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CD133+ cells derived from skeletal muscles of Duchenne muscular dystrophy patients have a compromised myogenic and muscle regenerative capability

Jinhong Meng[a,b], Francesco Muntoni[a,b], Jennifer Morgan[a,b,*]

[a] Dubowitz Neuromuscular Centre, Molecular Neurosciences Section, Developmental Neuroscience Programme, UCL Great Ormond Street Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK
[b] NIHR Great Ormond Street Hospital Biomedical Research Centre, 30 Guilford Street, London WC1N 1EH, UK

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ABSTRACT

Cell-mediated gene therapy is a possible means to treat muscular dystrophies like Duchenne muscular dystrophy. Autologous patient stem cells can be genetically-corrected and transplanted back into the patient, without causing immunorejection problems. Regenerated muscle fibres derived from these cells will express the missing dystrophin protein, thus improving muscle function.

CD133+ cells derived from normal human skeletal muscle contribute to regenerated muscle fibres and form muscle stem cells after their intra-muscular transplantation into an immunodeficient mouse model. But it is not known whether CD133+ cells derived from DMD patient muscles have compromised muscle regenerative function.

To test this, we compared CD133+ cells derived from DMD and normal human muscles. DMD CD133+ cells had a reduced capacity to undergo myogenic differentiation *in vitro* compared with CD133+ cells derived from normal muscle.

In contrast to CD133+ cells derived from normal human muscle, those derived from DMD muscle formed no satellite cells and gave rise to significantly fewer muscle fibres of donor origin, after their intra-muscular transplantation into an immunodeficient, non-dystrophic, mouse muscle.

DMD CD133+ cells gave rise to more clones of smaller size and more clones that were less myogenic than did CD133+ cells derived from normal muscle. The heterogeneity of the progeny of CD133+ cells, combined with the reduced proliferation and myogenicity of DMD compared to normal CD133+ cells, may explain the reduced regenerative capacity of DMD CD133+ cells.

1. Introduction

CD133+ cells are a rare population that resides within human skeletal muscle (Benchaouir et al., 2007; Meng et al., 2014; Negroni et al., 2009). We and others have shown that CD133+ cells isolated from normal human muscle contribute to muscle regeneration (Negroni et al., 2009) and form functional muscle stem cells after their intra-muscular transplantation in an immunodeficient mouse model (Meng et al., 2014), but CD133+ cells derived from DMD patient muscle have not been extensively investigated (Benchaouir et al., 2007; Meng et al., 2014).

The use of autologous, rather than heterologous, donor stem cells will reduce the possibility of their immunological rejection, although they will have to be genetically modified prior to their transplantation (Counsell et al., 2017; Meng et al., 2016; Young et al., 2016). But satellite cells from DMD muscle may not function correctly, due either to lack of dystrophin expression at the required time (Dumont et al., 2015), or the fact that continual activation of stem cells in pathological muscle may lead to their exhaustion (Blau et al., 1983; Sacco et al., 2010; Mouly et al., 2005). In addition, changes in the components of the stem cell niche (Smith et al., 2016; Alexakis et al., 2007; Sabatelli et al., 2012) (*e.g.* alterations in components of connective tissue, or of the muscle fibre) or signalling pathways (Jiang et al., 2014) may be deleterious to satellite cell function. It is not known whether any of these factors affect CD133+ cells.

We therefore decided to compare the myogenicity and muscle regenerative capacity of CD133+ cells derived from the muscles of 4 control and 4 DMD patients with different mutations in the *DMD* gene.

* Corresponding author at: Dubowitz Neuromuscular Centre, Molecular Neurosciences Section, Developmental Neuroscience Programme, UCL Great Ormond Street Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK.
E-mail address: jennifer.morgan@ucl.ac.uk (J. Morgan).

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Table 1
Muscle biopsies used for H&E and immunostaining.

Donor	Muscle	Age (years)	Mutation	H&E	IF of CD133
Normal	Quadriceps	3	None	Morphologically normal	ND
Normal	Quadriceps	9	None	Morphologically normal	ND
DMD	Quadriceps	5	Dystrophin point mutation, c.3804G > A (p.Trp 1268X), detected in exon 28	Features of DMD pathology	ND
DMD	Quadriceps	8	Dystrophin point mutation, c.9851G > A (p.Trp 3284X), detected in exon 68	Features of DMD pathology	Present
DMD	Quadriceps	4	Dystrophin point mutation, c.1388G > A (p.Trp 463X), detected in exon 12	Features of DMD pathology	Present

*ND: Not detected.

DMD CD133+ cells had impaired myogenic capacity both *in vitro* and *in vivo*. Clonal analysis suggested that this may relate to the increased proportion of slow proliferating colonies and low/non-myogenic colonies within the CD133+ cell -derived clones from DMD compared to normal muscles. We also found that human skeletal muscle -derived CD133+ cells are heterogeneous, containing cells of satellite cell/myoblast, myoendothelial, pericyte and fibroblast lineages.

Although CD133+ isolated from normal human muscle contribute to muscle regeneration *in vivo*, their counterpart from DMD patients are significantly less effective. This will have to be taken into consideration when thinking of using stem cells derived from patient muscle therapeutically.

2. Results

2.1. CD133+ cells derived from DMD muscles have variable myogenicity in vitro

We have shown that CD133+ cells isolated from normal human muscle are myogenic *in vitro* and can contribute to muscle regeneration in an *in vivo* mouse model (Meng et al., 2014; Meng et al., 2015). In order to investigate CD133+ cells from DMD muscle, we performed H&E and immunostaining of CD133 on skeletal muscle sections from either normal (n = 2) or DMD patients (n = 3). The details of muscle biopsies used in this experiment are listed in Table 1. As expected, normal muscles stained with H&E had little fat or fibrotic tissue, while DMD muscles had pathological changes typical of DMD (Fig. 1a, b). In line with our previous finding (Meng 2014), CD133+ cells were in the satellite cell position in muscle biopsies from 18-day old infants (Meng et al., 2014), but not in normal biopsies from individuals older than 2-years of age (Fig. 1c). However, in 2 out of 3 muscle biopsies from DMD patients, CD133+ cells were found outside the myofibres (Fig. 1d and Table 1). These data suggest that the composition of CD133+ cells in normal and DMD muscles may not be the same, thus there might be functional differences between normal and DMD CD133+ cells.

To investigate the myogenic capacity of CD133+ cells derived from the muscles of DMD patients, we isolated these cells from 4 DMD patients and 4 normal donors (Table 2) and expanded them in M10 medium. We performed FACS analysis of all the normal and DMD CD133+ preparations at mpds from 14.38–18.47 (Fig. 1e). As

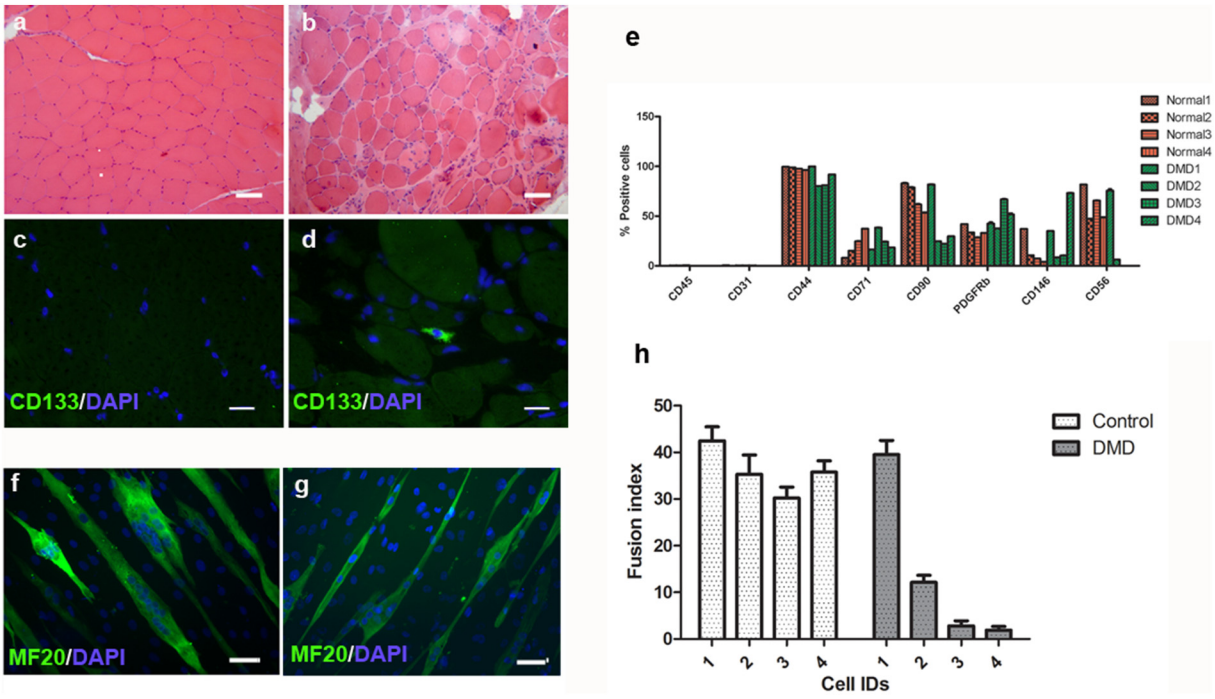

Fig. 1. Location of CD133+ cells within human skeletal muscle, characterization of CD133+ cell population and their myogenic capacity *in vitro*. a,b: H&E staining of normal and DMD human skeletal muscle sections show typical degeneration and regeneration muscle fibres in DMD muscle biopsy (b), in comparison to normal muscle biopsy (a). Scale bar = 25 μm. c, d: Immunofluoresent staining of CD133 on normal (c) and DMD (d) muscle sections. There were CD133+ cells present within DMD muscles. Scale bar = 10 μm. e: Characterization of CD133+ cell population by FACS analysis of cell surface markers. Skeletal muscle-derived CD133+ cells lack haematopietic stem cell and endothelial cell markers. All cell populations contained cells expressing mesenchymal stem cell and pericyte markers. Not all DMD cell populations contain CD56+ cells. f, g: *In vitro* myogenicity of CD133+ cells. Four normal and four DMD CD133+ cell preparations were induced to undergo myogenic differentiation *in vitro*. Myotubes were stained with an antibody to myosin heavy chain (MF20, green), nuclei were counterstained with DAPI (blue). f and g are representative images of the MF20 staining of DMD1 and DMD2 cells. Scale bar = 25 μm. h: fusion index (mean ± SEM) of each cell preparation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)