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Non-myeloablative Bone Marrow Stem Cell Transplantation for *mdx* Mice Myodystrophy Therapy

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Abstract

Background: *Mdx* mice are experimental model of cureless human monogenic disease Duchenne Muscular Dystrophy (DMD). Hope for a cure is associated with the use of stem cells therapy particular but not exclusively. Analysis of multiple experimental results shows what intramuscularly transplantation of different types of cells of different origins with stem cells properties can't convert mutant striated muscles fibers (SMF) into wild type SMF. It was concluded that only replacement of mutant bone marrow (BM) by wild type BM cells can convert mutant SMF into SMF of wild type. Unfortunately X-ray irradiation of *mdx* mice at a lethal dose of 11, 7 or 5 Gy followed by transplantation of wild C57BL/6 mice BM cells did not increase SMF dystrophin synthesis. The aim of this study was to analyse a dystrophin synthesis by *mdx* mice striated muscles after x-ray irradiation with the dose of 3 Gy followed by C57BL/6 bone marrow cells transplantation. Also we investigate the reparation of structure of diaphragm muscle fibers NMJs. To confirm the functional significance of observed structural changes of NMJs an investigation of resting membrane potential of diaphragm muscle fiber NMJs was also conducted.

Methods: 1-1.5 months old *mdx* mice were irradiated by x-ray at a dose of 3 Gy. Next day freshly prepared BM cells were injected intravenously in the amount of (15-20) x 10^6 cells per mouse. Animals were studied through 2, 4, and 6 months after transplantation. Each experimental group of mice included 3-8 animals. Mus. quadriceps femoris and diaphragm muscle fibers with their nerve-muscle junctions (NMJs) were under investigation.

For chimerism registration a special study was conducted using transplantation of GFP-positive C57BL/6 BM cells to *mdx* mice after 3 Gy irradiation. Through 6 months BM cells were separated from long bones and smears were prepared. After carbinol fixation smears were stained by propidium iodide and studied on confocal microscope LSM 5 Pascal (Carl Zeiss, Germany) to count the part of GFP-positive cells in relation to whole quantity of nuclear cells.

Results: We observed a stable growth of dystrophin synthesis after nonlethal X-ray irradiation at a dose of 3 Gy. The part of dystrophin positive SMF of M. quadriceps femoris increased from 1% up to 4% (2 months), 12% (4 months) and 27% (6 months) after transplantation. Growth of dystrophin synthesis was accompanied by the decrease of SMF death level, by increase of part of SMF without central nuclear up to 22%, by accumulation of MNJ branches and by reparation of resting membrane potentials. The part of GFP-positive cells between all cells with nuclear on the BM smears of chimeric GFP transplanted *mdx* mice at 6 months after transplantation was 3.3 ± 0.8 % that show for chimeric nature of mice.

Conclusion: Non myeloablative bone marrow cell transplantation of *mdx* mice after X-ray irradiation 3 Gy is accompanied by formation of chimerism, stable growth of dystrophin synthesis and reparation of structure and function of NMJs.

Keywords: *Mdx* mice; Myodystrophy; Non myeloablative bone marrow transplantation; Dystrophin; Nerve-muscle junctions; Resting membrane potential

Introduction

Use of myogenic cells for the treatment of Duchenne's disease was first suggested by Partridge et al. [1]. The development of the genetically authentic model of this desease – *mdx* mice allowed to test myoblasts and other types of stem cells in the therapy of this pathology [1]. In the experiments with GFP-marked cells it was shown that bone marrow (BM) stem cells take part in the regeneration of *mdx* mice striated muscle fibres (SMF) [2-6]. Our attention to this type of stem cells due to the data that BM, being the source of stem cells plays the leading part in the system of mammalian stem cells [7].

In the skeletal muscle stem cell niches of wild type mice as well as of the *mdx* mice [8] are sites for BM stem cells differentiation into satellite cells and other types of muscle stem cells [8-11] [10]. It is essential to say that cells expressing the hematopoietic marker CD45 are predominantly present in the BM but also reside in the skeletal muscle and participate in muscle repair [12,13] and in muscle of *mdx* mice after BM stem cells transplantation [6].

Except blood and muscle cells BM stem cells are also involved in the cell differentiation and regeneration in lungs, liver, skin, gastrointestinal tract and of epithelium of thyroid gland [6,14,15].

Irradiation is a mandatory preliminary step of BM stem cells

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transplantation in recipient, as it suppresses the immune system of the recipient, making it possible to replace mutant bone marrow with the bone marrow of wild-type. The aim of this work was to study the recovery of dystrophin synthesis after C57BL/6 mice BM transplantation in *mdx* mice irradiated at a non-lethal dose of 3 Gy [28,29]. To estimate the significance of change of dystrophin synthesis for physiology of striated muscles there was study of structure and function of nerve-muscle junctions of diaphragm muscle fibers.

Materials and Methods

Animals

C57BL/6 mice have been received from Rappolovo animal farm (St. Petersburg, Russia). *Mdx* mice were provided by Professor Partridge (Hammerssmith Hospital, UK) [1]. Mice were maintained in animal nursery (Institute of Cytology RAS) with standard feeding and light regimes.

Transplantation

1-1.5 months old *mdx* mice were irradiated by x-ray at a dose of 3 Gy using roentgen device rum-17 (Russia) using 0.5 mm CU + 1.0 Alu filters. Energy of X-ray was 45 R/min. Next day freshly prepared bone marrow (BM) cells were injected intravenously in dose (15-20) x 10^6 cells per mouse. Animals were studied through 2, 4, and 6 months after transplantation. Each experimental group of mice included 3-6 animals. Mus. quadriceps femoris and diaphragm muscle fibers with their nerve-muscle junctions (NMJs) were under investigation.

For chimerism registration a special study was conducted with transplantation of GFP-positive BM cells, friendly provided by Dr. V. Serikov (CHORI, Okland, USA) after 3 Gy irradiation. At 6 month after transplantation BM cells were separated from long bones and smears were prepared by propidium iodide and studied on confocal microscope LSM 5 Pascal (Carl Zeiss, Germany) to count the part of GFP-positive cells to whole quantity of cells. There were used 8 C57BL/6 and 45 *mdx* mice.

Immunohistochemistry

10 μm cryosections were cut with cryostat (Bright Co, Ltd, UK) after preliminary muscle freezing in liquid nitrogen. Dried sections were fixed in ethanol mixed with carbinol (1:1 v/v) for 1 min at -20°C or in 10% formalin for 30 minutes at room temperature [30].

For dystrophin staining sections fixed in ethanol/carbinol (1:1 v/v) solution were treated with 1% bovine serum albumin (BSA) for 30 min, washed with PBS for 5 min and incubated with rabbit polyclonal antibodies to dystrophin (Abcam, US) dilution 1 : 100 for 1 h. Samples were washed with FITS-labeled goat anti-rabbit antibody (Sigma, US), 1:150, 1 h. Sections washed with DAPI or propidium iodide, were mounted in glycerol, and assayed under LSM 5 Pascal microscope (Carl Zeiss, Germany). The number of SMFs with dystrophin and fraction of dystrophin-positive fibers in central muscle were counted on sections.

To estimate the number of dead SMFs and SMFs without centrally located nuclei sections were stained with hematoxylin-eosine, passed through ethanol and xylol grades and mounted in Canada balsam. Stained sections were visualized under Axiophot microscope (Carl Zeiss, Germany).

NMJs assessment

Muscle longitudinal and cross sections fixed with 10% formalin were treated with 1 μg/ml tetramethylrhodamine-α-bungarotoxin (TMR-α-BTX) (Biotium, US) for 1 h. Then the sections were washed three times with PBS for 5 min, mounted into reagent that reduced unspecific fluorescence (Biomeda Co, US) and visualized under LSM 5 Pascal (Carl Zeiss, Germany).

NMJ structure was examined in records of single NMJ. NMJ area was calculated on muscle cross sections with ImageJ software (National Institute of Health, US). The area of single acetyl-choline receptor (AChR) clusters that makes the NMJ and the number of AChR clusters in each NMJ were estimated on the longitudinal sections with ImageJ software. The area of each NMJ includes area of AChR and areas of gaps between single clusters. The data were statistically processed with Microsoft Excel. The differences between groups were assessed using Student's t-test. The differences were considered to be significant at $p < 0.05$ [30].

Membrane potential recording

The experiments were performed on freshly isolated diaphragm muscle as described previously [31,32]. A diaphragm strip with nerve stump was placed in a Plexiglas chamber. The chamber was continuously perfused with a physiological solution containing (mM): NaCl, 137; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 2; $NaHCO_3$, 24; NaH_2PO_4, 1; glucose, 11; pH 7.4. The solution was continuously bubbled with 95% O_2 and 5% CO_2 gas mixture and maintained at 28°C. The muscle was equilibrated for 1 hour prior to the start of recording. The resting membrane potential (RMP) were recorded intracellularly using standard microelectrode techniques. Recordings were made in extrajunctional membrane

Time after BMC transplantation, months	Dystrophin (+) SMF, %	Dead SMF, %	CN(−) SMF, %
Control, 2, (3)	1.1 ± 0.4	2.2 ± 0.6	10.5 ± 1.0
2, (4)	4.1 ± 0.9 (3)	1.4 ± 0.3 (4)	16.1 ± 1.7 (4)
4, (3)	12.4 ± 3.9 (3)	0.88 ± 0.2 (3)	20.6 ± 1.3 (3)
6, (5)	27.6 ± 6.7 (5)	0.7 ± 0.1 (5)	22.6 ± 1.9 (5)

BMC: Bone marrow cells; SMF: Striated muscle fibers; CN(-): Muscle fibers without central nuclei

Here and in Tables 2, 3, and 4 the number of animals indicated in parentheses

Table 1: Dystrophin synthesis by SMF of mdx mice M. quadriceps femoris after x-ray irradiation at a dose of 3 Gy followed by BMC transplantation (%, X ± m_x).

Mice	Explored region of synapsis	
	Junctional region	Extrajunctional region
mdx mice, 4 months after bone marrow transplantation, (3)	11.23 ± 3.3	0.0
nonirradiated *mdx* mice, 6 months old, (3)	1.3 ± 0.5	0.0

Table 2: *Mdx* mice diaphragm dystrophin-positive striated muscle fibers (%, X ± m_x) 4 months after bone marrow transplantation.

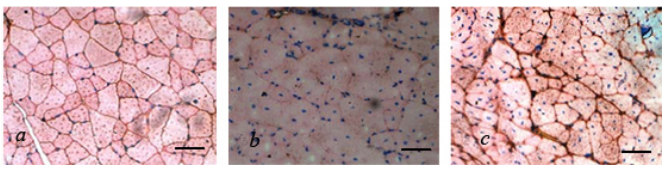

Figure 1: Transverse sections of M. quadriceps femoris of control C57BL/6 mice (a), of control mdx mice (b), and of chimeric mdx mice after 3 Gy irradiation and 6 months BMSCs transplantation (c). Immunostaining for dystrophin with biotin labeled second antibody and streptoavidin with peroxidase targeted. Nuclei were observed by Gimsa staining. Mag. Ob.10x, Oc.10x. Bar 60 mkm.

regions within 1–2 mm from visually identified terminal branches of the nerve, and directly at the nerve terminal (end-plate region, junctional membrane) of the same muscle. RMPs were recorded from 15 to 25 different fibers within each muscle. The entire protocol was repeated in muscles from different animals to obtain the average resting potential for that condition. Data are given as the mean ± SEM. Statistical significance of the difference between group's means was evaluated using a Student's t-test (ORIGIN 6.1. software).

Results

There was an accumulation of dystrophin positive *mdx* mice SMF through 2 months after syngenic bone marrow transplantation up to 4.1% in compare with 1.1% of control animals. Dystrophin synthesis accelerates 2 times for every 2 months and reach 27.7 ± 6.7% at 6 months after transplantation (Table 1 and 2) (Figure 1). We observed also the duplication of SMF without central nuclei up to 22.6% and loss of dead SMF up to 0.7 ± 0.1%. The obtained level of dystrophin synthesis (27.7 ± 6.7%) is consistent with data of other authors concerning 20% level of dystrophin-positive SMF as critical minimal working level for the function of dystrophin-deficient muscles [21,22]. Also we observed 11.2 ± 3.3% of dystrophin-positive SMF in diaphragm in four months after transplantation (Table 2) which is consistent with data obtained from skeletal muscle. The part of GFP-positive cells between all cells with nuclear on the BM smears of chimeric GFP transplanted *mdx* mice at 6 months after transplantation was 3.3 ± 0.8 % that indicates the chimeric nature of mice. All results point to the increase of differentiation level of *mdx* mice SMF after change of mutant bone marrow for bone marrow of wild type.

Study of NMJs structure and their resting potential was conducted for two reasons. We have shown early that local transplantation of BM stem cells to M. quadriceps femoris of unirradiated *mdx* mice partly restore the structure of NMJs [30]. To confirm the results we studied a structure of diaphragm NMJs on the transverse section of synapsis region of diaphragm SMFs by staning with Tetramethylrhodamine-α-bungarotoxin after general transplantation of BM cells. The synapsis of diaphragm *mdx* mice are formed by islets (75.8%) and branches (14%). In four months after BM transplantation we observed a significant increase in quantity of branches (43 %) and decrease in the part of islets in the diaphragm NMJs up to 53.7 ± 6.0 % (Figure 2 and Table 3). Development of branches and decrease of islets are considered to be the markers of the fractional reparation of NMJs structure. In case of local transplantation of BMSCs we did not observed the formation of branches. High concentration of islets and growth of square of NMJs taken place on the background of negligible dystrophin synthesis near 2% [30]. To estimate functional significance of the structural changes of diaphragm NMJs we studied the electrophysiological properties of NMJs through 4 months after BM transplantation (Table 4).

In the control C57B1/6 mice, the value of resting membrane potential (RMP) in the junctional (end-plate) region of NMJs was –81.4 ± 0.5 mV being more negative than that in the extrajunctional region of sarcolemma (–78.0 ± 0.4 mV) ($p < 0.01$). The value of the observed local hyperpolarization corresponds well to the previous results and is specific for intact muscle fibers [28,31]. In *mdx* mice the value of RMP was lower than in the control C57B1/6 mice. The observed depolarization is in agreement with the well-known facts [33]. Moreover, the values of RMP through overall membrane did not differ. However, in *mdx* mice after 4 months of BM cells transplantation, the values of RMP in the junctional and extrajunctional regions increased to the level of control C57B1/6 mice. In the end-plate region, the value of RMP increased by 5.6 mV while in the extrajunctional region, it increased by 2.6 mV. As a result, local hyperpolarization of the end-plate increased by 3.7 ± 0.9 mV ($p < 0.01$), which was characteristic of the control animals and is a reliable indicator of a normal functioning of a neuromuscular synapse after BM transplantation (Table 4).

Discussion

Originally our choice for the dose of 3 Gy for irradiation was

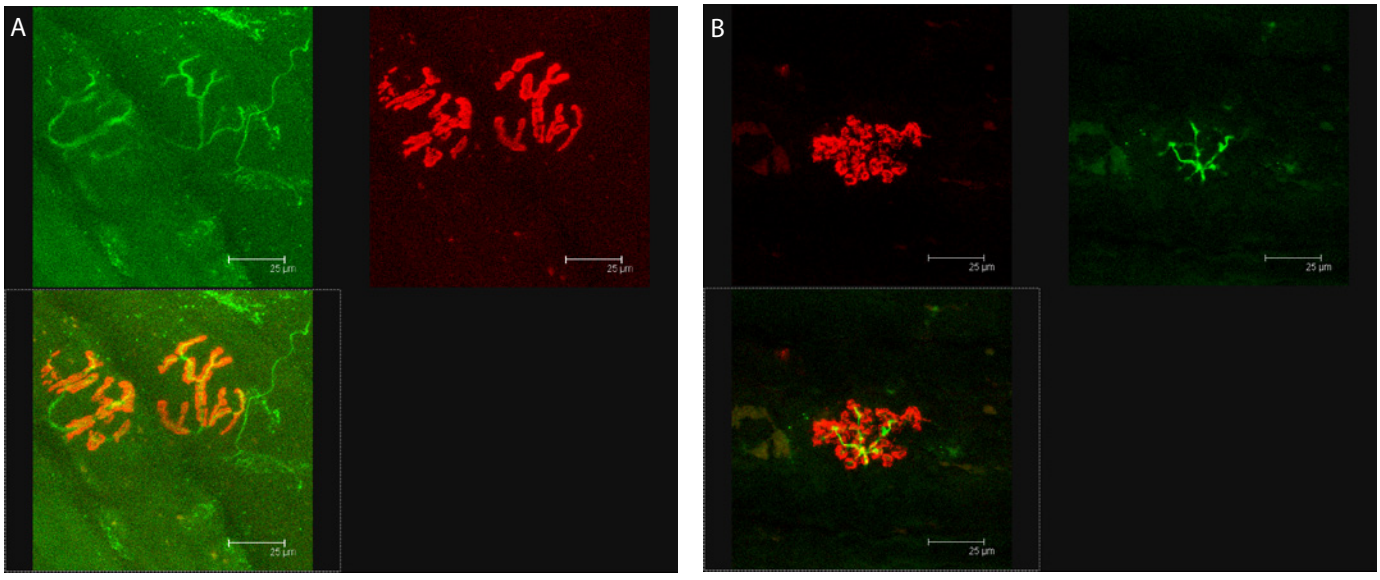

Figure 2: A. Diaphragm of C57BL/6 mice. Red –acetylcholine receptors; green – neurofilaments, NMJ consists from acetylcholine receptors organized as branches; bar -25 mkm
B. Diaphragm of mdx mice. Red colour – acethylcholine receptors, green colour – neurofilaments, NMJ is consists from acetylcholine receptors organized and formed by islets.

Mice	Parts of NMJs with		
	Branches, %	Plaque , %	Islets, %
control *mdx* mice, 4 months old (3)	14.1 ± 3.2	10.2 ± 1.0	75.8 ± 3.1
chimeric *mdx* mice, 4 months old, 2 months after transplantation (3)	23.5 ± 4.1	6.7 ± 2.5	69.8 ± 1.6
control *mdx* mice, 6 months old (3)	9.4 ± 1.6	3.3 ± 0.9	87.3 ± 2.5
chimeric *mdx* mice, 6 months old, 4 months after transplantation, (3)	42.6 ± 5.7	3.7 ± 1.0	53.7 ± 6.0

Table 3: Reparation of structure of diaphragm NMJs after x-ray irradiation at a dose of 3 Gy and bone marrow transplantation.

Mice	The resting membrane potential of diaphragm muscle, mV		
	Junctional region	Extrajunctional region	Delta
Control C57BL/6, 6 months old (8)	–81.4 ± 0.5 n = 173	–78.0 ± 0.4 n = 193	3.4 ± 0.6*
Mdx mice, 6 months old (6)	–75.1 ± 0.6 n = 106	–74.4 ± 0.5 n = 118	0.07
Mdx mice, 6 months old, 4 months after BMSCs transplantation (6)	–80.7 ± 0.7 n = 109	–77.0 ± 0.6 n = 97	3.7 ± 0.9*

* $p < 0.01$: Difference between extrajunctional and junctional regions of sarcolemma
n: number of muscle fibers.

Table 4: Reparation of the resting membrane potential of *mdx* mice diaphragm muscle after bone marrow cells transplantation.

triggered by Abedi et al. [5] and our results [6], as it was impossible to get high stable level of dystrophin synthesis by *mdx* mice SMF by varying lethal doses of X-ray irradiation. Early we described the positive influence of 3 Gy irradiation and change of mutant BM for bone marrow of wild type for dystrophin synthesis by SMF [28,29]. X-ray irradiation doses between 1.5 and 3 Gy induce the acquisition of stable mixed chimerism in recipients. Such type of nonmyeloablative stem cells transplantation permits rapid engraftment from sibling and related donors with minimal toxicity, induce stable mixed chimerism and donor specific transplantation tolerance [34,35]. There are also some descriptions of successful cure of patients with nonmalignant hematologic diseases and congenital immunodeficiencies [36,37]. Level of figures of chimerism depends on used methods. Low range of chimerism in our experiment (3.3 %) may be explained by imperfection of used methods of chimerism determination in comparison with results of other authors. In case of successful allo-BM transplantation of patient with Diamond-Blackfan anemia and Duchenne muscular dystrophy the mixed chimerism was observed with 8-10.4 % donor cells in the muscle biopsy too [38].

Our results demonstrates that transplantation of wild type BM cells into *mdx* mice after 3 Gy irradiation is more effective for dystrophin synthesis reparation then after irradiation at a dose of 5 Gy or higher. The growth of dystrophin was observed in M. quadriceps femoris and in SMF of diaphragm. Through 6 months the part of dystrophin positive SMF reached 27.6 ± 6.7 % which is a critical minimal working level for the function of dystrophin-deficient muscles (20%, [21,22]). Practically it means the cure of *mdx* mice after BM transplantation.

There are several reasons that may explain the success of BM cells transplantation after 3 Gy irradiation. We speculate that except suppression of immunological conflict the suppression of dystrophin synthesis after irradiation in lethal doses and bone marrow stem cells transplantation is caused by the disturbance of SMF sarcoplasm molecular systems that take part in regulation of nuclei differentiation of transplanted SMFs. Moreover, *mdx* mice have an altered expression level of 1735 genes from the studied 7776 genes, including genes of Notch-Delta and Neuregulin 3 signaling pathways that cause activation, proliferation and differentiation of satellite cells [39]. There is no doubt that after the radiation exposure in *mdx* mice at a lethal dose of 5 Gy or higher the disturbance of this signal paths increase even more. In our experiments the dose of 5 Gy was lethal for *mdx* mice [6].

We believe that the reparation of NMJs structure after BM transplantation, as well as dystrophin synthesis restoring, are the evidences of effectiveness of BM exchange in nonmyeablative bone marrow transplantation. We suggest that positive effects of BM cells exchange are connected with dystrophin synthesis. Local transplantation of Lin(-) BM cells in M. quadriceps femoris of *mdx* mice aggregates islets of bungarothoxin-positive substance for large NMJs but without branches. In this case the level of dystrophin synthesis did not exceed 2% [30]. The more extensive level of dystrophin synthesis in case of whole body irradiation 3 Gy followed by BM cells transplantation (Table 2) correlates with reparation of NMJs and branches enrichment. Our results support the conclusions of Kong and Anderson [40] and Banks et al. [41] for the importance of dystrophin participation in NMJs formation.

To confirm the nonrandomness of structural changes a study of electrophysiological properties of NMJs was conducted. We observed reparation of resting membrane potential of diaphragm muscle after BM exchange. Value of local hyperpolarization of the end-plate reached 3.7 ± 0.9 mV. It is typical for the wild-type animals and is a reliable indicator of normal functioning of a neuromuscular synapse [42,43] after BM transplantation (Table 4).

The use of of nonmyeablative bone marrow transplantation may be a next step in the cure of *mdx* mice and recipients with Duchenne muscular dystrophy disease.

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References

1. Partridge TA (1997) Models of dystrophinopathy, pathological mechanisms and assessment of therapy. In: The Dystrophin Gene, Protein and Cell Biology, Brown SC & Lucy TA (Eds), Cambridge University Press 310-331.
2. Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, et al. (1998) Muscle regeneration by bone marrow-derived myogenic progenitors. Science 279: 1528-1530.
3. Bittner RE, Schöfer C, Weipoltshammer K, Ivanova S, Streubel B, et al. (1999) Recruitment of bone-marrow-derived cells by skeletal and cardiac muscle in adult dystrophic mdx mice. Anat Embryol (Berl) 199: 391-396.
4. Gussoni E, Soneoka Y, Strickland CD, Buzney EA, Khan MK, et al. (1999) Dystrophin expression in the mdx mouse restored by stem cell transplantation. Nature 401: 390-394.
5. Abedi M, Greer DA, Foster BM, Colvin GA, Harpel JA, et al. (2005) Critical variables in the conversion of marrow cells to skeletal muscle. Blood 106: 1488-1494.
6. Mikhaïlov VM, Evtifeeva EV, Serikov VB, Perverzev AE, Karmanova AV, et al. (2006) Participation of bone-marrow stem cells in the differentiation of mdx mice striated muscle. Tsitologiia 48: 410-417.
7. Sun D, Martinez CO, Ochoa O, Ruiz-Willhite L, Bonilla JR, et al. (2009) Bone marrow-derived cell regulation of skeletal muscle regeneration. FASEB J 23: 382-395.
8. Dreyfus PA, Chretien F, Chazaud B, Kirova Y, Caramelle P, et al. (2004) Adult bone marrow-derived stem cells in muscle connective tissue and satellite cell niches. Am J Pathol 164: 773-779.
9. Fukada S, Miyagoe-Suzuki Y, Tsukihara H, Yuasa K, Higuchi S, et al. (2002)

Muscle regeneration by reconstitution with bone marrow or fetal liver cells from green fluorescent protein-gene transgenic mice. J Cell Sci 115: 1285-1293.

10. Kucia M, Ratajczak J, Ratajczak MZ (2005) Bone marrow as a source of circulating CXCR4+ tissue-committed stem cells. Biol Cell 97: 133-146.
11. Luth ES, Jun SJ, Wessen MK, Liadaki K, Gussoni E, et al. (2008) Bone marrow side population cells are enriched for progenitors capable of myogenic differentiation. J Cell Sci 121: 1426-1434.
12. Camargo FD, Green R, Capetanaki Y, Jackson KA, Goodell MA (2003) Single hematopoietic stem cells generate skeletal muscle through myeloid intermediates. Nat Med 9: 1520-1527.
13. Rosu-Myles M, Stewart E, Trowbridge J, Ito CY, Zandstra P, et al. (2005) A unique population of bone marrow cells migrates to skeletal muscle via hepatocyte growth factor/c-met axis. J Cell Sci 118: 4343-4352.
14. Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, et al. (2001) Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell 105: 369-377.
15. Mikhailov VM, Sokolova AV, Serikov VB, Kaminskaya EM, Churilov LP, et al. (2012) Bone marrow stem cells repopulate thyroid in X-ray regeneration in mice. Pathophysiology 19: 5-11.
16. Chretien F, Dreyfus PA, Christov C, Caramelle P, Lagrange JL, et al. (2005) In vivo fusion of circulating fluorescent cells with dystrophin-deficient myofibers results in extensive sarcoplasmic fluorescence expression but limited dystrophin sarcolemmal expression. Am J Pathol 166: 1741-1748.
17. Wernig G, Janzen V, Schäfer R, Zweyer M, Knauf U, et al. (2005) The vast majority of bone-marrow-derived cells integrated into mdx muscle fibers are silent despite long-term engraftment. Proc Natl Acad Sci USA 102: 11852-11857.
18. Lapidos KA, Chen YE, Earley JU, Heydemann A, Huber JM, et al. (2004) Transplanted hematopoietic stem cells demonstrate impaired sarcoglycan expression after engraftment into cardiac and skeletal muscle. J Clin Invest 114: 1577-1585.
19. Quattrocelli M, Cassano M, Crippa S, Perini I, Sampaolesi M (2010) Cell therapy strategies and improvements for muscular dystrophy. Cell Death Differ 17: 1222-1229.
20. Otto A, Collins-Hooper H, Patel K (2009) The origin, molecular regulation and therapeutic potential of myogenic stem cell populations. J Anat 215: 477-497.
21. Phelps SF, Hauser MA, Cole NM, Rafael JA, Hinkle RT, et al. (1995) Expression of full-length and truncated dystrophin mini-genes in transgenic mdx mice. Hum Mol Genet 4: 1251-1258.
22. Wells DJ, Wells KE, Asante EA, Turner G, Sunada Y, et al. (1995) Expression of human full-length and minidystrophin in transgenic mdx mice: implications for gene therapy of Duchenne muscular dystrophy. Hum Mol Genet 4: 1245-1250.
23. Rombouts WJ, Ploemacher RE (2003) Primary murine MSC show highly efficient homing to the bone marrow but lose homing ability following culture. Leukemia 17: 160-170.
24. Elwood N (2004) Telomere biology of human hematopoietic stem cells. Cancer Control 11: 77-85.
25. Hiyama E, Hiyama K (2007) Telomere and telomerase in stem cells. Br J Cancer 96: 1020-1024.
26. Zimmermann S, Martens UM (2008) Telomeres, senescence, and hematopoietic stem cells. Cell Tissue Res 331: 79-90.
27. Gadalla SM, Savage SA (2011) Telomere biology in hematopoiesis and stem cell transplantation. Blood Rev 25: 261-269.
28. Mikhailov VM, Sokolova FV, Timonina NT, Kravtsova VV, Krivoy II (2011) Bone marrow transplantation as method of treatment of mdx mice myodistrophy. Cell Ther Transplant 3: 12.
29. Mikhailov VM, Sokolova AV, Krivoi II, Kravtsova VV, Kaminskaya EV, et al. (2011) Bone marrow transplantation as mode of stem cells therapy of mdx mice dystrophy. J Cell Sci Ther 2: 98.
30. Sokolova AV, Zenin VV, Mikhaĭlov VM (2010) Structure of neuromuscular junctions and differentiation of striated muscle fibers of mdx mice after bone marrow stem cells therapy. Tsitologiia 52: 399-406.
31. Krivoi II, Drabkina TM, Kravtsova VV, Vasiliev AN, Eaton MJ, et al. (2006) On the functional interaction between nicotinic acetylcholine receptor and Na+,K+-ATPase. Pflugers Arch 452: 756-765.
32. Heiny JA, Kravtsova VV, Mandel F, Radzyukevich TL, Benziane B, et al. (2010) The nicotinic acetylcholine receptor and the Na,K-ATPase alpha2 isoform interact to regulate membrane electrogenesis in skeletal muscle. J Biol Chem 285: 28614–28626.
33. Miles MT, Cottey E, Cottey A, Stefanski C, Carlson CG (2011) Reduced resting potentials in dystrophic (mdx) muscle fibers are secondary to NF-κB-dependent negative modulation of ouabain sensitive Na+-K+ pump activity. J Neurol Sci 303: 53-60.
34. Tomita Y, Sachs DH, Sykes M (1994) Myelosuppressive conditioning is required to achieve engraftment of pluripotent stem cells contained in moderate doses of syngeneic bone marrow. Blood 83: 939-948.
35. Goebel WS, Yoder MC, Pech NK, Dinauer MC (2002) Donor chimerism and stem cell function in a murine congenic transplantation model after low-dose radiation conditioning: effects of a retroviral-mediated gene transfer protocol and implications for gene therapy. Exp Hematol 30: 1324-1332.
36. Slavin S, Nagler A, Naparstek E, Kapelushnik Y, Aker M, et al. (1998) Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. Blood 91: 756-763.
37. Amrolia P, Gaspar HB, Hassan A, Webb D, Jones A, et al. (2000) Nonmyeloablative stem cell transplantation for congenital immunodeficiencies. Blood 96: 1239-1246.
38. Nair V, Das S, Sharma A, Sharma S, Kaur J, et al. (2011) Successful bone marrow transplantation in a patient with Diamond-Blackfan anemia with co-existing Duchenne muscular dystrophy: a case report. J Med Case Reports 5: 216.
39. Turk R, Sterrenburg E, de Meijer EJ, van Ommen GJ, den Dunnen JT, et al. (2005) Muscle regeneration in dystrophin-deficient mdx mice studied by gene expression profiling. BMC Genomics 6: 98.
40. Kong J, Anderson JE (1999) Dystrophin is required for organizing large acetylcholine receptor aggregates. Brain Res 839: 298-304.
41. Banks GB, Chamberlain JS, Froehner SC (2009) Truncated dystrophins can influence neuromuscular synapse structure. Mol Cell Neurosci 40: 433-441.
42. Nikolsky EE, Zemková H, Voronin VA, Vyskocil F (1994) Role of non-quantal acetylcholine release in surplus polarization of mouse diaphragm fibres at the endplate zone. J Physiol 477: 497-502.
43. Kravtsova VV, Mikhailov VM, Sokolova AV, Mikhailova EV, Timonina NA, et al. (2011) Recovery of electrogenesis in skeletal muscles after cell therapy of myodystrophy in MDX mice. Dokl Biol Sci 441: 357-359.