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CD56+ Muscle Derived Cells but Not Retinal NG2+ Perivascular Cells of Nonhuman Primates are Myogenic after Intramuscular Transplantation in Immunodeficient Mice

Daniel Skuk* and Jacques P Tremblay

Axe Neurosciences, Research Center of the CHU de Quebec - CHUL, Quebec, QC, Canada

Abstract

Some reports attributed to pericytes and other perivascular cells (PCs), regardless of their origin, optimal properties for cell therapy in myology. The retina is an ideal tissue to obtain pericytes and one study reported that PCs from the mouse retina were myogenic *in vitro*. Given the importance of nonhuman primates (NHPs) for translational research, we compared the *in vivo* myogenicity of NHP retinal PCs and satellite cell derived myoblasts (SCDMs) by transplantation in immunodeficient mice. We used a protocol to culture retinal pericytes of large mammals with macaque retinas. By flow cytometry, 76%-78% of the cultured cells were NG2+. CD56+ SCDMs from another macaque were proliferated *in vitro*. Both Tibialis anterior muscles of 4 SCID mice were injected with 1x106 cells in saline (SCDMs in the right muscles and PCs in the left), using cardiotoxin to induce muscle regeneration. They were sampled 1 month later and analyzed by histology. In SCDM-grafted muscles, NHP nuclei were abundant, in large regions with numerous NHP-derived myofibers, and some of them were Pax7+. PC-grafted muscles showed no muscle regeneration, have few NHP nuclei in small regions devoid of myofibers, and no NHP-myofibers or Pax7+ NHP nuclei were observed. Therefore, NHP SCDMs, but not retinal NG2+ PCs, regenerated muscle *in vivo* in immunodeficient mice.

Keywords: Cell therapy; Myoblasts; NG2; Nonhuman primates; Pericytes; Perivascular cells; Skeletal muscle

List of abbreviations: DMEM: Dulbecco's Modified Eagle Medium; H&E: Hematoxylin and Eosin Stain; HBSS: Hank's Balanced Salt Solution; MCDB-120: Molecular, Cellular, and Developmental Biology-120 Culture Medium; NG2: Neural/Glial Antigen 2; NHP: Non Human Primate; PBS: Phosphate-Buffered Solution; PC: Perivascular Cell; SCID: Severe Combined Immunodeficiency; SCDM: Satellite Cell Derived Myoblast; TA: Tibialis Anterior (muscle)

Introduction

Cell therapy is a potential tool for a future treatment of skeletal muscle pathologies, mostly in a context of regenerative medicine. This therapeutic strategy requires the transplantation of mononuclear cells that are myogenic, that is, capable of [1]: (a) fusing with the myofibers of the host, to provide exogenous myonuclei coding for proteins able to fulfill a therapeutic role, (b) forming new myofibers, which can restore the muscle lost in degenerative myopathies, and (c) forming new satellite cells, i.e., healthy muscle-specific stem cells able to participate in subsequent myofiber regeneration. In clinical trials, CD56+ muscle derived cells, that is, Satellite Cell Derived Myoblasts (SCDMs), were so far the only cells that showed to be myogenic following transplantation in skeletal muscles of patients [2-4]. In spite of that, other cells were reported to fulfill some myogenic properties following transplantation under specific experimental conditions in mice and sometimes in dogs [5]. Some research teams assigned significant myogenic capacity for cell therapy in myology to Perivascular Cells (PCs) [6], essentially pericytes [7], mesoangioblasts [8,9] myoendothelial cells [10] and ß-4-integrin+ cells [11]. Mesoangioblasts/pericytes have been the most studied and they are considered to be essentially the same cell type in humans [12,13]. They were considered the most attractive PCs for cell therapy in myology, given the excellent results of gene complementation reported in mice and dogs by one group [8,9], although a clinical trial in patients gave negative results [14].

The retina is a tissue in which it is easy to obtain large amounts of pericytes for culture *in vitro*, due to the high density of capillaries and small vessels with high amounts of pericytes [15]. Indeed, the retina, with the central nervous system, has the highest density of pericytes in the body: 1 pericyte per endothelial cell, against 1 pericyte per 100 endothelial cells in the skeletal muscle [16]. Moreover, since the retina is dissected from a closed cavity anatomically separated from skeletal muscles, it ensures that the cell culture is devoid of satellite cells [17], a factor that could lead to errors when pericytes are isolated from skeletal muscle [17]. Importantly, one group reported that PCs from the mouse retina, which corresponded to pericytes and/or smooth muscle cells (most probably the first), were spontaneously myogenic *in vitro*, i.e., able to fuse with preexisting myotubes initiating the expression of muscle-specific genes [17].

Considering the crucial importance of Nonhuman Primates (NHPs) for translational preclinical research [18] in contrast to the poor clinical predictability of studies in mice [19,20], given close phylogenetic relationship of macaques and humans and therefore the greatest biological similarities among research animal models, and in view of our extensive experience using NHPs in the research of cell therapy in myology, we wanted to test the myogenicity of pericytes of macaques by transplantation in immunodeficient mice, anticipating potential later studies of allotransplantation in skeletal muscles of NHPs.

***Corresponding author:** Daniel Skuk, Axe Neurosciences (P-09300), CHUL, 2705 Boulevard Laurier, Québec (QC), G1V 4G2, Canada, Tel: +1(418)654-2186; Fax: +1(418)654-2207; E-mail: daniel.skuk@crchudequebec.ulaval.ca

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Material and Methods

Animals

Retinal cells were obtained from a cynomolgus monkey (Macaca fascicularis; female; 3 years old, weight 2.4 kg) in which euthanasia was performed for reasons unrelated to this study. Euthanasia was done by an intravenous overdose of pentobarbital (120 mg/kg) after anesthesia with intramuscular ketamine (15 mg/kg). Four Severe Combined Immunodeficiency (SCID) Balb/c mice (Charles River, Wilmington, MA) were used as recipients for cell transplantation. The Laval University Animal Care Committee authorized these procedures, conducted according to the guidelines set by the Canadian Council of Animal Care.

Retinal cell culture

We used the protocol specifically developed to culture retinal pericytes from large mammals by Bryan & D'Amore [15], given its simplicity and efficacy. We enucleated both eyeballs in aseptic conditions after monkey euthanasia, carefully dissecting away the remnants of extraocular muscles, and leaving the ocular globes 15 min in 20% povidone-iodine in sterile Hank's Balanced Salt Solution (HBSS), before transferring them to a laminar flow chamber. After several HBSS washes, we cut the ocular globes 2-3 mm behind the cornea with fine scissors to extract the vitreous body and retina. Then, we removed any remnant of pigment epithelium with fine forceps, we minced the retinas with fine scissors and we leave the minced retinas in suspension in DMEM (Invitrogen, Burlington, ON, Canada) with 5% calf serum (HyClone, Logan, UT) at 37°C until the next day. Then, the minced retinas were dissociated with 0.2% collagenase (Sigma, St-Louis, MO) in Phosphate-Buffered Solution (PBS) containing bovine serum albumin (100 µl) for 1 h at 37°C. We gentle pipetted the suspensions and we washed it through a 40-µm cell-filter (Sarstedt, Nümbrecht, Germany). We plated the cells that passed the mesh into two 25 cm^2 flasks (one for each eye) with DMEM with 5% calf serum. The cells were passaged once, proliferated until confluence, and frozen for storage in liquid nitrogen. After thawing, the cells were proliferated in the same medium with 10% calf serum for one week before transplantation.

SCDM culture

SCDMs were proliferated in vitro from a frozen cell line previously obtained in our laboratory from a skeletal muscle biopsy performed in another female cynomolgus monkey of the same age. Briefly, a muscle biopsy was minced with fine scissors into fragments of less than 1 mm3 and then dissociated with 0.2% collagenase (Sigma, St-Louis, MO) in HBSS for 1 h, followed by another dissociation in 0.125% trypsin (Gibco, Grand Island, NY) in HBSS for 45 min. The isolated cells were sub-cultured *in vitro* in MCDB-120 culture medium [21] with 15% fetal bovine serum (HyClone), 10 ng/ml basic fibroblast growth factor (Feldan, St-Laurent, QC, Canada), 0.5 mg/ml bovine serum albumin (Sigma), 1.0 µM dexamethasone (Sigma), and 5 µg/ml human insulin (Sigma). This cell line, previously prepared for other experiments, was transduced with the *LacZ* reporter gene as described [22] and with a micro-dystrophin V5 gene as previously reported [23].

Flow cytometry

An aliquot of the retinal cell populations were analyzed by flow cytometry to determine whether they express the neural/glial antigen 2 (NG2), the most commonly referenced pericyte marker [6,15]. The cells were incubated with 50 µg/mL of a phycoerythrin-conjugated mouse Monoclonal Antibody (mAb) anti-human NG2 (R&D Systems, Minneapolis, MN). The muscle cell line was previously known to be 91% CD56+, used as indicator of the percentage of SCDMs, by incubation with a phycoerythrin-conjugated anti-CD56 antibody (1/40, Beckman Coulter, Fullerton, CA) and analyzed by flow cytometry.

Cell transplantation

Both Tibialis Anterior (TA) muscles of the SCID mice were X-ray irradiated (20 Gy in one session), 9 days before transplantation. Irradiation was done using a 6-megavolt linear accelerator (Siemens, Malvern, PA), placing the distal part of the posterior limbs into the irradiation field and putting a 1-cm-thick bolus over them to deliver the maximum radiation energy in the TA. For transplantations, the cells were detached from the flasks using 0.1% trypsin in HBSS and washed 3 times with HBSS. Cell survival, estimated by a trypan blue exclusion test, was 99% for both cell types. The final cell pellets were resuspended in HBSS and aliquots of 1×10^6 cells in HBSS were prepared for transplantation as pellets in 1.5 ml microtubes. At the moment of each transplantation, the HBSS was removed and the cells were resuspended in 20 µl of HBSS containing 10-µg/ml cardiotoxin (Sigma-Aldrich), to be implanted in each TA through approximately 20 percutaneous microinjections done with glass micropipettes (Drummond Scientific, Broomall, PA). In each mouse, SCDMs were transplanted in the right TA and retinal cells in the left TA.

Sampling

Mice were killed by intracardiac perfusion with heparinized saline under deep anesthesia 4 weeks after cell transplantation. The TAs were dissected, mounted in embedding medium and snap-frozen in liquid nitrogen. Serial cross-sections of 10-12 µm were obtained in a cryostat at -25°C.

Histology

Hematoxylin and Eosin Stain (H&E) was done to observe the general structure of the muscles. Macaque dystrophin was detected with the MANDYS104 mouse mAb anti human/dog dystrophin (1:4; kindly supplied by Dr. Glenn E. Morris, Centre for Inherited Neuromuscular Disease, RJAH Orthopaedic Hospital, Oswestry, United Kingdom), which cross-reacts with cynomolgus but not with mouse dystrophin. The MANDYS104 mAb identifies the dystrophin region corresponding to aminoacids 1841 to 2254, which is absent from the transgenic micro-dystrophin [23,24] present in the grafted SCDMs. Therefore, the immuno-detection of the dystrophin corresponds to the native full-lenght dystrophin present in the SCDMs and not to the transgenic micro-dystrophin. Macaque nuclei were detected with a mouse anti-human lamin A/C mAb (1:100; Vector Laboratories, Burlingame, CA), which cross-reacts with cynomolgus but not with mice lamins. The transcription factor Pax7 was detected with a mouse mAb anti-human/mouse Pax7 (1:50; R&D Systems, Minneapolis, MN). For immunodetections, sections were incubated 10 min with 10% fetal bovine serum in PBS and then incubated 1 h at room temperature with the primary antibodies in PBS with 1% fetal bovine serum. For MANDYS104 and Pax7, slides were then incubated 30 min in a biotinylated anti-mouse IgG antibody (1:200; Dako, Copenhagen, Denmark), followed by 30 min in streptavidin-Cy3 (Sigma-Aldrich). For lamin A/C, an anti-mouse conjugated to Alexa 488 (1:300; Molecular Probes, Eugene, OR) was used as second antibody. Double incubations for co-detection of Pax7 and lamin A/C were done according to the following sequence: mouse anti-Pax7 → anti-mouse IgG biotin → streptavidin-Cy3 → mouse anti-lamin A/C → anti-mouse IgG Alexa Fluor 488. In this case, a 30-min incubation

with the blocking solution was also done before the second reaction. By following this sequence and similar cautions to those previously used for the detection of satellite cells among human nuclei in SCID mouse muscles using the same antibodies [25], we had no problem of interference between the two secondary anti-mouse IgG antibodies. We confirmed cross-reaction of specific anti-human antibodies with cynomolgus proteins (dystrophin and lamin A/C) previous to their use in this study.

Microscopic analysis

The muscle sections were analyzed using an Axiophot microscope with epifluorescence and bright field optics (Zeiss, Oberkochen, Germany). Pictures were taken with an A650 IS digital camera (Canon, Tokyo, Japan).

Results

Flow cytometry analysis of retinal PC cultures.

Two flow cytometry analyses showed respectively that 76% and 78% of the cells present in the cultures of retinal cells were NG2+ (Figure 1).

Macroscopic aspect of the cell-grafted muscles

At the time of sampling, the macroscopic observation showed that the right TAs had whitish patches, as we generally observed with this protocol of transplantation when the muscle regeneration is deficient and fibrosis developed. This was not observed in the left TAs, grafted with SCDMs.

Histological observations in the cell-grafted muscles

Histological analysis showed well-defined differences between the muscles grafted with SCDMs (Figure 2A-2E) and the muscles grafted with PCs (Figure 2F-2H).

Sections stained with H&E show that the SCDM-grafted muscles exhibited a pattern of remodeling and regeneration with abundant rounded myofibers of different sizes with internal nuclei in several cases (Figure 2A). NHP nuclei (i.e., labeled with the anti-human/cynomolgus lamin A/C mAb) were abundant in these muscles, diffusely distributed in a wide region, localizing in both the myofibers and in the interstitium (Figure 2B). Some of the NHP-derived nuclei expressed Pax7 (roughly 3.7% +/- 1.5%) and were generally in the periphery of myofibers (and example is shown in Figure 2C,2D). Large amounts of myofibers expressed NHP-derived dystrophin as detected with the MANDYS104 mAb (Figure 2E) and NHP-derived spectrin (not shown) in the SCDM-grafted muscles. We counted 425 +/- 281 myofibers expressing NHP-derived dystrophin (range 134-738) in the SCDM-grafted muscles.

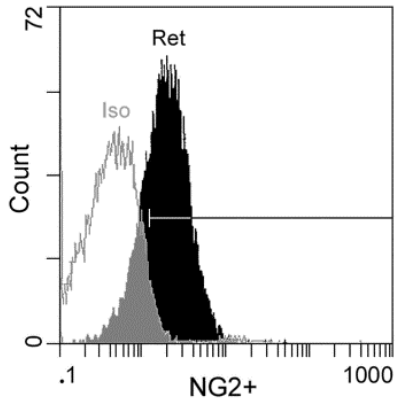

Figure 1: Flow cytometry analysis of one of the cultures of cynomolgus retina-derived cells following the protocol to isolate and proliferate retinal pericytes from large mammals developed by Bryan & D'Amore [15] shows 78% of the cells being positive for NG2 expression.

Conversely, in the PC-grafted TAs, sections stained with H&E show no evidence of muscle regeneration (Figure 2F,2G). These muscles exhibited circumscribed regions of non-muscular disorganized tissue with the aspect of focal fibrosis (Figure 2F,2G). There were NHP nuclei in the PC-grafted TAs, identified by the anti-human/cynomolgus lamin A/C mAb (Figure 2H), although they were less abundant than in the SCDM-grafted muscles and instead of being diffusely distributed in the muscle section they were concentrated in the regions devoid of myofibers that appeared as focal fibrosis (Figure 2F,2H). No NHP-derived nuclei expressed Pax7 in the PC-grafted muscles ($p=0.007$ in a paired t-test when compared with the SCDM-grafted muscles). No myofibers expressing dystrophin ($p=0.028$ in a paired t-test when compared with the SCDM-grafted muscles) or spectrin of NHP origin were detected in the muscles grafted with PCs.

Discussion

In this study, a population of NHP cells that was predominantly NG2+ (the most commonly referenced pericyte marker [6,15]), obtained by a technique specifically developed to culture retinal pericytes from large mammals [15], was no myogenic after intramuscular transplantation in SCID mice, that is, the cells were unable to participate in myofiber regeneration, form new myofibers or give rise to Pax7+ cells (i.e., potentially satellite cells). However, the cell transplantation protocol used here is optimal for these outcomes when the grafted cells are myogenic [25-27]. SCID mice are an excellent substrate to accept xenografts of myogenic cells from humans [25-28] and dogs [29], resulting in abundant myofibers expressing graft-derived proteins, as well as producing abundant satellite cells of human origin [25]. In fact, the population of predominantly CD56+ NHP muscle derived cells (SCDMs) implanted in the contralateral muscle, was able to regenerate abundant myofibers and to give rise to Pax7+ cells (some of which can be potentially satellite cells considering their anatomical location). Importantly, the SCDMs used in this study exhibited a good myogenic capacity following transplantation *in vivo*, even if they were extensively proliferated *in vitro* due to the fact that they were subjected to two genetic modifications as mentioned.

For the rest, the grafted NHP PCs/pericytes survived much less and/or proliferated much less than the NHP SCDMs, given the larger amounts of NHP nuclei observed in the SCDM-grafted muscles compared to the pericyte-grafted muscles. Moreover, grafted PCs/pericytes showed no migration capacity, since in PC-grafted muscles the NHP nuclei were concentrated in few restricted small regions, while the NHP nuclei were homogeneously dispersed in large areas of regeneration in the SCDM-grafted muscles.

This failure of retinal PCs/pericytes of macaques to be myogenic *in vivo* could be considered as contrasting to the myogenic properties reported by other researchers using the equivalent mouse cells *in vitro* [17]. In fact, this discordance may be due to:

A. Inter-species differences: primates (i.e., macaques and humans) and rodents diverged 75 million years ago and evolved in

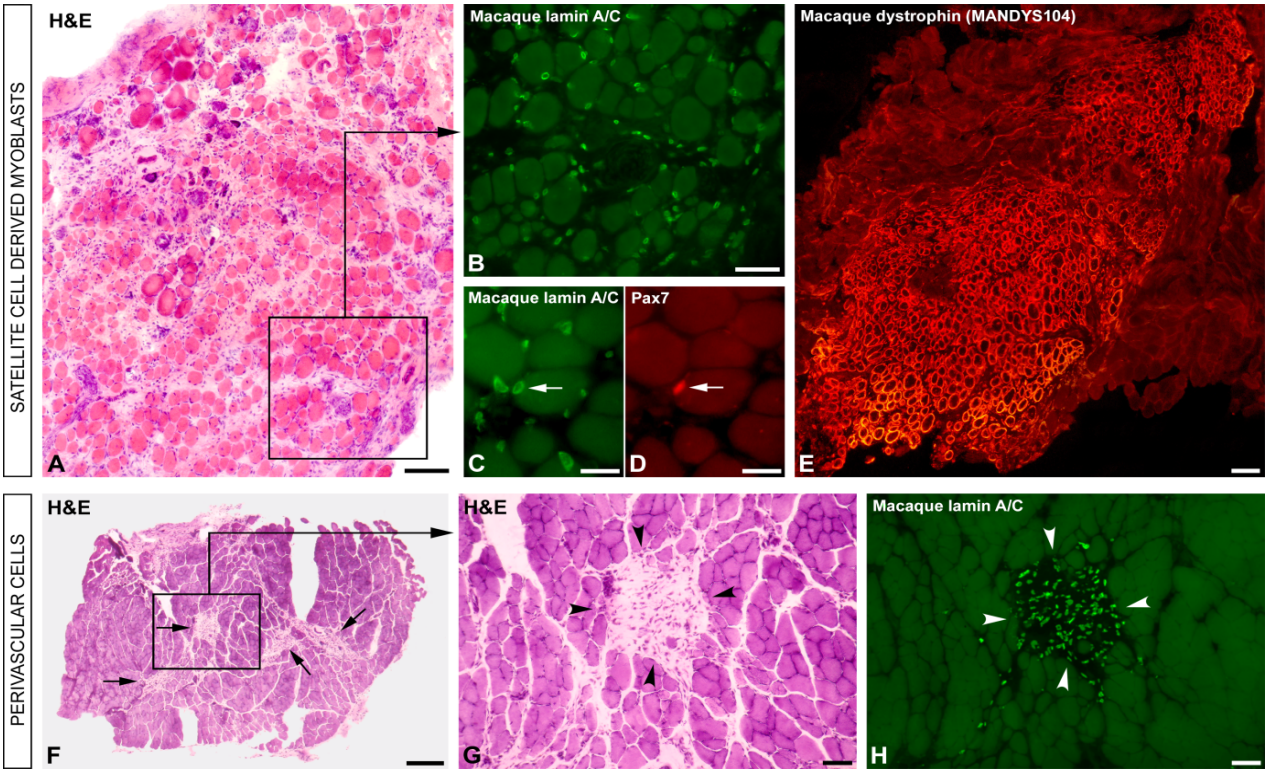

Figure 2: Cross-sections of muscles grafted either with NHP SCDMs (A to E) or NHP PCs/pericytes (F to H). A illustrates a section of a SCDM-grafted muscle stained with hematoxylin and eosin (H&E), showing the muscle remodeling and regeneration. The region in the square in A corresponds to the region in B, in which fluorescent immunodetection of lamin A/C in a serial section shows several NHP nuclei integrated as myonuclei or remaining in the connective tissue. C and D illustrate a monkey nucleus (green fluorescent immunodetection) in the periphery of a myofiber (arrow) that is also Pax7+ (red fluorescence co-immunodetection). E is a serial section of this shown in A (including a wider region) in which the fluorescent immunodetection of macaque dystrophin detected with the MANDYS104 monoclonal antibody shows that a large muscle region is filled with abundant myofibers formed by the grafted SCDMs. F shows the complete section of a muscle grafted with pericytes, which exhibits circumscribed regions of non-muscular disorganized tissue with the aspect of fibrosis (arrows). The region in the square is enlarged in G, to show, in a serial section stained for green fluorescent immunodetection of macaque lamin A/C, that the few remaining NHP nuclei are in these regions of non-muscular tissue (between arrowheads). Scale bars: 500 µm (F), 100 µm (A, E, G, H), 50 µm (B) and 20 µm (C, D).

different conditions, which determined that they develop several biological differences [30].

B. The myogenicity of retinal PCs/pericytes may be a phenomenon occurring only under specific experimental conditions *in vitro*.

In any case, it is clear that, beyond the anecdotal interest of experimental observations of the myogenicity of pericytes or other similar retinal PCs *in vitro*, the *in vivo* incapacity of these cells to regenerate the muscle tissue takes away all value from the point of view of cell therapy in a context of regenerative medicine applied to the skeletal muscle regeneration. In principle, these results in SCID mice do not encourage the possibility to test these cells using allotransplantation conditions in NHPs.

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References

1. Skuk D, Tremblay JP (2015) Cell therapy in muscular dystrophies: many promises in mice and dogs, few facts in patients. Expert Opin Biol Ther 1-13. [PubMed]
2. Skuk D, Goulet M, Roy B, Chapdelaine P, Bouchard JP, et al. (2006) Dystrophin expression in muscles of Duchenne muscular dystrophy patients after high-density injections of normal myogenic cells. J Neuropathol Exp Neurol 65: 371-386. [PubMed]
3. Skuk D, Goulet M, Roy B, Piette V, Cote CH, et al. (2007) First test of a "high-density injection" protocol for myogenic cell transplantation throughout large volumes of muscles in a Duchenne muscular dystrophy patient: eighteen months follow-up. Neuromuscul Disord 17: 38-46. [PubMed]
4. Skuk D, Roy B, Goulet M, Chapdelaine P, Bouchard JP, et al. (2004) Dystrophin expression in myofibers of Duchenne muscular dystrophy patients following intramuscular injections of normal myogenic cells. Mol Ther 9: 475-482. [PubMed]
5. Vilquin JT, Catelain C, Vauchez K (2011) Cell therapy for muscular dystrophies: advances and challenges. Curr Opin Organ Transplant 16: 640-649. [PubMed]
6. Crisan M, Corselli M, Chen WC, Peault B (2012) Perivascular cells for regenerative medicine. J Cell Mol Med 16: 2851-2860. [PubMed]
7. Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, et al. (2008) A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 3: 301-313. [PubMed]
8. Sampaolesi M, Torrente Y, Innocenzi A, Tonlorenzi R, D'Antona G, et al. (2003) Cell therapy of alpha-sarcoglycan null dystrophic mice through intra-arterial delivery of mesoangioblasts. Science 301: 487-492. [PubMed]
9. Sampaolesi M, Blot S, D'Antona G, Granger N, Tonlorenzi R, et al. (2006) Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs. Nature 444: 574-579.
10. Zheng B, Cao B, Crisan M, Sun B, Li G, et al. (2007) Prospective identification of myogenic endothelial cells in human skeletal muscle. Nat Biotechnol 25:1025-1034. [PubMed]
11. Liadaki K, Casar JC, Wessen M, Luth ES, Jun S, et al. (2012) beta4 integrin marks interstitial myogenic progenitor cells in adult murine skeletal muscle. J Histochem Cytochem 60: 31-44. [PubMed]
12. Dellavalle A, Sampaolesi M, Tonlorenzi R, Tagliafico E, Sacchetti B, et al. (2007) Pericytes of human skeletal muscle are myogenic precursors distinct from satellite cells. Nat Cell Biol 9: 255-267.
13. Tonlorenzi R, Dellavalle A, Schnapp E, Cossu G, Sampaolesi M (2007) Isolation and characterization of mesoangioblasts from mouse, dog, and human tissues. Curr Protoc Stem Cell Biol. [PubMed]
14. Cossu G, Previtali SC, Napolitano S, Cicalese MP, Tedesco FS, et al. (2015) Intra-arterial transplantation of HLA-matched donor mesoangioblasts in Duchenne muscular dystrophy. EMBO Mol Med [PubMed]
15. Bryan BA, D'Amore PA (2008) Pericyte isolation and use in endothelial/pericyte coculture models. Methods Enzymol 443: 315-331. [PubMed]
16. Shepro D, Morel NM (1993) Pericyte physiology. Faseb J 7: 1031-1038. [PubMed]
17. Kirillova I, Gussoni E, Goldhamer DJ, Yablonka-Reuveni Z (2007) Myogenic reprogramming of retina-derived cells following their spontaneous fusion with myotubes. Dev Biol 311: 449-463. [PubMed]
18. Anderson DJ, Kirk AD (2013) Primate models in organ transplantation. Cold Spring Harb Perspect Med 3: a015503. [PubMed]
19. Perrin S (2014) Make mouse studies work. Nature 507: 423-425. [PubMed]
20. Mak IW, Evaniew N, Ghert M (2014) Lost in translation: animal models and clinical trials in cancer treatment. Am J Transl Res 6: 114-118. [PubMed]
21. Ham RG, St. Clair JA, Webster C, Blau HM (1988) Improved media for normal human muscle satellite cells: serum-free clonal growth and enhanced growth with low serum. In Vitro Cell Dev Biol 24: 833-844. [PubMed]
22. Skuk D, Goulet M, Paradis M, Tremblay JP (2011) Myoblast transplantation: techniques in nonhuman primates as a bridge to clinical trials. In: Soto-Gutierrez A, Navarro-Alvarez N and Fox IJ (eds). Methods in bioengineering: cell transplantation. Artech House: Boston. Pp: 219-236.
23. Quenneville SP, Chapdelaine P, Skuk D, Paradis M, Goulet M, et al. (2007) Autologous transplantation of muscle precursor cells modified with a lentivirus for muscular dystrophy: human cells and primate models. Mol Ther 15: 431-438. [PubMed]
24. Wang B, Li J, Xiao X (2000) Adeno-associated virus vector carrying human minidystrophin genes effectively ameliorates muscular dystrophy in mdx mouse model. Proceedings of the National Academy of Sciences of the United States of America 97: 13714-13719.
25. Skuk D, Paradis M, Goulet M, Chapdelaine P, Rothstein DM, et al. (2010) Intramuscular transplantation of human postnatal myoblasts generates functional donor-derived satellite cells. Mol Ther 18:1689-1697. [PubMed]
26. Skuk D, Furling D, Bouchard JP, Goulet M, Roy B, et al. (1999) Transplantation of human myoblasts in SCID mice as a potential muscular model for myotonic dystrophy. J Neuropathol Exp Neurol 58: 921-931. [PubMed]
27. Gekas J, Walther G, Skuk D, Bujold E, Harvey I, et al. (2010) In vitro and in vivo study of human amniotic fluid-derived stem cell differentiation into myogenic lineage. Clin Exp Med 10:1-6. [PubMed]
28. Huard J, Roy R, Guerette B, Verreault S, Tremblay G, et al. (1994) Human myoblast transplantation in immunodeficient and immunosuppressed mice: evidence of rejection. Muscle Nerve 17: 224-234. [PubMed]
29. Ito H, Vilquin JT, Skuk D, Roy B, Goulet M, et al. (1998) Myoblast transplantation in non-dystrophic dog. Neuromuscul Disord 8: 95-110. [PubMed]
30. Mestas J, Hughes CC (2004) Of mice and not men: differences between mouse and human immunology. J Immunol 172:2731-2738. [PubMed]