SHP2 ცილის როლი და მახასიათებლები პოსტნათალური კუნთის განვითარებაში

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აბსტრაქტი

SHP-2 (კოდირებული PTPN11-ით) არის საყოველთაოდ ექსპრესირებადი ცილა-თიროზინფოსფატაზა, რომელიც საჭიროა უჯრედის ზედაპირის სხვადასხვა რეცეპტორების მიერ სიგნალის გადაცემისთვის. SHP-2 მუტაციების მქონე ადამიანებს უვითარდებათ ნუნანის სინდრომი ან LEOPARD სინდრომი, რომლებიც ხასიათდება გულ-სისხლძარღვთა, ნევროლოგიური და ჩონჩხის დარღვევებით. Shp2 არის მნიშვნელოვანი სასიგნალო აგენტი ზრდის ფაქტორებისა და ციტოკინებისთვის. Ptpn11-ის როლის გამოსაკვლევად თაგვების პოსტნატალურ მიოგენეზში, ჩვენ გავაანალიზეთ იმუნოჰისტოქიმიური ღეროვანი უჯრედების მარკერების გამოყენებით: Pax 7, Myo D, Myf 5 და Myo G ველური ტიპის და Shp2 ნოკაუტ თაგვებისგან იზოლირებულ კუნთოვან უჯრედებში. ჩვენ გამოვიყენეთ SHP-2 თაგვის მუტანტი, რომელშიც SHP-2-ის ექსპრესიის დაკარგვა გამოწვეული იყო მრავალ ქსოვილში წამლის შეყვანის საპასუხოდ. ჩვენი კვლევები უჩვენებს, რომ ყველა მარკერი ხარისხობრივად იკლებს დაბადებიდან 14 დღის ჩათვლით თაგვის კუნთის უჯრედებში. ჩვენ ვაჩვენეთ, რომ ორი SH2 დომენის მქონე შიდაუჯრედული თიროზინ ფოსფატაზა Shp2 კრიტიკულ როლს თამაშობს თაგვის კუნთის განვითარებაში დაბადების შემდეგ. ჩვენი მონაცემები აჩვენებს მოლეკულურ განსხვავებას საკონტროლო და Sh2 ნოკაუტის პოსტნათალურ მიოგენურ ღეროვან

უჯრედებში და მიუთითებს Ptpn11-ის სასიგნალო ფუნქციაზე სატელიტური უჯრედების აქტივობაში. ეს დასკვნები ასახავს SHP-2-ის მნიშვნელოვან როლს მოზრდილებში კუნთების ზრდასა და რემოდელირებაში და ავლენს ზოგიერთ უჯრედულ და მოლეკულურ მექანიზმს. მოსალოდნელია, რომ მოდელი იქნება შემდგომი გამოყენებული იმის გასაგებად, თუ როგორ არეგულირებს SHP-2 კუნთების მორფოგენეზს, რამაც შეიძლება გამოიწვიოს ახალი თერაპიის შემუშავება მალფორმაციების სამკურნალოდ ადამიანის პაციენტებში SHP-2 მუტაციებით.

საკვანძო სიტყვები: თიროზინ ფოსფატაზა Ptpn11 (Shp2), Pax 7, Myo D, Myf 5, Myo G, კუნთის უჯრედი, განვითარება, ღეროვანი უჯრედები.

THE FEATURES AND ROLE OF SHP2 PROTEIN IN POSTNATAL MUSCLE DEVELOPMENT

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Abstract

SHP-2 (encoded by *PTPN11*) is a ubiquitously expressed protein tyrosine phosphatase required for signal transduction by multiple different cell surface receptors. Humans with carry germline SHP-2 mutations develop Noonan syndrome or LEOPARD syndrome, which are characterized by cardiovascular, neurological and skeletal abnormalities.Shp2 is an important signaling agent for growth factors and cytokines. To investigate the Ptpn11 in postnatal myogenesis of mice we analyzed using immunohistochemistry stem cell markers: Pax 7, Myo D, Myf 5 and Myo G in muscle cells isolated from wild type and Shp2 knockout mice. We used a conditional SHP-2 mouse mutant in which loss of expression of SHP-2 was induced in multiple tissues in response to drug administration. Our findings indicate that all these markers gradually

decreased from birth to day 14 in mouse muscle cells. Here we show that Shp2, an intracellular tyrosine phosphatase with two SH2 domains, plays a critical role in mouse muscle development after birth. Our data demonstrate a molecular difference in the control and Sh2 knockout postnatal myogenic stem cells, and assign to Ptpn11 signaling a key function in satellite cell activity. These findings illustrate an essential role for Shp-2 in muscle growth and remodeling in adults, and reveal some of the cellular and molecular mechanisms involved. The model is predicted to be of further use in understanding how Shp-2 regulates muscle morphogenesis, which could lead to the development of novel therapies for the treatment of malformations in human patients with SHP-2 mutations.

Key words: tyrosine phosphatase Ptpn11 (Shp2), Pax 7, Myo D, Myf 5, Myo G, muscle cell, development, stem cells.

Introduction

Ptpn11 (Shp2) is a tyrosine phosphatase that is an important and positive mediator of multireceptor signaling [1]. (SHP2) plays an important role in major signaling pathways, mediates its multiple physiological functions during the development and maintenance of homeostasis, and leads to important pathological outcomes when unregulated[2]. Shp2 is a ubiquitously expressed protein that plays a key regulatory role in cell proliferation, differentiation, and growth factor (GF) signaling. Ptpn11 functions downstream of receptor tyrosine kinases are highly conserved in evolution and can be observed in invertebrates and vertebrates [3,4]. It interacts with several molecules in the cell and regulates important signaling events, including the RAS/ERK, PI3K/AKT, JAK/STAT, and PD-1 signaling pathways downstream of multireceptor tyrosine kinases (RTKs) when stimulated by activating factors. stimulated cytokines [5]. In addition, Ptpn11 is involved in PI3K, Jak/Stat, Mapk/p38, NF-kB, and NFAT signaling depending on the cell type and receptor [6]. Ptpn11 is important for myogenesis as it acts during the migration of embryonic progenitor cells from somites to the limbs, where it mediates signals provided by Met and its adapter molecule Gab1[7]. Dysregulation of SHP2 expression or activity causes various developmental diseases, as well as hematological and solid cancers. In addition, increased expression or activity of SHP2 also reduces the sensitivity of cancer cells to anticancer drugs [8].

Mutations in the PTPN11 gene encoding the protein tyrosine phosphatase Shp2 cause Noonan syndrome and LEOPARD syndrome, complex hereditary diseases involving cardiac and vascular defects. However, the function of Shp2 in blood vessels, especially vascular smooth muscle cells (VSMCs), remains largely unknown. Shp2 is required for myocyte proliferation

during cardiovascular development and vascular remodeling through TGFβ1-regulated collagen synthesis [9]. Molecular differences in the control of cell cycle termination in fetal and postnatal myogenic stem cells and the assignment of the Ptpn11 signaling pathway to a key role in the activity of satellite cells [10].

Therefore, we aimed to study a role of Ptpn11 (Shp2) protein in postnatal muscle development.

Material and methods

Animals: To study the effect of deleting SHP-2 in a muscle cell line, we used Ptpn11fl/fl mice carrying the Cre transgene under the control of the MyoD promoter (Ptpn11fl/fl MyoD-Cre mice). The Myo-D promoter in these mice controls Cre expression in MSCs, at least in the limbs and head during development. In Ptpn11fl/fl and Ptpn11fl/+ MyoD-Cre hybrid mice, the frequency of Ptpn11fl/fl Myo-D-Cre pups were similar to that of Ptpn11fl/+, Ptpn11fl/fl- and Ptpn11fl/+ Myo-D -Cre hatchlings. educated. Thus, loss of SHP-2 expression in muscle does not lead to embryonic death. However, Ptpn11fl/fl Myo D-Cre mice were phenotypically abnormal. Despite being the same size at birth, all MSC-SHP-2 KO mice were stunted and significantly smaller than their counterparts by 2-3 days after birth.

Immunohistology: Immunohistology quantification and cell counts Immunohistology was performed as described[24]. The main antibodies used were: anti-Pax7 guinea pig, anti-Pax7 mouse (DHSB, Iowa City, USA), anti-BrdU rat (Biorad, Hercules, USA) anti-Ki67 rabbit (Leica, Wetzlar, Germany), goat anti-collagen (ColIV, Millipore, Billerica, USA) goat anti-desmin. For cell counts with Pax7, MyoD and MyoG antibodies, cell nuclei were always examined with DAPI (Sigma Aldrich, Munich, Germany) counterstain.

More than 100 cells/animal and cells from at least three animals/genotype were quantified.

Cell and myofiber culture: Primary myogenic progenitor cells were seeded at a density of 40,000 cells per well and cultured for one day in DMEM/F12 (1:1) supplemented with 15% FCS in coated Labtek 16-well chambers (Nunc, Roskilde, Denmark). with 10% Matrigel (Sigma-Aldrich, Munich, Germany). For the in vitro differentiation assay, cells were seeded at a concentration of 120,000 cells per well, grown overnight in DMEM/F12 (1:1) supplemented with 15% FCS and changed through differentiation medium. (DMEM supplemented with 2% horse serum). Cell where Isolation from Mouse Hind Limb as described [25].

Statistics: Three or more animals were used per genotype and experiment. Data were analyzed using an unpaired two-tailed t-test. p values less than 0.05 were considered significant. Results are presented as the arithmetic mean ± standard error of the mean (S.E.M.). n.s.: not significant; $p>0.05$;

Results: Loss of SHP-2 expression conditional knockout mice does not result in embryonic lethality (data not shown). The Shp2 conditional knockouts (cKO) were smaller than WT littermates (Fig. 1) Although of similar size at birth, all MSC SHP-2 KO mice showed reduced growth and were visibly smaller than littermates by 2-3 days postpartum. In addition, SHP-2 KO mice exhibited other skeletal abnormalities that included shortened and malformed limbs, and pectus excavatum or pectus carinatum.

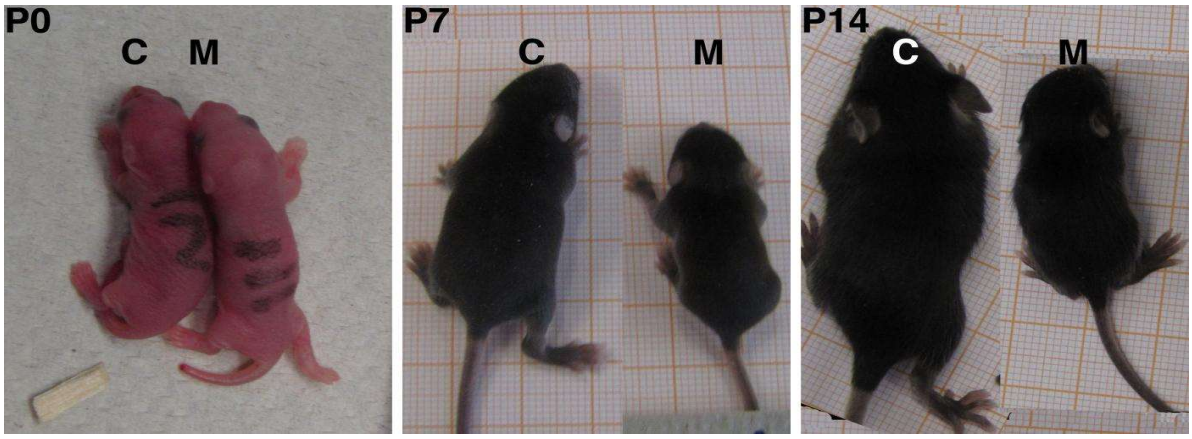
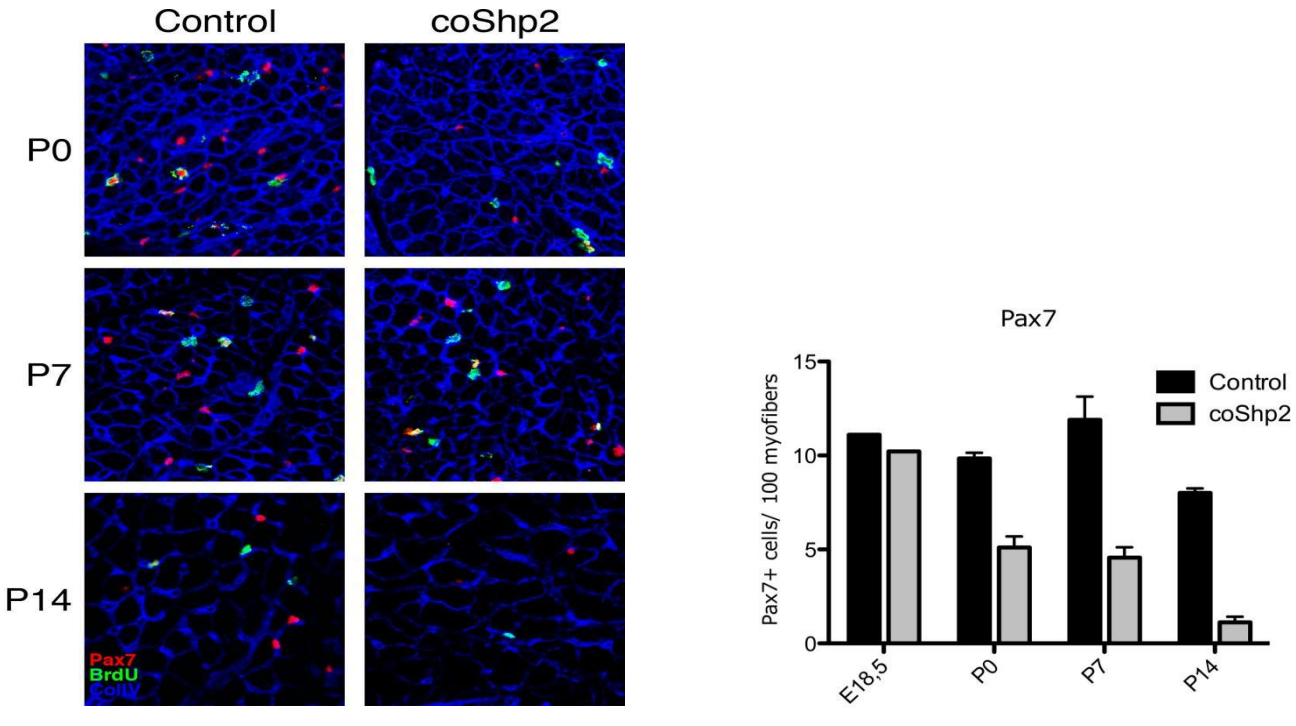


Figure 1

Photos of Postnathal day 0 (P0), Postnathal day 7 (P7) and Postnathal day 14 (P14) mice, left are controls indicated as C (wilt type), right are Shp2 mutants indicated as M.



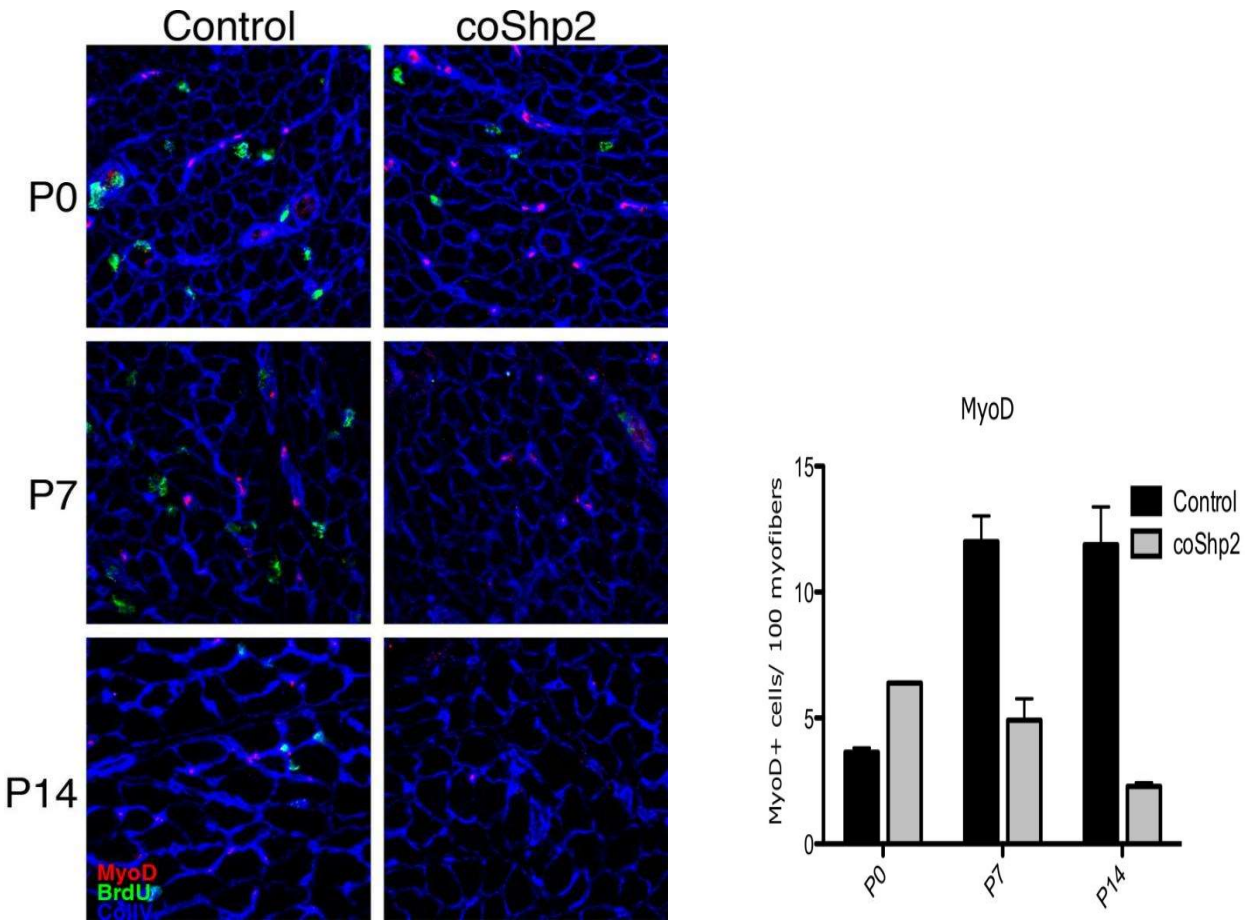
A B

Figure 2

Immunohistological analysis of Pax7 (red), BrdU (green) and collagen IV (ColIV, blue) in muscles (Fig. 2 A) Satellite cell marker Pax 7 is decreased from postnathal day 0 to postnathal day 14 in SHP-2 KO mice, there is no change in Embryo Day 18 stage between control and SHP-2 KO mice. There is no change postnathal day 0- postnathal day 7 in Pax 7 expression in WT mouse, only at day 14 it is decreased. There is significant difference in Pax 7 expression between WT and SHP-2 KO mice (Fig. 2 B).

A) Immunohistochemistry of Pax 7, in muscle cells isolated from control and mutant mice at P0, P7 and P14 stages. B) Evluation of Pax 7 fluorescence intensivity.

Immunohistological analysis of MyoD (red), BrdU (green) and collagen IV (ColIV, blue) in muscles (Fig. 3 A) Stem cell marker Myo D is decreased from postnathal day 0 to postnathal day 14 in SHP-2 KO mice. There is increase of Myo D in expression of postnathal day 7 and day 14 versus to P0 in WT mouse. There is significant difference in Myo D expression between WT and SHP-2 KO mice (Fig. 3 B)

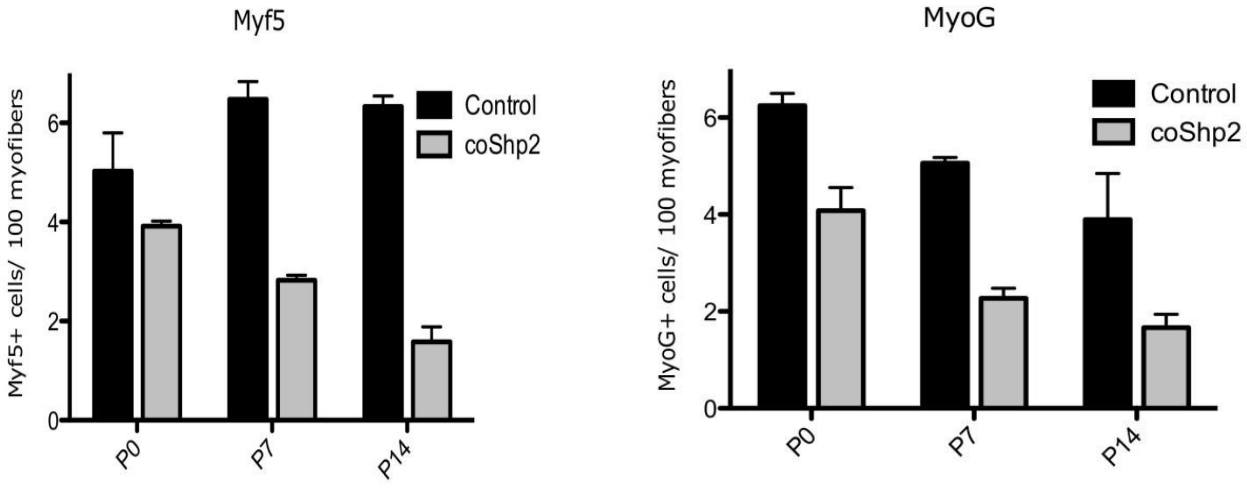


A B

Figure 3

A) Immunohistochemistry of Myo D, in muscle cells isolated from control and mutant mice at P0, P7 and P14 stages. B) Evaluation of Myo D fluorescence intensity.

Skeletal myogenesis marker Myf 5 is decreased from postnathal day 0 to postnathal day 14 in SHP-2 KO mice. There is no significant change in Myf 5 expression in postnatal day 7 and day 14 versus to P0 in WT mouse. (Fig. 4 A) Sceletal muscle marker Myo G is decreased from postnathal day 0 to postnathal day 14 in SHP-2 KO mice. There is decrease in Myo G expression in SHP-2 KO mice compared to WT (Fig. 4 B).



A B

Figure 4

A) Evaluation of Myf5 fluorescence intensivity. B) Evluation of Myf5 fluorescence intensity.

Discussion

The tyrosine phosphatase Ptpn11 is an important transducer of signals provided by growth factors and cytokines [6,11]. Here we show that Ptpn11 plays a central role in myogenesis. Loss of Ptpn11 satellite cells of postnatal myogenesis in a resting state. This results in vigorous muscle growth and kyphosis. Interestingly, patients with hypomorphic mutations in Ptpn11 (Leopard or Noonan syndrome) also have kyphosis, and functional muscle deficits may contribute to the development of this phenotype [9].

Like primary stem cells, satellite cells can regenerate muscle cells. PAX-7 has been shown to affect myogenesis by regulating satellite cell function [12]. The transcription factor PAX7 is an established marker for satellite cells in adult skeletal muscle. Pax7-/- mice have been reported to lack satellite cells and be characterized by significantly reduced muscle regeneration. The muscles of these mice were also characterized by smaller fibers containing fewer nuclei than control mice [13].

MyoD and myogenin are muscle regulatory factors involved in muscle development and differentiation [14,15]. In mature muscle, MyoD is mainly expressed in rapidly contracting mature muscle fibers [16] and regulates rapid muscle development [17]. On the other hand, myogenin is mainly expressed in slow muscle fibers [18].

Myogenic regulatory factors (MRFs) are important regulators of skeletal myogenesis. The myogenic factor Myf5 defines the onset of myogenesis in developing mammals. Mice lacking Myf5 and MyoD do not form myoblasts and are characterized by a complete absence of skeletal muscle at birth. The myogenic factor Myf5 defines the onset of myogenic development in mammals and its central role in embryonic muscle development is well established [19]. Therefore, it makes sense to address the role of Myf5 in adult myogenesis during skeletal muscle regeneration, which became possible after circumventing the perinatal lethality of the original Myf5 knockout strains [20].

The knockout experiments in mouse embryos revealed a central role for MYoG in myogenesis. MyoG null mutants in the homozygous state were stillborn and exhibited various musculoskeletal abnormalities [21,22]. When MyoG, like another transcription factor, is activated, it can affect the cell differentiation process in adulthood [23].

The tyrosine phosphatase Ptpn11 is an important transducer of signals emitted by growth factors and cytokines [24,25]. Here we show that Ptpn11 plays a central role in myogenesis. Loss of Ptpn11 puts satellite cells of postnatal myogenesis into a resting state.

Conclusion

Taken together, these results identify Shp2 as a critical signaling molecule in the coordinated regulation of progenitor cell proliferation and myogenic stem cell differentiation. Shp2 is essential for myogenesis. Loss of of Ptpn11 decreases satellite cell number and provides evidence for an unexpected molecular difference in regulation of proliferation in fetal and postnatal myogenic progenitors cells.

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