REVIEWS

A New Approach to the Therapy of Duchenne Muscular Dystrophy with Early Precursors of Myogenesis

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 132, No. 12, pp. 604-613, December, 2001
Original article submitted October 30, 2001

Recent fundamental and applied studies and clinical observations allowed researchers to develop new methods for the correction of gene defects during hereditary diseases. Early diagnostics of these disorders at the level of structural genes is now performed by routine methods. New approaches to the therapy of hereditary diseases were elaborated. Gene therapy suggests substitution of defective genes for their normal analogues. During cytotherapy early precursors of various cells are transplanted to correct functional defects in organs and tissues.

Compensation of gene defects in X-linked progressive Duchenne muscular dystrophy (DMD) a common, severe, and non-curable human disease, attracts much attention. It was shown that 1 of 3500 newborn boys (more rarely girls) is characterized by progressive damages to skeletal muscles. These patients lose the ability to move by the end of the first decade of life. They die by the end of the second decade of life due to total atrophy of skeletal muscles, heart failure, and respiratory failure. At the end of the 20th century molecular and genetic studies revealed DMD gene. Mutations in this gene lead to blockade of dystrophin synthesis in skeletal muscles and myocardium. The more benign type of X-linked Becker muscular dystrophy and DMD are allelic. During this disease dystrophin synthesis is partially preserved. There are sample data on mutations in the DMD gene and their realization in the phenotype [5].

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Biochemical, biophysical, and electrophysiological studies showed that dysfunction of muscle fiber membranes plays an important role in the pathogenesis of DMD [3]. Therefore, DMD was attributed to sarcolemmopa-thies [45]. The mechanisms underlying the formation and realization of mutational changes during DMD are not reviewed here [1]. We consider only some aspects of functioning of mature muscle fibers and their embryonic precursors. In muscle fibers dystrophin performs various functions and serves as a skeleton. Dystrophin is a rod-shaped threaded subsarcolemmal dimer. Its N-terminal part is bound to cytoplasmic F-actin, which forms the cytoplasmic network and provides rigidity of the cytoskeleton. The C-terminal part of dystrophin is associated with the transmembrane protein complex (dystrophin-associated proteins, DAP), basal membrane (merosin), and extracellular matrix (via α- and β-dystroglycans, Fig. 1). Thus, all elements of a muscle fiber (cytoskeleton, membrane, and extracellular matrix) are integrated into a functional unit resistant to deformations and mechanical damages. Dystrophin is responsible for organization of the DAP membrane complex that includes not only dystroglycans, but also sarcoglycans, syntrophins, and minor proteins dystrobrevins. These proteins are involved in synaptogenesis, play a role in transmembrane transport of K^+ and Na^+, and mediate the effects of various enzymes, including NO synthetases and kinases. Dysturbances in dystrophin synthesis during DMD leads to the loss of mechanical rigidity in muscle fibers, disintegration of the DAP complex, changes in electrochemical properties of the sarcolemma, retention of Ca^{2+} and loss of K^+ from the

0007-4888/01/1326-1131$25.00

cytoplasm, intensification of lipid peroxidation, development of micronecroses, and destruction of muscle fibers. It should be emphasized that disturbances accompanying DMD are determined by a defect in 1 of 4 dystrophin domains (Fig. 2). The most severe morphological and clinical manifestations of the disease are caused by changes in the molecular structure of C-terminal cysteine-rich (CR) domains associated with DAP. Mutations in the N-terminal actin-binding domain (AB), rod-like domain (ROD), and WW domain positioned between the ROD and C-terminal domains are accompanied by less severe clinical manifestations. These relationships between dystrophin domains, DAP, and cytoskeleton are typical of only skeletal muscles and myocardium that express full-length dystrophin molecules. Expression of DMD gene in other tissues is alternative, which is related to the presence of at least 8 gene promoters. Their specific activity in tissues contributes to the formation of short dystrophin molecules (apo-dystrophins). The main product of DMD genes in nonmuscle tissues is apo-dystrophin-1 (DP71), which contains the C-terminal domain, a fragment of the cysteine-rich domain, and 7 additional amino acids. The concentration of this protein in some tissues is comparable with the content of dystrophin in muscles not containing DP71. Apo-dystrophin-2 (DP116) and DP260 are predominantly expressed in peripheral nerves and retina, respectively. However, embryonic tissues express other apo-dystrophins. Fetal muscles contain not only full-length dystrophin, but also apo-dystrophin-1. The intensity of its expression is high in cultured differentiating myoblasts. Immature muscle fibers, myotubes, include additional transcriptional elements involved in the regulation of DMD gene expression (5-kb enhancer) [42]. This determines high activity of dystrophin promoter during myoblast differentiation. Activity of this promoter in mature myofibrils decreases by 30 times. Previous studies showed that in transgenic animals characterized by the absence of dystrophin in muscles and ectopic expression of apo-dystrophin-1, DAP is not disintegrated, and clinical manifestations of the disease are less pronounced [19].

Embryonic muscle cells express dystrophin-related peptides utrophin and dystrobrevin (DAP). Dystrophin and related proteins are formed from a common precursor gene and characterized by high homology. However, genes for dystrophin-related proteins are localized in other chromosomes. For example, the utrophin gene is present in chromosome 6. Therefore, damages to the DMD gene do not disturb biosynthesis of dystrophin-related peptides. Utrophin can interact with DAP and possesses similar activities. During early embryogenesis utrophin is synthesized in the sarcolemma of skeletal muscles. However, utrophin is completely substituted for dystrophin on the 26th week of pregnancy. Experiments on mdx mice showed that utrophin compensates dystrophin deficiency in muscles. Utrophin is synthesized in adult mdx mice, and clinical manifestations of dystrophinopathy are absent in these animals. The compensatory effect of utrophin was confirmed in experiments on mice deficient in DMD and utrophin genes. These animals were characterized by severe muscular dystrophy, cardiopathy, developmental retardation, and early death [30].

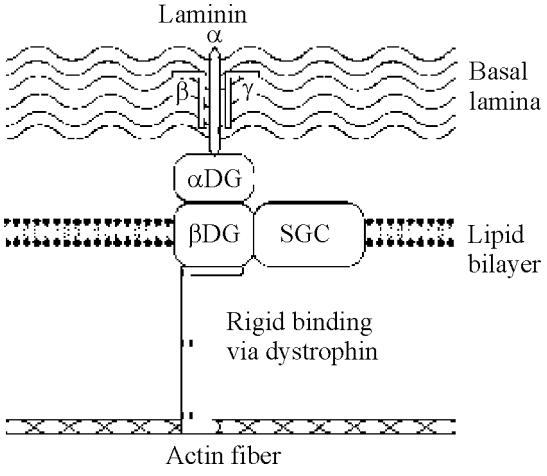

Fig. 1. Dystrophin firmly bind the intracellular and extracellular protective layers. α-DG and β-DG: α- and β-dystroglycans, respectively; SGC: sarcoglycan C.

These data indicate that early myogenic precursors hold much promise for the therapy of DMD. We review possible approaches to this therapy with various embryonic structures.

Early Myogenic Precursors

Satellite cells. The early developmental stages of mammalian skeletal muscles occur in somites and begin from the appearance of myogenic precursors. After fusion their progeny (myoblasts) form multinuclear myofibrils.

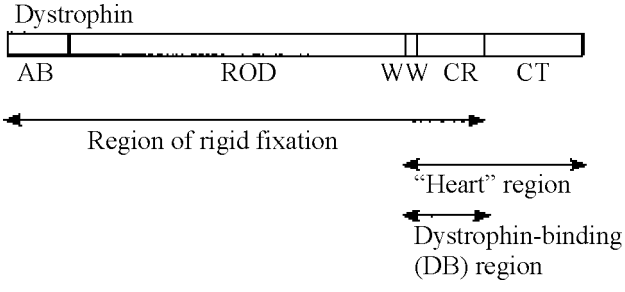

Fig. 2. Model of dystrophin. AB, CR, and ROD: actin-binding, C-terminal cysteine-rich, and rod-like domains, respectively.

Until recent time muscle satellite cells (SC) responsible for the growth, reparation, and maintenance of skeletal muscles were believed to serve as the very early precursors of postnatal myogenesis. SC are oval-shaped mononuclear cells localized on the surface of microtubes until the formation of the basal lamina. The total count of SC decreases with age [31]. In newborn mice contain 32% muscle cell nuclei are SC nuclei, while in adult animals their ratio decreases to 5% [12]. These changes reflect fusion of SC with newly formed and preexisting myofibrils. In adult skeletal muscles SC are mitotically resting cells. They are activated and undergo numerous cycles of proliferation in response to stress (traumas or physical training associated with body weight gain) [8]. Myogenic precursor cells developed from activated SC intensively proliferate with the formation of symplasts [32]. In adult muscles the total number of resting SC undergoing repeated degeneration and regeneration remains practically unchanged, which confirms their self-renewal [55]. During DMD the number and proliferative potential of SC decrease, which is probably related to chronic regenerative processes [25].

Myogenesis proceeds in the following stages: commitment of cells, cell cycle progression, initiation of differentiation, mutual recognition, spatial orientation, adhesion, and fusion [56]. During embryonic myogenesis information carried by extracellular morphogens and matrix and via cell-cell interactions is received by cells through signal molecules, transduced into the nucleus and, together with intracellular signals, provides expression of tissue-specific genes. Studies of myogenic regulators responsible for the expression of mHLH genes (Myo D, myogenin, and Myf-5) and experiments on mutants with defects in these genes revealed molecular mechanisms underlying activation of specific gene expression during differentiation and development of the muscle tissue.

The Myo D family of bHLH transcription factors is involved in commitment and differentiation of embryonic myoblasts during their development. Primary myogenic regulators Myf-5 and Myo D are necessary for determination of myoblasts, while secondary factors myogenin and myogenin-regulating factor-4 (MRF-4) control terminal differentiation [9,47]. Expression of these factors during SC activation, proliferation, and differentiation is similar to that realized during embryogenesis of skeletal muscles. Resting SC do not express these factors. The intensity of Myo D expression progressively increases over 12 h after experimental muscle injury until the appearance of the cell proliferation marker (proliferating cells nuclear antigen). Myogenin is expressed during fusion and differentiation of myoblasts [65]. Resting SC express tyrosine kinase c-Met, a receptor for hepatocyte growth factor (HGF). Activated SC entering the cell cycle initially express Myf-5 or Myo D, while then these cells express both factors. Proliferation is followed by cell differentiation. These cells express myogenin, MRF-4, and myosin. Intensive expression of Myo D is necessary for the entrance of SC into the proliferative phase that precedes terminal differentiation. In the absence of Myo D the count of myogenic precursors in-creases. This is associated with their self-renewal, but not with expression of progeny cells. It was hypothesized that SC form stem cells developed into myo_genic precursors [46].

SC first appear in the limbs of mouse embryo at gestational age of 17.5 days [21]. SC in adults are divided into subclasses by the type of mature muscle fibers, into which their progeny are integrated. They form fibers genetically identical to original muscle tissue [54].

Vessels that supply blood to skeletal muscles contain precursor cells differentiating into SC. Myogenic cells in the dorsal aorta express myogenic and endothelial markers, which are also present in adult SC [21].

The dorsal aorta is populated with migrating angioblasts originating from the paraxial mesoderm in somites. This population develops from the mesoderm and is presented by diffusely distributed cells, which undergo fusion with the formation of blood vessels [23].

Resting SC contacting mature muscle fibers express c-Met and M-cadherin, but not markers of committed myoblasts (Myf-5, Myo D, and desmin) [17]. Probably, SC are pluripotent stem cells. It can be assumed that the development of committed myoblasts depends on microenvironmental conditions and presence of growth factors. Muscle stem cells in adult mice repopulate the hematopoietic compartment and develop into skeletal muscle myocytes after intravenous administration to irradiated recipients [14,27,34].

Muscle stem cells belong to another population of committed myogenic cells. This population is presented by SC precursors. The expression of myogenic regulatory factors in these cells decreases before their entrance into the resting phase. Self-renewal of SC continues until the start of myogenic regulatory factor expression and does not depend on this process. Selective Myf-5 expression in myogenic cells probably promotes self-renewal of stem cells in skeletal muscles. The count of resting SC increases due to dedifferentiation of committed cells. Previous studies showed that adhesion molecules,

including M-cadherin, are involved in the distribution of SC along the muscle fibers [41]. Their precursors probably express tissue-specific molecules, which are normally present on committed myogenic precursors.

Traumas, denervation, physical training, or strain can provoke SC activation [20]. This process is realized via various mechanisms, including inflammatory reactions and release of growth factors (Fig. 3). Resting SC carrying VCAM-1 molecules on the surface can interact with leukocytes that infiltrate tissues and express the specific VLA-4 contra-receptor. This results in the initiation of myogenic differentiation in SC and recruitment of immune cells into the regenerative process [38]. Neutrophils and activated monocytes migrate into focuses of tissue damages over the first hours after muscle injury. After 48 hours the regenerating muscle tissue contain primarily macrophages, which phagocytize necrotized cells and secrete soluble factors inducing SC proliferation [48]. The rate of myogenesis is much lower in the absence of macrophages [43]. The leukemia-inhibiting factor is released from damaged muscles before infiltration of tissues with leukocytes. This factor is secreted by muscle and resident non-muscle cells, and then macrophages producing interleukin-6. Both factors stimulate proliferation of myogenic precursors. Insulin-like factor-1 induces expansion of myogenic precursors and muscular hypertrophy during regeneration of skeletal muscles. Fibroblast growth factor-6 also promotes proliferation of myogenic precursor cells. HGF acts as a potent mitogen and chemoattractant for SC both *in vitro* and *in vivo* [11]. Resting SC express HGF receptor, protooncogene *c-met*. The intensity of HGF expression in regenerating muscles is high. This factor is responsible for activation of SC and/or expansion of myogenic precursors before the formation of new myofibrils. The activation of SC is associated with binding of VLA-4 and VCAM-1 molecules with infil-

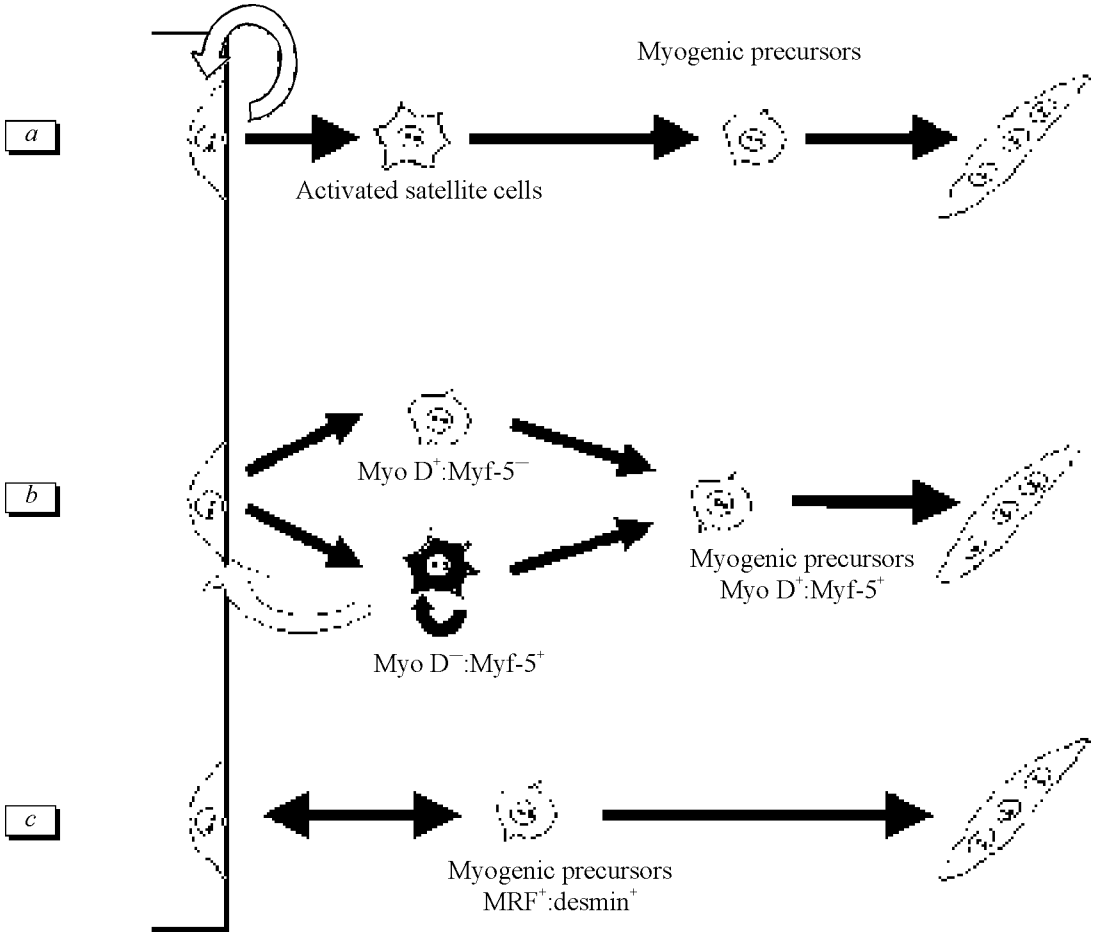

Fig. 3. Self-renewal of muscle satellite cells. *a*) Asymmetric cell division. Asymmetric division of stem cells results in the formation of 2 daughter cells: committed myogenic precursor and uncommitted pluripotent stem cell similar to the mother cell. *b*) Myf-5-dependent self-renewal. Self-renewal of satellite cells can be associated with Myf-5. In this model primary activation of Myf-5 without expression of Myo D determines the activation of satellite cells, and stem cells undergo self-renewal. *c*) Dedifferentiation of committed myogenic precursors expressing Myo D, Myf-5, and other markers of myoblasts. Activation of resting satellite cells. MRF: myogenin-regulating factor.

trating polymorphonuclear leukocytes and resident SC, respectively. HGF activates SC through autoimmune c-Met receptor expressed by resting SC. HGF can be produced by undamaged muscle fibers in response to physiological stimulation. Destruction of the basal lamina and extracellular matrix in muscle fibers leads to the release of HGF. Activation of resting SC is followed by expression of Myo D and/or Myf-5 and generation of daughter myogenic precursors. Various growth factors, including HGF, platelet-derived growth factor, leukemia-inhibiting factor, interleukin-6, fibroblast growth factor, and insulin-like factor-1 play an important role in expansion of myoblast precursors. The growth factor produced by infiltrating macrophages is involved in proliferation of myogenic precursors. After stimulation of proliferation myoblast precursors enter the terminal phase of differentiation into myocytes. This process depends on the expression of myogenin and MRF-4 before fusion with existing or newly formed muscle fibers. Insulin-like factor-1 increases the number of muscle fibers independently on proliferation of myogenic precursors.

Administration of recombinant HGF into the muscle tissue leads to the activation of resident SC [59]. HGF induces morphological transformation of mouse C2C12 myoblasts (SC derivatives), but blocks their differentiation [7]. Activation of *c-met* is probably required for proliferation of myogenic precursors, but terminal differentiation and fusion of cells with the formation of multinuclear myofibrils proceed after blockade of the signal pathway. Previous studies revealed reciprocal expression of c-Met and HGF in growing myoblasts and myotubes. Myoblasts and newly formed myotubes express c-Met and HGF, respectively. HGF produces strong chemotactic effects on cultured myoblasts [13], which indicates that this factor is involved in migration of activated SC into distal regions of damaged muscles [36]. Other authors reported that c-Met and HGF are simultaneously expressed in proliferating myoblasts [7]. Thus, expansion of myoblasts is probably realized by the autocrine pathway. It can be suggested that HGF is released from the extracellular matrix after destruction of the basal lamina.

Other myogenic precursors. In the past years SC were believed to be monopotent. The concept regarding differentiation of stem cells is currently undergoing revision. Experiments with transfer of genetically labeled bone marrow cells to immunodeficient mice showed that donor cells migrate into focuses of muscle injury, undergo *in situ* myogenic differentiation, and contribute to regeneration of muscle fibers [27]. Bone marrow progeny cells from donors are integrated into the myocardial tissue and skeletal muscles in recipient mdx mice (experimental model of DMD). The donor cell marker was also found in the endothelium. These data indicate that bone marrow stem cells differentiate into myogenic and other cells. However, adoptive transfer of these cells is not accompanied by the increase in SC count. Progeny of hemopoietic stem cells intravenously administered to irradiated mdx mice also contribute to regeneration of muscle fibers. M. A. Goodell *et al*. obtained hemopoietic stem cells on a FASC device [29]. The method is based on isolation of stem cells characterized by weak fluorescence after labeling with a Hoechst 33342 stain. These cells intensively express proteins responsible for drug resistance [29]. Twelve weeks after intravenous transplantation of hemopoietic stem cells to lethally irradiated mdx mice dystrophin expression was detected in 45% muscle fibers. SC did not include donor cell nuclei. Stem cells isolated from muscles by this method differentiate *in vitro* into desmin-positive myoblasts. After intravenous injection stem cells from the muscle tissue are involved in regeneration of myofibrils. Donor cell nuclei were found not more than in 9% recipient muscle fibers. As differentiated from bone marrow hemopoietic stem cells, these myogenic precursors develop into SC in regenerating muscle fibers in recipients. They normalized hemopoiesis in lethally irradiated recipients [37]. These data indicate that the muscle tissue contains pluripotent stem cells, which can be isolated (with high degree of purity) after labeling with Hoechst 33342 stain. Neural stem cells not only develop into neurons, oligodendrocytes, and astrocytes [57], but also repopulate the hemopoietic system [4,15]. Bone marrow cells introduced into the brain parenchyma or ventricles can differentiate into the astroglia and microglia [24]. These data indicate that all tissues include pluripotent stem cells that differentiate into various cells depending on microenvironmental conditions.

Embryonic stem cells. The very early myogenic precursors hold much promise as the source of donor material. Embryonic stem cells are of particular interest in this respect. They undergo intensive proliferation *in vitro* without differentiation [45], but can form 3 embryonic layers even after long-term culturing [61]. Myogenic differentiation of mouse embryonic stem cells was modeled during culturing of blastocyst BLC6 cells. These cells differentiate into embryoid bodies and, under certain conditions, express bHLH genes for myogenic regulators. The sequence of these events is similar to that observed during embryogenesis. Newly formed myocytes express functional nicotinic cholinoceptors and possess physiological activity of skeletal muscle cells [53].

Much recent progress in construction of embryonic stem cells from human blastocysts opened new perspectives in obtaining great amounts of cells for transplantation. Banks of embryonic stem cells typed by HLA antigens can be used for adequate donor-recipient matching.

These data indicate that embryonic, bone marrow, and muscle stem cells and SC can be used for cytotherapy of hereditary diseases. Biological materials obtained from early embryos and fetuses at early gestational age hold much promise as the source of myogenic cells.

Introduction of New Biotechnological Approaches to the Therapy of DMD into Clinical Practice

New approaches to the treatment of patients with DMD include gene and cell therapy. Transfer of somatic genes by nonviral DNA vectors carrying dystrophin cDNA [6] and RNA/DNA oligonucleotides [52] is followed by low efficient transgenes expression. Dystrophin gene transfer by adenoviral vectors to mdx mice did not prevent immune incompatibility, while gene transfer to adult muscle cells was ineffective. Vectors of adeno-associated viruses are the only system based on the use nonpathogenic viruses with impaired replication [39]. These vectors are nontoxic, provide efficient and long-term gene expression in various tissues *in vivo*, and do not induce the host immune response [64]. They cross extracellular barriers, which ensures effective transduction of myofibrils with different degrees of maturity. Recently, this method was used for the therapy of mdx mice with muscular dystrophy. Dystrophin minigenes controlled by the tissue-specific promoter in adeno-associated viruses were constructed [63]. After intramuscular injection most myofibrils expressed 2 minigenes, which was accompanied by structural restoration of DAP. This therapy normalized morphology of myofibrils and cell membrane integrity in mdx mice.

Gene defects in mdx mice were corrected by overexpression of the autosomal structural dystrophin analogue utrophin [60], which is present in various tissues, including the sarcolemma and developing or regenerating striated muscles. The efficiency of this replacement was associated with the similarity of functional domains in dystrophin and utrophin. The search for agents, which can be introduced intramuscularly and can enhance transcription of the endogenous utrophin gene in dystrophin-deficient muscles, attracts much attention [16].

Apart from direct genetic modification of dystrophin-deficient muscle tissues by viral vectors, *ex vivo* gene transfer and transplantation of transfected cells to recipients hold much promise in this respect. This approach suggests the use of cellular reservoirs, which would provide regeneration of dystrophic muscles and normalize tissue-specific expression of dystrophin. The major limitation of gene therapy of skeletal muscles is that viral vectors can not effectively transduce mature myofibrils. Gene transfer by a fixed amount of viral particles during genetic modification of cells *ex vivo* is more efficient than direct gene transfer into muscle tissues *in vivo* [28]. It should be emphasized that in patients with DMD, which reach the age of 10, myofibrils lose the ability to regenerate and become refractory to direct transduction by viruses [51].

The source of myogenic cells for further transplantation should be selected with due consideration for their maturity, differentiation capacity, and ability of these cells or their progeny to be integrated into recipient skeletal muscles. Genetically unmodified cells from healthy donors can be used for the correction of dystrophin deficiency.

The ability of myoblasts to incorporate into skeletal muscles during regeneration in postnatal ontogeny suggests that these cells can be used for transplantation to patients with primary hereditary myopathies. Transplantation of exogenous myoblasts involved in the formation of new muscle fibers during reparation and regeneration should be followed by genetic modification of recipient muscle tissue. Intramuscular administration of immortalized myogenic C2C12 cells to mdx mice recovered muscle fibers expressing dystrophin [50]. In the beginning of the 1990s an attempt was made to introduce this approach into clinical practice. Myogenic cells from donors were *in vitro* grown and transplanted into damaged muscles in patients with DMD [40,44]. Transplantation of myoblasts to patients with DMD was followed by temporal recovery of dystrophin-positive muscle fibers and normalization of contractile activity in skeletal muscles. P. K. Law *et al*. [44] observed pronounced clinical effects and stable expression of dystrophin in muscle of patients with DMD. Treatment of mdx mice with immortalized syngeneic cells produced good results. Recent studies showed that most myogenic precursors die soon after injection to recipients. Myoblasts died over the first 2-3 days after transplantation to isogenic [35], congenic [26], immunodeficient, and immunosuppressive recipients [10,33]. Therefore, early death of donor cells is not associated with apoptosis and specific immune reactions. Cell death is partly related to the nonspecific immune response to tissue injury, since treatment with anti-LFA-1 antibodies [33] and transfection of the interleukin-1 receptor antagonist

gene into donor cells prevent this process [51]. However, some donor cells that undergo slow division in tissue culture, but rapidly proliferate after transplantation, are characterized by self-renewal and generation of myogenic progeny. These presumptive stem cells are present in the muscle tissue in newborn mice. Their count increases after consecutive short-term passages on plastic plates with removal of nonadherent cell fractions. Passage 6 fractions is superior to unfractionated cells and myogenic precursors by viability in recipients and capacity to form new muscle fibers. Cells of these fractions are fused to myofibrils expressing rapid and slow myosin isoforms. However, fusion of myoblasts from individual myofibrils with muscle fibers leads to expression of only slow myosin isoform [51]. Functionally similar cells express the inhibitor of apoptosis Bcl-2 [22] and, therefore, they can be divided phenotypically in the early and late stages of myogenesis. These myogenic precursors express stem cell antigens Sca-1 and CD34, migrate from the circulation into muscle tissues in mdx mice (after intraarterial injection), play an important role in the formation of myofibrils, and restore dystrophin expression in damaged muscles. The impairment of blood vessels in the muscle tissue 48 h after transplantation of Sca-1- and CD34-positive cells markedly improves graft survival [62]. These data suggest that there are 2 types of muscle regeneration. Slow regeneration is realized after destruction of muscle fibers and involves only SC. Rapid regeneration is observed during severe tissue injuries and involves not only SC, but also stem cells localized on the external surface of vessels or migrating from the circulation into the damaged region. Self-renewing pluripotent hematoangiomyogenic stem cells are present at least during embryogenesis and early stages of postnatal ontogeny [18,21]. Previous studies revealed myogenic precursors in the dorsal aorta, embryonic aortogonadomesonephric region, liver, and fetal bone marrow. Pluripotent stem cells also possess other properties. During embryogenesis they migrate from the vascular bed into developing tissues, adapt to specific microenvironmental conditions, and differentiate into muscle SC or bone marrow hemopoietic and stromal stem cells. Therefore, embryonic and fetal tissues hold much promise as the source of very early myogenic precursors [2]. The optimum conditions for *in vivo* expansion and specific differentiation of pluripotent stem cells should be elaborated.

Conclusion

Much progress was recently made towards the correction of various hereditary diseases. Evaluation of the molecular and genetic mechanisms for DMD (dystrophin gene defects and associated destructive changes in skeletal muscles) gave impetus to research concerning the correction of this hereditary disease. Viral vectors providing stable expression of dystrophin minigenes in mdx mice (DMD model) were constructed. The methods for *ex vivo* genetic modification of myogenic cells and transfer of transfected cells into damaged muscle tissues were elaborated. It was shown that bone marrow and muscle stem cells and SC are the promising source of myogenic precursors. Moreover, embryonic stem cells can be used to obtain myogenic precursors.

Evaluation of the nature of early myogenic precursors allowed researchers to develop new method for the therapy of primary muscular dystrophies, including DMD. The enriched population of primary myogenic precursors is necessary for effective cytotherapy of these diseases. The factors and optimum conditions for selective expansion of myogenic precursors in dystrophic muscle tissues should be elaborated *in vivo*. Extensive studies of stem cell biology contribute to the development of methods for gene and cell therapy of hereditary diseases, including DMD.

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