

# Transplantation of human umbilical cord-derived mesenchymal stem cells for the treatment of Becker muscular dystrophy in affected pedigree members

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**Abstract.** The regeneration of muscle tissue has been achieved using multipotent mesenchymal stem cells in mouse models of injured skeletal muscle. In the present study, the utility of multipotent human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) in the treatment of Becker muscular dystrophy (BMD), a genetic disease where muscle tissue fails to regenerate, was examined in members from a pedigree affected by BMD. The disease status was evaluated in 4 affected pedigree members (III1, II2, II3 and III2; aged 50, 46, 42 and 6 years, respectively). The transplantation of the hUC-MSCs (performed on 3 patients, I2, II3 and III2) was performed by infusion with an intravenous drip over a 30-min period, and the patients were evaluated at 1, 3, 4 and 12 weeks following the procedure. The evaluation was based on physical characteristics, as well as on molecular testing for serum creatine kinase (CK) and lactate dehydrogenase (LDH) levels and a histological examination of muscle biopsies. The patients suffered no adverse reactions in response to the transplantation of the hUC-MSCs. At 1 week following transplantation all 3 patients showed improvement in the muscle force of the limbs, muscle size and daily activity. The walking gait of patient III2 had improved by 1 week post-transplantation and reached a normal status by 12 weeks. Serum CK and LDH levels were decreased relative to the baseline levels. A histological examination of muscle biopsies displayed no obvious tissue regeneration. In conclusion, the treatment of patients with BMD using hUC-MSCs was safe and

of therapeutic benefit that lasted for up to 12 weeks. hUC-MSCs are, therefore, a potential cell therapy-based treatment option for patients with muscular dystrophies.

## Introduction

Duchenne and Becker muscular dystrophies (DMD and BMD) are common X-linked recessive neuromuscular degenerative diseases. The majority of patients with DMD and BMD are male, although females are the carriers, and the frequency of occurrence of DMD and BMD in newborn males is estimated to be 1/3,500 and 1/12,000, respectively (1). The molecular basis for DMD/BMD is a mutation of the human dystrophin gene, and many family pedigrees sustain a number of functionally inactivating mutations which include large fragment deletions (55-65%), duplications (5-15%), point mutations (35%) and small insertions/deletions (indels). Dystrophin is the intermediate component linking the cytoskeleton to the extracellular matrix (ECM), and the protein plays an important role in maintaining membrane stability and the function of muscle cells (2-5). The membrane of muscle cells can be easily destroyed in the absence or dysfunction of dystrophin, which eventually leads to the replacement of muscle tissue by adipose and connective tissues.

Some mutations of the human dystrophin gene produce proteins possessing partial function. This version of the disease is clinically referred to as BMD, and 92% of patients are diagnosed as BMD (6,7). The clinical manifestations of DMD/BMD include the degeneration of muscle fibers, the progressive atrophy of proximal limb muscle, pseudohypertrophy of the gastrocnemius muscle, motor retardation or delay, a 'waddling' gait and Gowers' sign (8). Symptoms in individuals affected by DMD are often already apparent by the age of 3 years, and patients eventually succumb to the disease at a young age (approximately 20 years) due to respiratory or heart failure (9). The symptoms of BMD are relatively milder than those of DMD, with disease onset beginning anywhere from the age of 5 to 20 years and death occurring at the age of 40-50 years (9).

Despite an extensive molecular understanding of the diseases, treatment options to date remain limited for DMD/BMD and patients still succumb to the diseases. Therefore, new therapies

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are urgently required (10). To date, glucocorticoids are the only drug that effectively delays the progression of DMD, maintains the stability of lung tissue and lung function, and reduces the mortality of patients with DMD due to heart failure (11-14). In 2010, glucocorticoids were included in the DMD treatment guidelines by the DMD Care Considerations Working Group of Centers for Disease Control and Prevention (15). However, the mechanisms underlying glucocorticoid function in alleviating some symptoms or delaying the progression of DMD remain unknown (16). Furthermore, several side-effects in the chronic use of glucocorticoids are undesirable, including weight gain, Cushingoid symptoms/features, high blood pressure, cataracts, bone mineral density reduction, vertebral compression fracture, growth retardation and delayed puberty (17).

Multipotent human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) (18) have been explored as a potential, innovative cell-based therapy for tissue regeneration in various conditions or diseases. hUC-MSCs are capable of differentiating into multiple cells types, such as osteogenic, chondrogenic, adipogenic and myogenic cells (19), as well as into neuron-like cells (20) and germ-like cells (22) *in vitro*. Cells isolated from the umbilical cord tissue have also been shown to be more effective in rescuing photoreceptors and visual function, and to be capable of sustained population doublings without karyotypic changes, thus proving useful in retinal diseases (21). Cultured mesenchymal stem cells (MSCs) from the human umbilical cord artery (UCA), umbilical cord vein (UCV), whole umbilical cord (UCC) and saphenous vein segments have exhibited a myofibroblast-like morphology and mechanical function *in vitro* (23). The cells are abundant, easy to harvest and to culture and have lower immunogenicity. Based on all of these properties, in this study, hUC-MSCs were transplanted into 3 pedigree members affected BMD, and the responses to this cell therapy were evaluated based on physical parameters and molecular and histological examination.

## Materials and methods

**Ethics statement.** All protocols in the present study were approved by the Ethics Committee of the Shandong Cancer Hospital (Shandong, China). Prior informed consent was obtained from all the subjects participating in the study.

**Clinical data.** The pedigree chart of the family with BMD from the Shandong Cancer Hospital is presented in Fig. 1, and our group has previously reported the underlying pathogenic mutations of this family (24). Three male family members (III1, II2 and II3) with an age of onset of 12 to 15 years displayed clinical manifestations typical of BMD, including the degeneration of muscle fibers, the progressive atrophy of proximal limb muscles, the pseudohypertrophy of gastrocnemius muscle, motor retardation, Gowers' sign and a 'waddling' gait and all eventually lost the ability to walk. One family member (III2) included in this study had not yet reached the age of onset, but was symptomatic. All patients had received no prior treatment, including glucocorticoids.

Each patient underwent routine blood work and an electrocardiogram prior to the procedure. Clinical evaluation was carried out prior to the procedure (control; 0 week) and at 1,

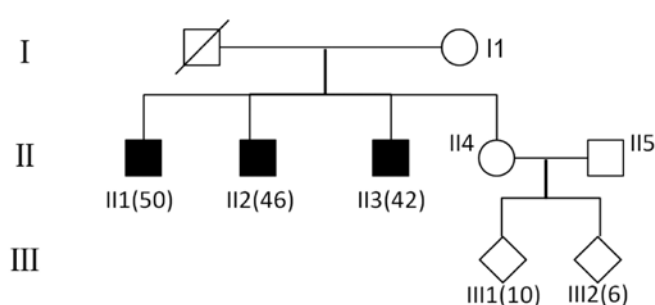


Figure 1. Pedigree structure of the family with Becker muscular dystrophy (BMD). Black squares represent male BMD cases; circles represent females. Rhombi indicate young males who had not yet reached the age of BMD onset by the start of the study. I1, II1-II5, III1 and III2 are coded patient identification numbers. The age of the family member at the time of sample collection is indicated in parentheses.

3, 4 and 12 weeks post-transplantation of hUC-MSCs. The evaluation of the patients was based on physical, as well as molecular testing: i) myodynamia of the 4 limbs according to the Medical Research Council scale for muscle strength; ii) performance according to the Barthel index of Activities of Daily Living; iii) muscular size based on upper and lower limb circumference; iv) subjective symptoms and signs; and v) levels of serum creatine kinase (CK), lactate dehydrogenase (LDH) and creatine kinase isoenzymes (CK-MB).

**Transplantation of hUC-MSCs.** The hUC-MSCs were purchased from Tianjin Amcellgene Engineering Co., Ltd. (Tianjin, China), where the stem cells had been prepared according to the guidelines issued by the US Food and Drug Administration (FDA; Silver Spring, MD, USA). The pack size was 20 ml/IU cells ( $1 \times 10^7$  cells/IU; batch number 201206JF11). Quality control of the hUC-MSCs included an assessment based on *in vitro* morphology, differentiation potential, tumorigenicity and proliferation. Immunophenotyping confirmed that >90% of the cells were positive for CD166, CD105, CD13 and CD29, and negative for CD34, CD45, CD86 and HLA-DR. All assays were conducted and certified by Tianjin Amcellgene Engineering Co., Ltd.

The patients received 5 mg dexamethasone intravenously 10 min prior to transplantation. The cells were transplanted by an intravenous drip into the left forearm vein, and the infusion was completed within 30 min. Patients II2 and II3 received 5 IU and patient III2 received 3 IU.

## Results

**Clinical status prior to the transplantation of hUC-MSCs.** Three family members were examined for disease characteristics. All exhibited normal intelligence. Upon physical examination, the 2 adult patients (II2 and II3) exhibited low muscular tension, muscle weakness, striated muscle atrophy, a winged scapula, failure of straightening both lower limbs, diminished tendon reflexes and were negative for Babinski syndrome. The pediatric patient (III2) had not yet reached the age of onset of this BMD pedigree, but was symptomatic with an abnormal gait and a winged shoulder. In the present study, 3 patients including patients II2, II3 and III2 received a

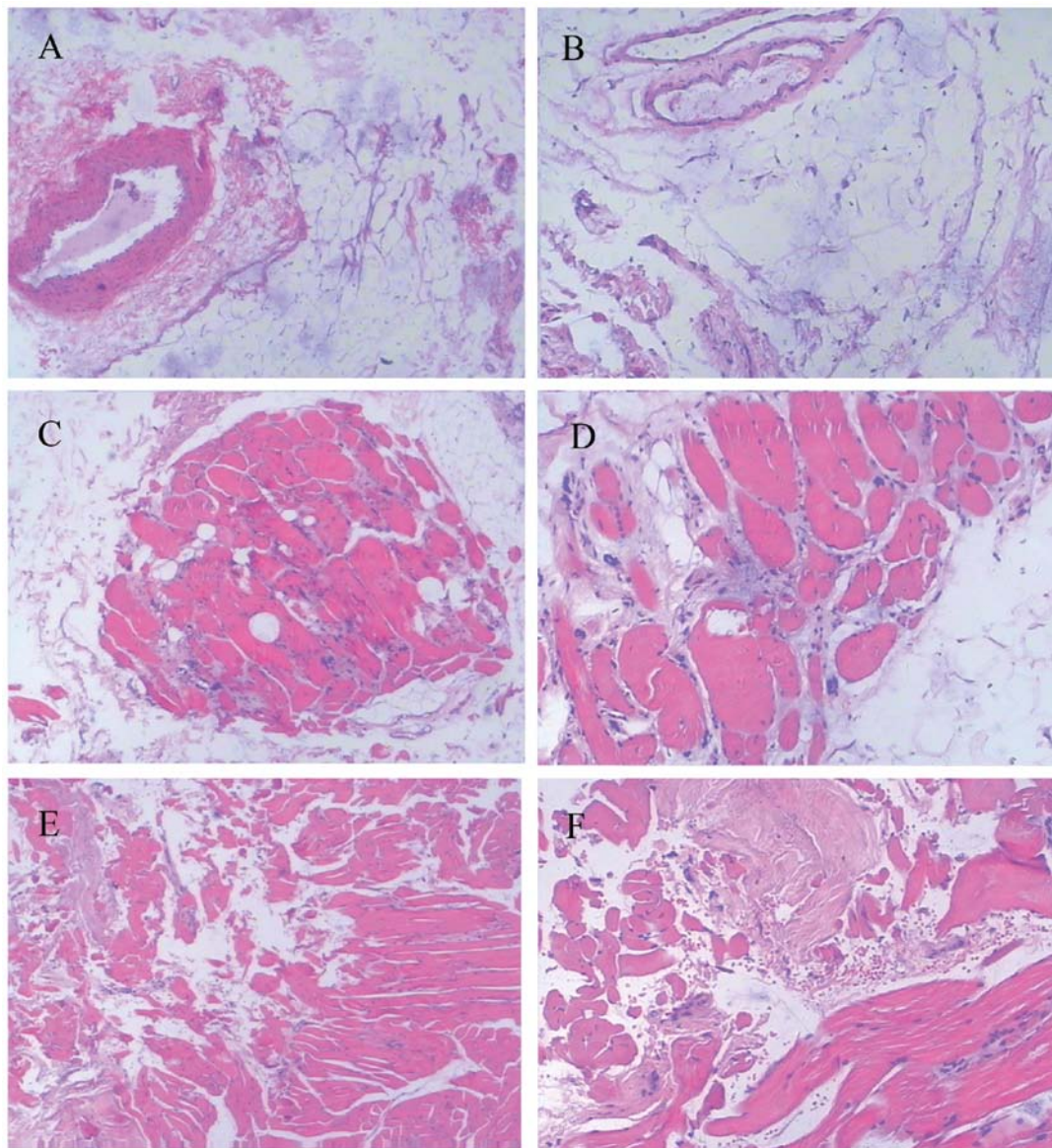


Figure 2. (A-D) Muscle biopsy analysis of patients II3 and II2. Representative images of striated muscle sections from (A and B) patient II3 and (C and D) patient II2. (A and C, x40 magnification; B and D, x100 magnification). Adipose tissue and blood vessels are visible in (B and D). (E and F) Muscle biopsy analysis of patient III2. Histological examination of striated muscle sections. (E, x40 magnification; F, x100 magnification). Eosinophilic material deposition is present in (F).

transplantation of hUC-MSCs. Eosinophilic material deposition was detected by an electromyogram and in the muscular biopsies from patients II2, II3 and III2 (Fig. 2). Myogenic damage was apparent in 3 patients (II2, II3 and III2) with pathological manifestations, including necrotic muscle fibers, dark (slow) muscle fibers in skeletal muscle, reactive inflammatory cell invasion and hyperplasia of fibrous tissue (Fig. 2). Based on a clinical evaluation, all patients included in this study were physically symptomatic, and their disease status was confirmed by histological analysis (Table I).

**Functional assessment following the transplantation of hUC-MSCs.** Three family members (II2, II3 and III2) received an infusion of hUC-MSCs (5, 5 and 3 IU, respectively) and were examined at 1, 3, 4 and 12 weeks following treatment. Patients II2 and II3 showed an improved muscle force of the limbs and an increased muscular size. Physical improvement

was already observed within 1 week. All 3 patients showed an increased muscle strength, appetite and food intake (Table I). In addition, the walking gait of patient III2 was gradually improved from this point on and was normal by 12 weeks post-transplantation (Table I). Serum levels of CK, AST and LDH are usually higher in patients with DMD/BMD. The degree of enzyme elevation in muscle disease largely reflects the underlying disease process, and is predominantly due to myonecrosis or membrane defects (25); thus, increases in CK and LDH levels are generally an indication of muscle damage. The serum levels of CK and LDH in patients II2 and II3 following the infusion of hUC-MSCs were decreased relative to the baseline levels (week 0; Fig. 3). The cell therapy, therefore, reduced muscle damage over this time frame in these 2 patients. By contrast, while the CK activity in patient III2 had initially decreased, the levels had increased by week 3 and then continued to rise sharply up to 12 weeks. However,

Table I. Subjective symptoms and signs of each patient from 0 to 12 weeks post-transplantation of hUC-MSCs.

	0 wpt	1 wpt	3 wpt	4 wpt	12 wpt
II2	Gowers' sign, winged shoulder, 'waddling' gait, muscle atrophy (+)	Similar to wpt 0. Felt increased strength. Appetite increased.	Similar to wpt 1.	Similar to wpt 1. Right hand could make a fist.	Similar to wpt 4.
II3	Gowers' sign, winged shoulder, 'waddling' gait, muscle atrophy (+)	Similar to wpt 0.	Similar to wpt 0.	Similar to wpt 0. Felt higher skin temperature. Appetite increased.	Similar to wpt 4.
III2	Gowers' sign, winged shoulder, 'waddling' gait, toe walking, ambulatory hesitancy, muscle atrophy (+)	Appetite increased 1/3. Felt increased strength. Gait improved. Absence of toe walking.	Similar to wpt 1. Appetite increased 1/3.	Similar to wpt 3.	With normal gait.

II2, II3 and III2 represent each candidate in the pedigree of the present study. hUC-MSCs, human umbilical cord-derived mesenchymal stem cells; wpt, weeks post-transplantation.

Table II. The functional assessment results of each patient from 0 to 12 weeks post-transplantation of hUC-MSCs.

Test	Site	0 wpt	1 wpt	3 wpt	4 wpt	12 wpt
Muscle force (II2/II3/III2)	Left arm	II\IV	II\IV	IV	IV	II\IV
	Right arm	0\IV	0\IV	0\I+V	0\IV	0\IV
	Left leg	0\IV	IV	IV	IV	IV
	Right leg	0\IV	0\IV	0\IV	0\IV	0\IV
ADL scoring (II2/II3/III2)		45\48\100	45\56\100	45\56\100	45\56\100	45\50\100
Muscle size (cm) (II2/II3/III2)	Left arm	16\19\6	16\19\7	16\19\7	16\19\7	16\19\6.5
	Right arm	16\18\6	16\18\7	16\18\7	16\18\7	16\18\7
	Left thigh	27\29\26	25\29.5\26	27\29.5\26	27\29.5\26	27\29\27
	Left calf	23\25\24	24\25\25	24\25\24	24\25\24	23\25\25
	Right thigh	25.5\28\26	26\29\27	25.5\29\26	25.5\29\26	25.5\28\27
	Right calf	22.5\25\24	23\25.5\23.5	22.5\25.5\24	22.5\25.5\24	22.5\25\25

II2, II3 and III2 represent each candidate in the pedigree of the present study. hUC-MSCs, human umbilical cord-derived mesenchymal stem cells; wpt, weeks post-transplantation.

the muscle size and muscle strength of patient III2 remained roughly stable over this time frame (Table II). Finally, a histological analysis of the muscle biopsies revealed that the muscle tissue remained essentially unaltered. At week 12 of evaluation, the muscle biopsies from patients II2 and II3 displayed adipose tissue deposition and degeneration, whereas eosinophilic material deposition was evident in the biopsy from patient III2 (Fig. 4). No obvious muscle regeneration was evident in the biopsies of any of the patients (Fig. 4).

## Discussion

Human muscular dystrophies are devastating progressive degenerating diseases with no current remedy. Mouse models of dystrophic disease have been elegantly exploited to demonstrate the potential clinical utility of hUC-MSCs in these diseases.

Previously, when hUC-MSCs were injected into the caudal veins of mice with limb-girdle muscular dystrophy type 2B (SJL mice), the cells had engrafted into the recipient dystrophic muscle and even expressed some human muscle proteins (26). These results highlight the effects and therapeutic potential of hUC-MSCs. In this study, we therefore infused cells into 3 patients with promising results, although the mechanisms responsible for these effects remain to be elucidated.

Efficacy has not been demonstrated in all studies utilizing hUC-MSCs for the treatment of dystrophic disease. In a different mouse model of muscle injury, only a limited fraction of hUC-MSCs were in fact found to express markers of pluripotent and myogenic cells, such as octamer-binding transcription factor 4 (OCT-4) and Nanog and only a minority of cells actually participated in new muscle fiber and myotube formation (27). Therefore, these results indicated that hUC-MSCs

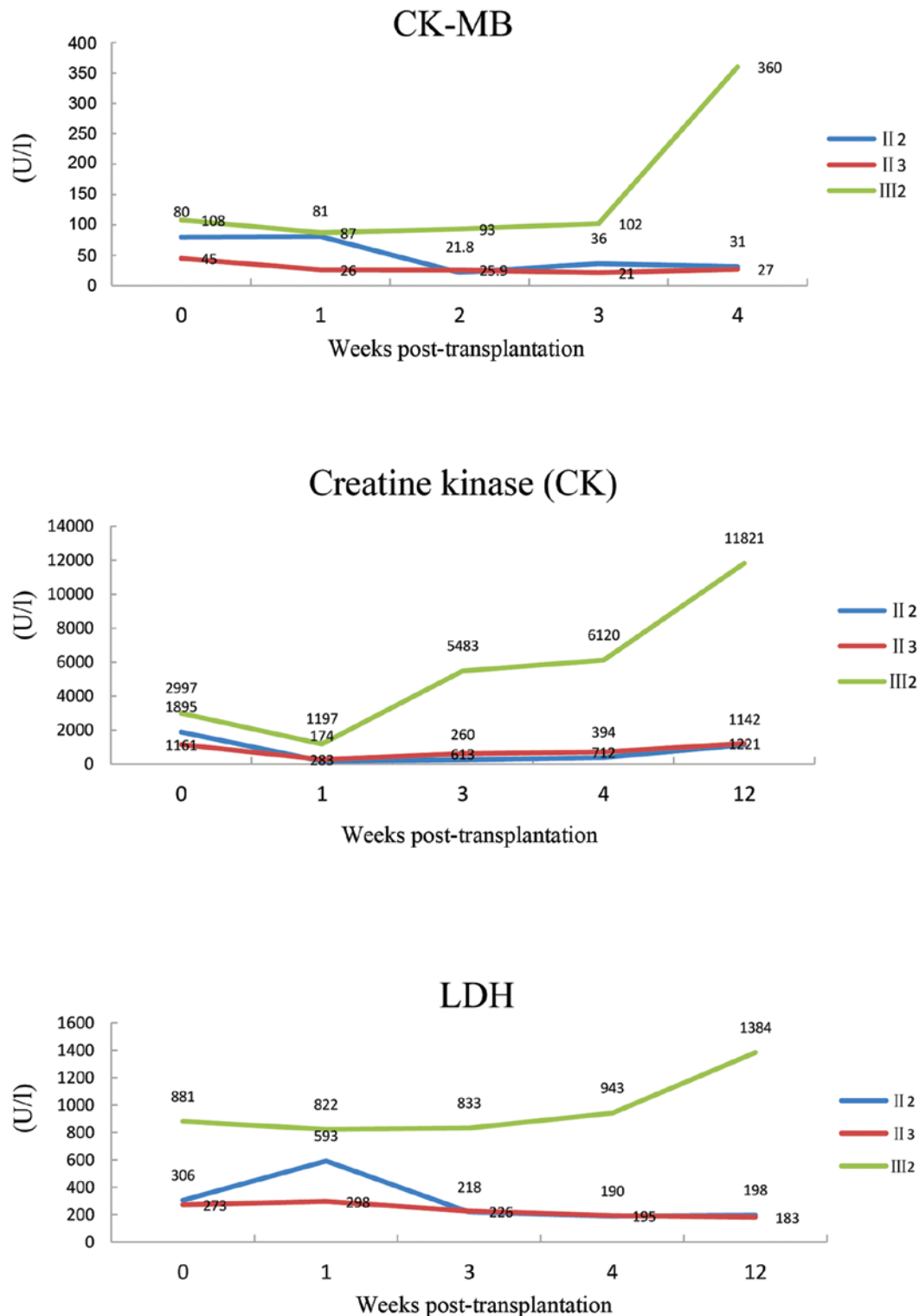


Figure 3. Values of serum creatine kinase (CK), creatine kinase isoenzyme (CK-MB) and lactate dehydrogenase (LDH) in each patient from 0 to 12 weeks post-transplantation of human umbilical cord-derived mesenchymal stem cells (hUC-MSCs).

possessed only limited potential for promoting granulation. Nevertheless, the transplanted hUC-MSCs promoted increased muscle mass and exerted a beneficial effect on regeneration of the injured muscle despite their inability to incorporate directly into regenerating muscle fibers. Similar trends were also observed in the present study. For example, the patients with long duration of the disease (II2 and II3) only showed

limited enhanced muscle force and muscle mass following the transplantation of hUC-MSCs, possibly due to severe muscle atrophy. By contrast, the muscle mass, muscle force, and the physical signs and subjective symptoms of pediatric patient III2 markedly improved.

The mechanism responsible for the degeneration of muscle tissue in DMD/BMD may originate in part from the depletion



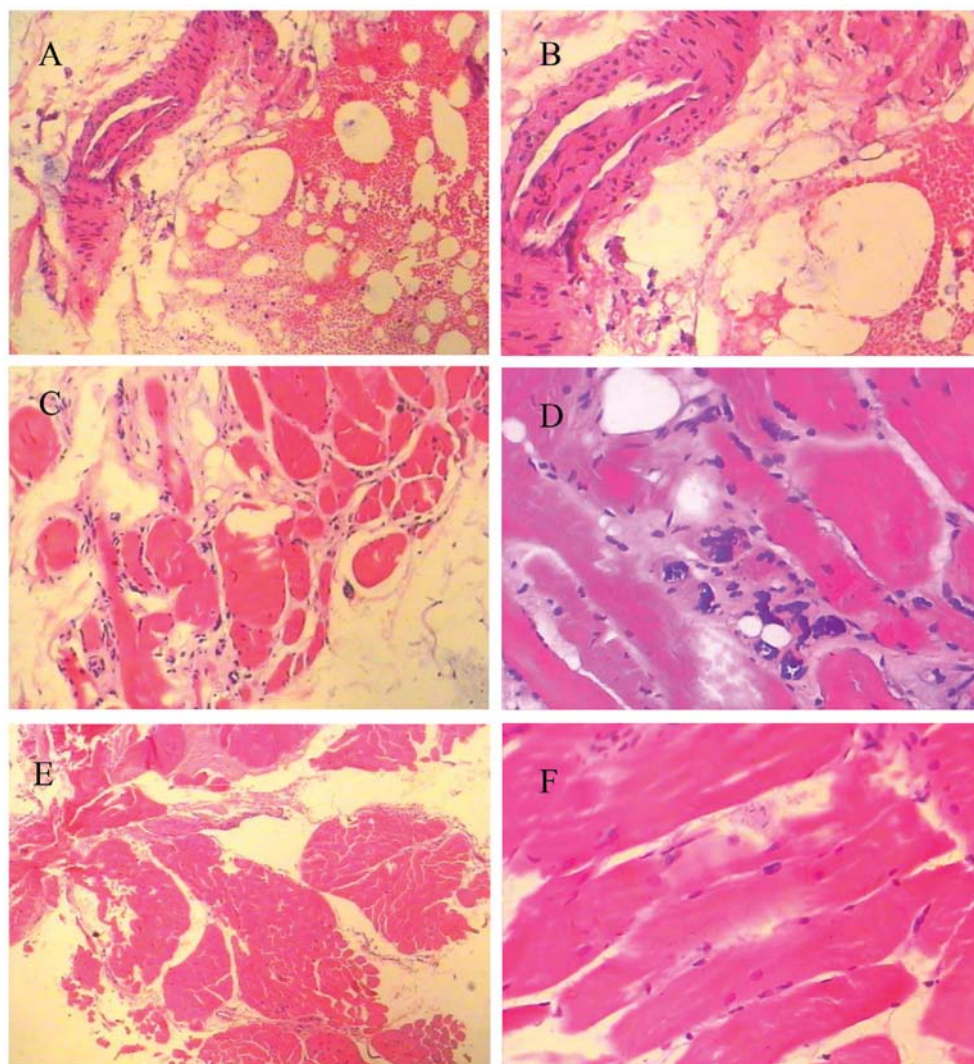


Figure 4. Muscle biopsy analysis of patients II2 II3 and III2 at 12 weeks following transplantation of human umbilical cord-derived mesenchymal stem cells (hUC-MSCs). Representative images of striated muscle sections from (A and B) patient II2, (C and D) patient II3 and (E and F) III2. (A, C and E, x40 magnification; B, D and F, x100 magnification). Adipose tissue deposition and degeneration were detected in (B and D). Eosinophilic material deposition was detected in (F). All 3 patients displayed no obvious muscle regeneration.

of specific cell types crucial for regeneration. Studies have clearly identified myosatellite and muscle precursor cells, located between the myolemma and the basal layer of muscle fibers, as vital for the rebuilding of injured muscle (28). Cycles of proliferation and differentiation, and the fusion of activated myosatellite cells occur in response to muscle trauma in order to rebuild functional fibers. In parallel, myosatellite cells are normally replenished through self-renewal. The skeletal muscle of patients with DMD/BMD experiences degeneration and regeneration repeatedly, to such a degree that myosatellite cells become depleted/exhausted. This scenario eventually results in the complete failure of muscle regeneration.

However, the mechanisms responsible for the positive effects of hUC-MSCs on muscle regeneration are less clear. It has been suggested that hUC-MSCs function similar to bone marrow-derived MSCs, the biological activities of which include paracrine signaling by trophic factors, the secretion of growth factors and cytokines, and the regulation of the proliferation and migration of endogenous cells (such as satellite cells and other muscle-related cells) (27,28). The source of the beneficial trophic

and/or growth factors may be either endogenous cell types or the transplanted MSCs or both. Some of these factors, such as basic fibroblast growth factor, (bFGF), insulin-like growth factor 1 (IGF-1), hepatocyte growth factor (HGF) and interleukin-6 (IL-6), are known to take part in the differentiation of myoblasts and the regeneration of muscle tissue (29,30). It is also possible that in addition to acting directly on myoblasts, these factors may promote the regeneration of injured or diseased muscle indirectly by enhancing the formation of blood vessels (31).

Although hUC-MSCs have exhibited a therapeutic effect in DMD/BMD, MSCs derived from different tissue sources have not been uniformly effective in clinical practice (23). MSCs derived from the umbilical cord have a significant advantage over those derived from bone marrow, placenta, or other tissues, as hUC-MSCs are easy to acquire and culture, and the cells have a high proliferation rate (32) and low immunogenicity (33). Moreover, hUC-MSCs are not tumorigenic and will not give rise to teratomas (34,35), and thus overall, fewer bioethical issues are involved in their collection or use (36). Low immunogenicity immunoregulation (33,35,37), paracrine signaling,

migration and the genomic stability of hUC-MSCs (38) have made them ideal candidates for therapies and favorable clinical prospects.

In conclusion, in the present study, to the best of our knowledge, the first transplantation of hUC-MSCs was performed in patients for the treatment of BMD. Our results revealed that no adverse reactions were detected in any patient for up to 12 weeks following transplantation, and that treatment was noticeably helpful to a patient with a shorter course of disease and less injury to muscle tissue. Although the prospective efficacy requires a longer follow-up period, the therapy used in this study provides a novel promising approach and a basis for the treatment of patients with DMD/BMD using cell therapy. The elucidation of the specific mechanisms underlying the efficacy of hUC-MSCs in patients with BMD may provide necessary insight into the development of more effective therapies for BMD.

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