of the forkhead/winged helix family have a signature motif that encodes a DNA binding domain and function as transcription factors to exert important regulatory functions during development (i.e. cell specification and lineage segregation) and with respect to stem cells and/or tissue repair (4–13). For example, Genesis (Foxd3) is expressed selectively in embryonic stem cells (5), TWH (Fox1) is a regulator of neural progenitor cells (35), and a protein related to HNF3 (FOXM1) has been identified in regenerating hepatocytes (36). Other forkhead/winged helix proteins have also been implicated in the regulation of cell cycle progression through interactions with cyclin-dependent kinase inhibitors. For example, overexpression of AFX (FOXO4) in multiple cell lines results in a G\(_1\) arrest of the cell cycle, which is dependent on the cyclin-dependent kinase inhibitor p27 (37). It has also been proposed that the tumor-suppressing role of PTEN is mediated through inhibition of protein kinase B on FKHR (FOXO1a) and FKHR-L1 (FOXO3a). Both PTEN and activated FKHR/FKHR-L1 were demonstrated to induce expression of p27 while having no effect of p21\(^{CIP}\) (8). Our current data concerning Foxk1, however, provide direct evidence for a specific role in the regulation of the cell cycle for members of this extended gene family in the regulation of progenitor (or stem) cell function.

In summary, these studies establish a functional role for Foxk1 in the regulation of the myogenic progenitor cell population and support the hypothesis that p21\(^{CIP}\) is a downstream target gene. These studies further provide a mechanistic understanding of the regulation of the myogenic progenitor cells and will advance the use of cell transfer technologies for therapeutic applications in the treatment of myopathies.

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