

240th ENMC workshop: the involvement of skeletal muscle stem cells in the pathology of muscular dystrophies 25 -27 January 2019, Hoofddorp, the Netherlands

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Jennifer Morgan , Gillian Butler-Browne , Francesco Muntoni , Ketan Patel , on behalf of the skeletal muscle stem cells involvement in pathology study group

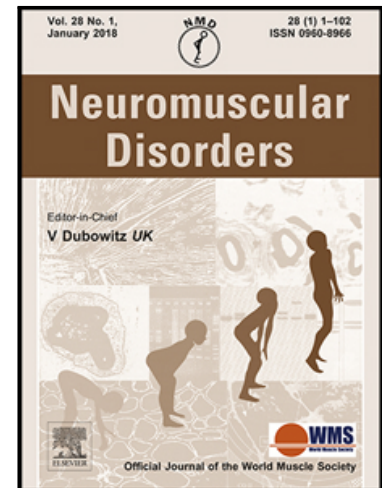
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Highlights

- Satellite cells are dysfunctional in several neuromuscular disorders
- Some muscles are more susceptible than others to disease and ageing
- In vitro and in vivo model systems have shed light on many of the processes involved in satellite cell function and dysfunction, but the drawbacks of each model system must be considered.
- Skeletal muscle pathology in mouse models of neuromuscular disease are affected by genetic background
- A single cell approach will be useful to identify dysfunction in subsets of cell populations

Workshop report

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1. Introduction

Twenty six participants representing patients, funding agencies and basic and clinical scientists involved in research into skeletal muscle stem cells and muscular dystrophies from France, Germany, Italy, India, UK, Australia, Spain, USA, The Netherlands and Switzerland met in Hoofddorp, The Netherlands on 25 – 27 January 2019. The meeting was held under the auspices of the ENMC and ENMC main sponsors, with the additional support of Muscular Dystrophy UK.

The central aim of this workshop was to determine whether there could be some intrinsic problems with satellite cell function (e.g. activation and myoblast proliferation, differentiation and fusion) and consequently provide evidence for satellite cell involvement in pathology in

major classes of muscular dystrophy and congenital myopathies. This is most likely to manifest as impaired muscle formation during development in the congenital myopathies. However, some problems in muscle formation may be due to extrinsic factors in the microenvironment of the satellite cells/myoblasts, such as altered composition of the extracellular matrix.

Although they vary in many features including severity, muscle groups affected, age of onset and heart involvement, muscular dystrophies are characterised by progressive skeletal muscle weakness and wasting. They are generally caused by mutations in genes that have been historically thought to be expressed either only within the muscle fibre to produce components of the dystrophin-associated protein complex, or within cells lying outside the myofibre (e.g. by fibroblasts (reviewed [1])). Skeletal muscle needs to grow, maintain itself for a lifetime and also repair itself in response to injuries or increased load. In muscular dystrophies, skeletal muscles undergo chronic intrinsic necrosis that gives rise to myogenesis and regeneration, in order to replace the necrotic portion of the myofibres. The satellite cell is the stem cell that is involved in regeneration as well as skeletal muscle growth and postnatal myogenesis and possibly hypertrophy. It sits in a niche between the myofibre sarcolemma and basal lamina both of which regulate satellite cell quiescence, activation and self-renewal.

Expression of several genes responsible for muscular dystrophies but also in congenital myopathies were not initially detected in satellite cells. These findings have major implications in that they support the idea that muscular dystrophies do not arise from the malfunctioning of satellite cells and that correcting other compartments should be sufficient to alleviate the disease. However with the advent of powerful detection methods it is becoming clear that this conclusion is not generally sustainable. This is best exemplified by discoveries made in the field of Duchenne Muscular Dystrophy (DMD). The involvement of the satellite cell in DMD was thought to be a secondary response to the lack of dystrophin in the myofibre as well as an excessive proliferation of the satellite cells which in human have limited replicative potential due to telomere shortening. The dystrophin-deficient myofibre undergoes necrosis and

satellite cells become activated, proliferate and then contribute to muscle regeneration and reconstitution of the satellite cell pool. In DMD, it was long held that the satellite cells fail in their myogenic capacity and ability to proliferate after repeated episodes of muscle necrosis and regeneration. Muscle fibers become progressively replaced by fibrosis an adverse environment that also can impair myogenesis.

Recent findings show that dystrophin is expressed transiently in satellite cells and this is proposed to control their asymmetric divisions, so that satellite cells from the dystrophin-deficient *mdx* mouse model of DMD give rise to a disproportionate number of stem-like satellite cells at the expense of differentiation-competent myoblast progenitors [2]. This observation raised the possibility of DMD being a muscle stem cell disease. But this may be too simplistic, as there appears to be excellent myogenesis and new muscle formation by dystrophic satellite cells during growth of *mdx* mice, and in tissue culture, that is maintained in the *mdx* mouse, even after persistent intrinsic myonecrosis of limb muscles over many months and after experimental muscle injury [3], suggesting that dystrophin is not critical for satellite cell function under the experimental conditions evaluated.

The satellite cell dysfunction in mouse models of dystroglycanopathy [4] and collagen VI deficiency [5] may be due to defects in the environment (niche), rather than intrinsic defects in the satellite cells themselves. But the extent to which members of the DAPC are expressed in satellite cells and whether their expression within satellite cells is vital for satellite cell function, is not clear.

Satellite cells also seem to have some direct effects on their environment and any disturbance in this may lead to muscle pathology: it is proposed that satellite cells signal to fibroblasts to hold them in quiescence and loss of satellite cells may increase muscle fibrosis.

Mutations in genes that regulate satellite cell/myoblast activity are implicated in some congenital muscular dystrophies [6] [7]. Here, altered behaviour of myoblasts prior to them

fusing to form myotubes let alone myofibres during embryogenesis and early growth results in impaired muscle formation evident at birth.

It is essential that we accurately assess the contribution of satellite cells to pathology of diverse muscular dystrophies, since current therapies mostly aim at correcting abnormalities solely in the myofibre. This, if not addressed, will have major implications on the long term efficacy of any treatment; if a defect in satellite cells is either the main, or a contributory, cause of a disease, then treating the myofibre will at best be partially successful.

2. Defects in muscle regeneration in different neuromuscular conditions

Duchenne muscular dystrophy: Grounds

Since skeletal muscle satellite (stem) cell, also called myoblast, transfer therapy has been intensively investigated for over 30 years as a possible gene replacement strategy for DMD, this talk first summarised the many persisting problems with clinical applications of this muscle stem cell therapy approach that need to be overcome. Key issues include the source of 'normal donor' skeletal muscle stem cells, expansion in tissue culture, best route of delivery, massive initial death of injected cultured myogenic cells, and limited proliferation and migration *in vivo*, before fusion with the host dystrophic muscle cells. These major problems need to be realistically addressed when proposing stem cell therapies for muscular dystrophies. Another therapeutic strategy for delivering myogenic stem cells, is the exciting field of tissue engineering that aims to build new muscle constructs to replace muscles that are unable to regenerate due to some impaired intrinsic capacity of muscle stem cells; while this holds great promise for many creative tissue culture studies of muscle cells, there remain serious challenges for clinical applications [8].

The critical issue of identifying whether there is any intrinsic problem with satellite cell capacity for myogenesis in DMD specifically, and other muscular dystrophies was then addressed. This can be identified by some marked problems with muscle formation during development (congenital manifestation): not evident in *mdx* mice, nor conspicuous for DMD baby boys.

Then it is essential to determine histologically the extent of any ongoing intrinsic myofibre necrosis (thus the need for regeneration) in growing and adult muscles; however, care is needed when assessing biopsies since many morphological changes in muscles that can occur over time for other reasons (e.g. split myofibres, central myonuclei) can sometimes be misinterpreted as resulting from necrosis/regeneration [9]. If there is extensive intrinsic necrosis, as evident in dystrophic rodent and dog models of DMD, then the capacity for sustained new muscle formation and regeneration needs to be determined: yet it is widely reported that limb muscles of mdx mice have excellent sustained new muscle formation. However, one has to be cautious about concluding that the same properties exist in the human condition without further evidence. The capacity for myogenesis (proliferation and fusion) of the satellite cells can also be examined experimentally; *in vitro* studies show excellent myogenesis (equivalent to normal muscles) even for satellite cells cultured from old mdx mice aged 15 months [3], and *in vivo* studies using experimental muscle injury in mdx mice show very similar regenerative capacity for old mdx and normal muscles. Thus overall, in animal models and possibly in DMD, these many observations strongly indicate no significant intrinsic problem with myogenesis of dystrophic skeletal muscle stem cells *in vivo*. This clearly contrasts with recent claims [2] that need to be carefully re-considered in the context of this large body of evidence for excellent intrinsic regeneration of dystrophic muscles. Instead, the central problem for DMD maybe the repeated necrosis of dystrophic muscles over many years, exacerbated by growth for almost 2 decades. While necrosis initially results in good regeneration of dystrophic muscles, over time the persistent bouts of asynchronous damage and inflammation progressively change the extracellular matrix composition with increasing collagen deposition and fibrosis that can impair myogenesis and stop regeneration, leading to severe loss of skeletal muscle mass and function in DMD boys.

Satellite cell behavior in myotonic dystrophy: Furling

Myotonic dystrophy type 1 (DM1 or Steinert disease), one of the most common neuromuscular diseases in adults, is caused by an unstable expansion of CTG repeats within

the 3'UTR of the *DMPK* gene. DM1 is an RNA dominant disorder in which nuclear-retained *DMPK* transcripts containing CUG expansions alter the function of RNA binding proteins. Abnormal binding of MBNL proteins to pathologic CUG tract leads to their sequestration within nuclear CUGexp-RNA foci and subsequently, to their functional loss that is associated with RNA metabolism alterations including alternative splicing misregulations. Interestingly, DM1 splicing changes in *CLCN1*, *BIN1* and *DMD* pre-mRNAs are associated with symptoms such as myotonia, muscle weakness and dystrophic process, respectively. At the clinical level, five forms of the disease (late, adult, juvenile, infantile and congenital) are recognized based on the occurrence and onset of symptoms as well as the size of the CTG tract, which correlates with severity of symptoms. In addition to myotonia, DM1 skeletal muscles displayed progressive weakness and atrophy as well as histological features including increased variation of fiber diameter, central nuclei, sarcoplasmic masses, nuclear clumps and ring fibers. Although an increased number of satellite cells are found in the more affected muscles, several studies support altered behavior of DM1 satellite cells that could impact their regenerative capacity and/or muscle mass maintenance. Thus, DM1 muscle precursor cells have a reduced proliferative capacity *in vitro* due to an early activation of the p16-dominant pathway leading to premature replicative senescence [10]. Moreover, expression of mutant RNAs harboring large CUG expansions alters the myogenic differentiation process of DM1 myoblasts. Defective differentiation/fusion was observed in myoblasts isolated from muscles of severe congenital DM1 patients showing muscle immaturity or delayed muscle maturation. Altogether these results suggest that satellite cell functions are progressively impaired by unstable CTG expansions, a mechanism that could contribute to the gradual muscle atrophy in DM1 pathology. Additional studies are required to determine the role of extrinsic and/or intrinsic signals on impaired behavior of satellite cells in DM1.

Satellite cells, DUX4, PAX7 and facioscapulohumeral muscular dystrophy: Zammit

Facioscapulohumeral muscular dystrophy (FSHD) is characterized by a descending skeletal muscle weakness and wasting. This implies that the normal repair functions performed by muscle satellite cells are compromised in FSHD. Both FSHD1 (MIM 158900) and FSHD2

(MIM 158901) are linked to the hypomethylation of D4Z4 repeats on chromosome 4q35. Each D4Z4 repeat contains an open reading frame for a retro-transposed transcription factor, double homeobox chromosome 4 (DUX4). Such epigenetic de-repression in FSHD leads to expression of DUX4 from the most distal D4Z4 unit, with DUX4 message then stabilized by a polyA signal located in the flanking pLAM region [11]. DUX4 is a homeodomain-containing transcription factor, and its aberrant expression is considered the primary cause of FSHD pathology [11, 12].

Investigation into how DUX4 drives FSHD pathology has mainly focused on expression of DUX4 target genes. However, the homeodomains of DUX4 have high homology to those of PAX7 and are interchangeable, suggesting an alternate hypothesis whereby ectopic DUX4 expression in FSHD interferes with PAX7 function. DUX4 and PAX7 mutually inhibit each other in activating their respective transcriptional target genes in human cells [13], while, PAX7 can rescue DUX4-mediated apoptosis in murine myoblasts.

Banerji and Zammit have recently found that the repression of PAX7 transcriptional target genes is a hallmark of FSHD, proposing a model whereby DUX4 causes pathology not only by directly activating its own target genes, but also by interfering with the ability of PAX7 to regulate its transcriptional gene target cohort [13]. Indeed, PAX7 target gene repression in FSHD correlates with disease severity, independently of DUX4 target gene expression. At the single cell level, PAX7 target gene repression can efficiently discriminate FSHD cells, even when no DUX4 target genes are detectable [14]. Thus, PAX7 target gene repression is a major biomarker for FSHD. Since DUX4 can be expressed in primary FSHD patient-derived proliferating myoblasts, such interference of PAX7 function by DUX4 would affect satellite cell mediated repair in FSHD.

Zammit and colleagues also explored FSHD pathomechanisms, particularly the pathways that control mitochondria: of interest considering alterations in mitochondrial structure and function in FSHD muscle, and the known sensitivity of FSHD cells to oxidative stress. Notably, they identified suppression of mitochondrial biogenesis in FSHD, in particular via PGC1alpha, the co-factor and activator of ERRalpha. Myoblasts from FSHD patients either form hypotrophic

myotubes, with a thin, elongated morphology, or myotubes with an unusual distribution of myonuclei and dysregulation of the microtubule network. Known ERRalpha agonists and safe food supplements Biochanin A, Genistein or Daidzein can rescue this hypotrophic myogenic differentiation in FSHD. Thus suppression of the PGC1alpha-ERRalpha axis leads to perturbed myogenic differentiation, which can effectively be rescued by readily-available food supplements, which may lead to a regenerative therapy approach for FSHD.

Oculopharyngeal muscular dystrophy: Butler-Browne

The regenerative capacity of skeletal muscle declines with age – this decline has been partly attributed to extrinsic environmental influences and diverse intrinsic potential of the muscle stem cells themselves. Age-related muscle decline can be exacerbated in patients with late onset degenerative disorders. A progressive loss of function of pharyngeal muscles is frequently encountered in ageing subjects (a condition called achalasia) as well as in several neurological or neuromuscular disorders. Among them, oculopharyngeal muscular dystrophy (OPMD) is a late-onset autosomal dominant degenerative muscle disorder, characterized by weakness of eyelids and pharyngeal muscles that typically appear from the fifth decade, leading respectively to ptosis and dysphagia. The genetic mutation - a short polyalanine expansion in the ubiquitous PABPN1 protein [15] – was found over 20 years ago but the pathophysiological mechanisms leading to the specific alterations of the myogenic program in these muscles remains to be determined. Butler-Browne reported that, although sharing similar functions, all muscles are not equal in terms of structure and presence of satellite cells, and among them pharyngeal and eyelid muscles - *per se* show atypical features, such as hypotrophic muscle fibers surrounded by connective tissue that are usually considered to be pathological in the other skeletal muscles (limb muscles). Surprisingly, during ageing Butler-Browne recently demonstrated that pharyngeal muscles have an increased proportion of muscle stem cells - 6% compared to the 1% or less classically observed in the limb muscles of 'old' subjects. In addition, they observed the presence of many satellite cells in the interstitial space. In the pharyngeal muscles of OPMD patients the number of muscle stem cells is even higher (12%) compared to control subjects of the same age, whereas there is no

signs of ongoing regeneration. Different hypotheses could explain this increased percentage of muscle stem cells both inside and outside of their niche, including extrinsic and/or intrinsic signals. Butler-Browne presented the different techniques used on human muscle samples: gene expression profile studies, co-culture experiments, xenograft models and CyToF to decipher the potential mechanisms involved. Preliminary results demonstrate both intrinsic and extrinsic signals are involved. The study of the specific signature of pharyngeal muscle stem cells which Butler-Browne has observed will further our understanding of what makes this muscle more susceptible to diseases and ageing and why the large number of muscle stem cell present in these muscles do not help muscle regeneration. Disorders affecting these muscles will represent particularly informative models to better understand skeletal muscle stem cell homeostasis in adults and in ageing.

POGLUT1 mutation: Paradas

Paradas discussed recessive mutations in the *POGLUT1* gene that have been associated with a muscular dystrophy due to decreased Notch signalling and reduced pool of quiescent satellite cells, demonstrated in adult skeletal muscle from patients [16]. A direct consequence is a slow proliferation and differentiation shown by the mutant human myoblasts, which causes an alteration of the muscle repair process. A secondary consequence is a disruption of the progressive alpha-dystroglycan glycosylation that physiologically occurs during myogenesis [17], potentially participating in the myopathic process. So, the critical role of this O-glycosyltransferase 1 protein, *POGLUT1*, in the maintenance of a correct pool of satellite cells has been uncovered, reinforcing the Notch signalling pathway as a potential therapeutic target for this myopathy. Primary myoblast culture and *Drosophila* have been demonstrated to be useful experimental models to study the effects of *POGLUT1* mutations on satellite cells. Generation of mouse models are in progress, showing promising preliminary results.

Congenital myopathies: Jungbluth

Jungbluth reviewed the evidence for satellite cell involvement in the congenital myopathies (CMs), a heterogeneous group of early-onset neuromuscular disorders conventionally defined

by the abundance of certain structural abnormalities – cores, nemaline rods and central nuclei – on muscle biopsy. In contrast to the congenital muscular dystrophies (CMDs), the plasma membrane integrity is typically preserved, reflected in normal or only mildly elevated CK levels. More than 20 genes have been implicated to date, encoding proteins most commonly involved in various aspects of excitation-contraction coupling, the process whereby a neuronal impulse is translated into intracellular calcium release and muscle contraction. Mutations in the skeletal muscle ryanodine receptor (*RYR1*) gene encoding the principal sarcoplasmic reticulum calcium release channel are the most common cause and have been associated with a wide phenotypical spectrum, ranging from severe CMs with antenatal onset to induced episodic manifestations – malignant hyperthermia and (exertional) rhabdomyolysis – in otherwise healthy individuals.

The evidence for satellite cell involvement is strongest in Early-onset Myopathy with Areflexia, Respiratory Distress and Dysphagia (EMARDD) due to recessive mutations in *MEGF10*, encoding a protein highly expressed in nerve and skeletal muscle tissues, (in particular in activated satellite cells) [7, 18]. The *MEGF10* protein plays a role in satellite cell proliferation, differentiation and fusion into multinucleated fibres, as supported by the typical muscle biopsy appearance of small and poorly fused myofibers in *MEGF10*-deficient states. *MEGF10* is also involved in satellite cell self renewal and maintenance of the satellite cell quiescent state. An important role in muscle regeneration is evidenced by the dystrophic phenotype in the *MEGF10* KO mouse, and worsening of dystrophic features in the *MEGF10*/mdx double KO when compared to the respective single mutants. Exaggerated satellite cell loss with aging and repeated injury, probably due to a shift to myogenic commitment and a consequently inadequately maintained muscle satellite cell pool, has also been observed in recessive *SEPN1*-related myopathies, a group of CMs characterized clinically by predominant axial involvement with early scoliosis and respiratory impairment, and increases in connective tissue, fibre type disproportion and cores (“Multi-minicore Disease, MmD”) on muscle biopsy. *SEPN1* encodes Selenoprotein N, a member of a protein group mediating the effects of Selenium, whose biological functions are not fully understood but appear to include an

important role in redox regulation. Reflective of the reported satellite cell abnormalities, SeIN deficiency has been associated with loss of regenerative capacity, muscle mass and strength on the organismal level [19]. A reduction of absolute satellite cell numbers has also been reported in muscle biopsies from affected humans and animal models of X-linked myotubular myopathy (XLMTM), specific qualitative studies have not yet been performed [20]. XLMTM is due to X-linked recessive mutations in *MTM1*, encoding myotubularin with important roles in membrane trafficking and other intracellular processes. XLMTM is a severe and often lethal early-onset myopathy with clinical features of marked weakness, severe respiratory and bulbar involvement, as well as the presence of numerous centralized nuclei and predominance of atrophic type 1 fibres on muscle biopsy.

These findings, in conjunction with the recent identification of mutations in *PAX7* and *MYMK* (see below), suggest that there is a distinct CM subgroup where satellite cell pathology is crucially involved. These CMs are often characterized clinically by marked axial involvement and severe respiratory impairment, and predominance of atrophic type 1 fibres and increases in connective tissue on muscle biopsy, indicating a consistent phenotype. Level of satellite cell assessment (in particular regarding activation states and cell fate commitment) has been variable and requires further standardization. The involvement of satellite cells in other CMs, and in particular their role in the commonly observed disturbances of muscle growth, remains to be elucidated.

Pathological pathways in dystrophic models of DMD and LGMD: van Putten

van Putten started by pointing out that despite the many similarities in disease pathology between muscular dystrophy patients and mouse models (i.e. primary defect, chronic cascades of muscle degeneration, regeneration, severe inflammation response and the development of fibrosis), there are also many differences. These include amongst others differences in growth speed, distribution of bodyweight on limb muscles, a lack of development of fat in mice and a general disease stabilization in adulthood. Only in animal models lacking multiple structural muscle proteins, life span is dramatically reduced. It

remains important to keep these differences in mind when conducting basic science experiments or pre-clinical studies with animal models; they remain models which each have their own limitations.

van Putten then focussed on the muscle pathology in *mdx* mice, which is characterized by cycles of degeneration and regeneration from the age of 3 weeks onwards, after which it stabilizes in adulthood due to their relatively good regenerative capacity. Several attempts have been undertaken to exacerbate disease pathology by backcrossing *mdx* mice on another genetic background. Based on findings in the gamma-sarcoglycan model for LGMD where pathology became worse when crossed on a DBA2/J genetic background [21, 22], D2-*mdx* mice were generated. She discussed the 'On Mouse And Measures' initiative of Charley's Fund where all available data of this novel DMD model were collected. In short, pathology is characterized by early onset of degeneration and regeneration. Overall muscle pathology (necrosis, fibrosis, inflammation) is more apparent in the D2-*mdx* than in the BL10-*mdx* model due to alterations in *Ltbp4* (causing fibrosis), *Anx6* (impairing regeneration) and genes in the *dyscalc* locus (causing calcification) and potentially other as yet unidentified mutations. In both models, severity differs between muscles, with diaphragm being most severely affected and tibialis anterior least severely affected. Functional differences (in hanging wire tests) exist between genders, with females outperforming males, and BL10-*mdx* outperforming D2-*mdx* mice. D2-*mdx* mice have lower counts of centralized nuclei, active regeneration (BrdU and eMHC staining). A striking difference between the strains is extensive calcified muscles in D2-*mdx* mice [23]. These seem to directly be linked to muscle damage, differ in abundance between muscles and these calcifications ameliorate with age (very abundant in 10 week old mice, but much reduced numbers at 34 weeks of age). The reason for this is still unknown.

Lastly she discussed muscle pathology in two mouse models for LGMD (*sgca*-null for LGMD2D and *sgcd*-null for LGMD2F). A striking difference between the strains is that muscle function in *sgca*-null males and females is affected to the same extent, while in *sgcd*-null mice, females outperform males [24]. Also, the extent of pathology differs between muscles and

ages, although again diaphragm is most severely affected in these strains. In these models, muscle regeneration is active in the young age (8 weeks), but decreases in adult mice (24 weeks).

3. Models of satellite cell in involvement in muscle regeneration

The workshop dedicated one session to discuss models to study satellite cell function in a spectrum of models. In the *mdx* mouse, Partridge presented alternative approaches to investigate growth and repair of skeletal muscle in animal models. This consisted of monitoring the rates of increase in number of myonuclei in fibres extracted from the extensor digitorum longus (EDL) muscle and of measuring the amounts of muscle generated by satellite cells labelled with BrdU over known time periods.

The former protocol was used to establish the rates of input from myogenic cells over the periods of growth in the immediate postnatal period, in the juvenile growth period in normal wild-type mice and the initial period of muscle repair and regeneration in different strains of dystrophic mouse. It showed that the contribution of satellite cells to muscle growth during the 3-4 week postnatal period was closely comparable in dystrophic *mdx* and in non-dystrophic wild-type mice. It also showed, unexpectedly, that the addition of new myonuclei to normal muscle continued at a slow rate, during growth up to 14 weeks of age and that in the *mdx* mice, myonuclei were added to each fibre at twice this rate to achieve a doubling of myonuclei per unit volume.

The BrdU labelling investigations showed that, during the early phase of the disease, *mdx* mouse muscle undergoes a progressive replacement of some 5-10% of its muscle fibres every 3 days, and fully regenerates each necrotic lesion on the basis of 3 days of myogenic cell proliferation. This measure of myogenesis demonstrates a clear distinction between the very rapid and complete repair of muscle lesions in the C57Bl-mdx mouse from a barely detectable repair process in the DBA/2J-mdx mouse. The latter dystrophic mouse model also manifests a severe widely distributed calcification of areas of necrosis that are eventually

resolved, accompanied by an early onset of endomysial fibrosis. These features make the DBA-mdx mouse an interesting new animal model in which to analyze inhibition of myogenic repair and accompanied by calcification and fibrosis.

Cell transplant models: Morgan

Morgan reviewed pre-clinical work on freshly-isolated satellite cells derived from either normal, *mdx* or Large^{myd} mice (models of DMD and dystroglycanopathy respectively). When transplanted into pre-irradiated muscles of immunodeficient *mdx* mice, freshly isolated donor satellite cells behave as stem cells, contributing to muscle regeneration and functionally reconstituting the satellite cell pool. Although satellite cell numbers decline over the lifespan and the vast majority are activated in growing, 2-week old muscles [25], donor satellite cells derived from mice from 2 weeks to 2 years of age engraft equally well when transplanted into young immunodeficient *mdx* irradiated muscles. The host muscle environment has a profound effect on donor satellite cell-mediated muscle regeneration, with irradiation being the only host muscle pre-treatment that mediated significant engraftment [25]. The environment in which satellite cells reside has a profound effect on their performance, but when removed from the pathological environment, satellite cells from Large^{myd} mice function well *in vitro* [4] and satellite cells derived from aged *mdx* mice contribute as well to functional satellite cells as do satellite cells derived from young adult normal mice [3].

Drosophila models: Chaturvedi.

Drosophila have recently become a viable model for studying muscle repair with the demonstration of satellite cells in adult muscles. Their mononucleate morphology, adjacency to muscle fibers, proliferation upon physical trauma, fusion of their progeny with damaged muscles, and lineage shared with adult mature muscle, motivate further inquiry into their function. Specifically, their role in muscle repair remains to be assessed through i) satellite cell specific ablation and ii) satellite cell transplantation experiments. Notably, the transcription

factor Zfh1 is the only identified marker of satellite cells in *Drosophila*. Interestingly, recent work has documented the expression of Zfh1 in mouse satellite cells and evidenced that this gene is required for muscle regeneration [26]. Expression of the fly homolog of the mammalian satellite cell marker Pax7, has not been reported in *Drosophila*. A rigorous molecular comparison and contrast with mammalian satellite cells will become possible through transcriptomic analyses.

To examine changes in morphology that may result from physical damage or dystrophy, Chaturvedi has standardised a protocol to visualise adult *Drosophila* muscles in situ in 3-dimension, through MicroCT scanning. Quantitation of these images have revealed definite and consistent differences in muscle morphology within, and between, male and female *Drosophila* with age.

At this workshop, many aspects human myopathies, specifically mutations in dystrophy patients were highlighted. Further, aspects of *Drosophila* muscle anatomy that need further exploration for an accurate comparison with human muscular dystrophies became clear. Taken together, the identification of adult satellite cells in *Drosophila*, the new technical ability to accurately measure morphology with the plethora with in vivo genetic perturbation techniques, have all positioned *Drosophila* as a good *in vivo* model system for human myopathy and muscle repair.

Zebrafish models: Wood

Wood presented work on three aspects of neuromuscular disease research using zebrafish as an animal model. Firstly he discussed that the dystrophin-associated glycoprotein complex (DGC) is highly conserved in zebrafish. The small tropical fresh water zebrafish has been used in muscular dystrophy research since 2003 when the DMD fish *sapje* was first characterised. Since this time, the most common forms of muscular dystrophy have been identified from ENU mutagenic screens or modelled with CRISPRs, TALENs and ZFNs, the field has moved away from antisense morpholino technology despite several well controlled studies [27]. A point of interest was that fish do not have central nucleation on pathology with

the exception of fish models of laminopathies. Briefly discussed was the fact that FSHD is a particularly problematic muscular dystrophy to model in fish.

Secondly he highlighted that transgenic muscle stem cell and progenitor lines exist. The Tol2 system has been used to create Tg(pax3a:EGFP), TgBAC(pax7a:EGFP), Tg(met:mCherry-2A-KALTA4), and Tg(myf5:EGFP) myf5 lines. Zebrafish line Tg(ubb:LOX2272-LOXP-RFP-LOX2272-CFP-LOXP-YFP) crossed onto a tamoxifen inducible mesogenin cre Tg(mgcn1:CreERT2) line can be used to show in the fish model normal cell division in muscle is asymmetric but in regenerating fiber there is symmetric division at the injury site [28], in confirmation of the work on Prof. Terry Partridge's mouse model presented earlier in the session. Currently not many DGC lines have been crossed onto these transgenic lines, despite general enthusiasm, due to the time consuming nature of these experiments, that would require additional grant funding. This study would establish overall stem cell contribution to muscular dystrophy pathology and highlight novelties between muscular dystrophy models.

Lastly he had a case for fish being a good model to study fiber detachment in muscular dystrophies. Due to optical transparency and *ex-utero* development it is possible to study basement development in various muscular dystrophy fish models, CMD models are particularly well suited to the zebrafish system. This may be critical in understanding basement membrane failure in fiber detachment and fibrosis. It was noted that Evans blue dye is particularly effective to investigate sarcolemma integrity. Physiology measurements such as passive and active force are achievable in fish models [29]. A question arose around whether it is possible to exacerbate the phenotype. Four options were identified: cellulose, electroshock and caffeine in larvae, in adult a swim tunnel can be employed. Zebrafish can measure key functional swimming parameters. A final point was made around understanding the role tenocytes play in fiber stability and cross talk with muscle progenitor cells and how little we know about this cell types contribution to muscular dystrophy.

4. Extracellular Matrix and Fibroblast influence on Satellite cell activity

Collagen VI as a key extracellular regulator of muscle homeostasis and regeneration: Bonaldo

Collagen VI (COL6) is an extracellular matrix protein with a unique structural organization and playing a remarkably broad range of functions in several tissues, including skeletal muscle. Although COL6 expression in muscles is mainly provided by interstitial fibroblasts, its proper deposition is critical for myofiber homeostasis, and mutations of COL6 genes are causative for human congenital myopathies such as Bethlem myopathy and Ullrich congenital muscular dystrophy. In the last two decades, a number of studies in COL6 null (*Col6a1*^{-/-}) mice have shed light into the pathophysiological mechanisms of COL6-related disorders. COL6 deficiency affects key intracellular pathways, leading to defective regulation of autophagy, accumulation of dysfunctional organelles and spontaneous apoptosis [30, 31].

Based on the close contact of COL6 microfibrillar network with myofiber sarcolemma and its distinctive deposition in muscle endomysium, further studies revealed that this matrix molecule plays a pivotal role for satellite cells. Indeed, COL6 is a component of the satellite cell niche, which is expressed by quiescent satellite cells in a regulated manner and is required for the proper self-renewal of satellite cells both in physiological conditions and during muscle regeneration. *In vivo* and *in vitro* studies in *Col6a1*^{-/-} mice demonstrated that COL6 is involved in the fine regulation of the biomechanical properties of skeletal muscle, and this is one mechanism through which COL6 influences the maintenance of satellite cell stemness [5]. In unpublished work with myoblast and satellite cell cultures, Bonaldo's team found that treatment with soluble COL6 is able to counteract myogenic differentiation and increase the incidence of Pax7-positive cells differentiation, pointing at a biochemical effect on signaling pathways. Altogether, these data indicate that COL6 has a dual function for skeletal muscle on one hand it is critical for the regulation of the mechanical properties of muscle, and on the other hand it transduces specific signals with satellite cells.

Separate work revealed that COL6 regulates macrophage recruitment and polarization. In unpublished studies, Bonaldo reported that COL6 ablation impairs macrophage recruitment and M2 polarization after muscle injury, pointing at a further independent role of this matrix protein in muscle regeneration.

These findings strengthen the concept that COL6 plays multiple functions for skeletal muscles, and besides influencing myofiber homeostasis and regulating satellite cell activities it also plays a critical role in the inflammatory process and tissue repair during muscle regeneration. A major point arising during the discussion of this presentation was indeed related to the scarce consideration thus far given to macrophages in muscle regenerative processes both in physiological and pathological conditions, and the need to increase our knowledge about the involvement of inflammatory cells in the pathology of muscular dystrophies.

Laminins: Ruegg

LAMA2-related Muscular Dystrophy (*LAMA2* muscular dystrophy) or merosin-deficient Congenital Muscular Dystrophy 1A (MDC1A) is a severe, early onset and fatal muscular dystrophy that is caused by mutations in the gene coding for laminin- $\alpha 2$. Muscles of *LAMA2* muscular dystrophy patients undergo severe muscle degeneration and show a high degree of fibrosis. Laminins are a family of heterotrimeric molecules that are major components of the extracellular matrix. Laminin- $\alpha 2$ is mainly found in the basement membrane of the skeletal muscle and in the endoneurium of the peripheral nerve. The lack of laminin- $\alpha 2$ results in the compensatory expression of the developmental laminin- $\alpha 4$. Biochemical studies have shown that laminin- $\alpha 4$ lacks the binding to the laminin- $\alpha 2$ receptors, such as α -dystroglycan and $\alpha 7$ integrin. In addition, laminin- $\alpha 4$, in contrast to laminin- $\alpha 2$, does not self-polymerize. Two specifically designed linker proteins can alleviate both of these shortcomings of laminin- $\alpha 4$. One linker, termed mini-agrin, binds to the coiled-coil region of laminin and to α -dystroglycan. The second linker, a chimera between the LN domain of laminin- $\alpha 1$ and the laminin-binding domain of nidogen, allows self-polymerization. When expressed in skeletal muscle fibers, both linkers are incorporated into the muscle basement membrane and confer stable association of laminin- $\alpha 4$ with the basement membrane and the binding to the plasma membrane of the muscle fibers. As a consequence, the two linkers improve the health of *LAMA2* mice to a large degree as shown in recent proof-of-principle studies in mice [32, 33]. Another feature of the muscle of *LAMA2* muscular dystrophy patients is a strong impairment in muscle regeneration

despite the high extent of muscle degeneration. This raises the question whether this regeneration deficit is based on cell-intrinsic alterations of muscle satellite cells to regenerate or on changes in the environment. Interestingly, the two linkers although not expressed in muscle satellite cells, greatly improve cardiotoxin-induced muscle regeneration in *LAMA2* muscular dystrophy, suggesting rather extracellular cues being important to improve regeneration. In summary, the presented experiments are strong evidence that *LAMA2* muscular dystrophy is based on the failure to properly assemble muscle basement membrane and to connect it to the muscle fiber membrane. The fact that the cDNAs encoding the two linkers are small enough to be incorporated into AAV vectors, make it possible to translate this strategy into the clinic.

5. Regulation of satellite cells in development, postnatally and in neuromuscular diseases

Oscillations of MyoD and Hes1 proteins regulate the maintenance of activated muscle stem cells: Birchmeier

The balance between proliferation and differentiation of muscle stem cells is tightly controlled, ensuring the maintenance of a cellular pool needed for muscle growth and repair. Notch signaling provide important cues that regulate this balance. In mice, mutation of components of the Notch signaling pathway result in deficits in muscle growth, a loss of the muscle stem cell pool and severe regeneration deficits [34] [35, 36]. In humans, mutation of the *POGLUT1* (protein O-glucosyltransferase 1) gene which encodes an enzyme involved in posttranslational Notch modification, causes an autosomal recessive limb-girdle muscular dystrophy.

Birchmeier showed during her presentation that the direct Notch target gene *Hes1* controls the balance between proliferation and differentiation of activated muscle stem cells in both, developing and regenerating muscle of mice. She showed that *Hes1* is expressed in an oscillatory manner in activated stem cells where it drives the oscillatory expression of *MyoD*. *MyoD* expression oscillates in activated muscle stem cells from postnatal and adult muscle under various conditions: when the stem cells are dispersed in culture, when they remain associated with single muscle fibers, or when they reside in muscle biopsies. When myogenic

cells differentiate, MyoD oscillations become unstable and instead long periods of sustained MyoD expression are observed. Similarly, when stable MyoD oscillations are experimentally disturbed, for instance by ablation of the Hes1 oscillator, activated muscle stem cells differentiated with higher probability. This precocious differentiation interfered with the maintenance of the muscle stem cell pool, and impaired muscle growth and repair. Analysis indicates that the oscillatory expression of MyoD allows activated myogenic stem cells to remain in a proliferative state. However, when MyoD oscillations become unstable and are replaced by sustained MyoD expression, cells are driven out of the proliferating state and differentiate [37]. Thus, oscillatory MyoD expression allows for the amplification of the activated stem cell pool to ensure correct muscle growth and regeneration. Birchmeier investigated a mechanism that causes muscular dystrophy when disturbed in humans (i.e. aberrant Notch signaling), and demonstrates the importance of the balanced proliferation/differentiation of muscle stem cells in muscle growth and repair.

Postnatal regulation of satellite cells: Relaix

In recent years, it has been established that quiescence is not a dormant state - as previously thought - but rather a dynamic one, that is maintained by the activity of several different pathways. Stem cells in most adult tissues are sustained quiescent under homeostatic conditions by the combination of factors that are provided by the specialised microenvironment, also known as niche. Identifying the diverse factors of the niche and their modes of interaction is a major challenge and a prerequisite for the use of muscle stem cells in regenerative medicine. One major obstacle for the study of quiescent cells is that any perturbation of the niche triggers a fast activation, which is manifested by swift transcriptional and epigenetic changes. To overcome this, Relaix has developed a novel protocol that permits the isolation of quiescent satellite cells in their *in vivo* state. Based on this new approach, we have performed a series of high throughput and single cells experiments that identify the major players of the quiescence and activation networks, including in preclinical models of neuromuscular disorders.

Several new approaches to the treatment of DMD are on the pathway to approval and development. A critical step is first determining efficacy or safety by using an animal model of the disease. These are most useful in preclinical study when they recapitulate the clinical condition as observed in human patients. Indeed, dissimilarities between the disease in humans and animals may be one cause of the high failure rate, where therapies appear to improve a condition in the model, but show poor clinical efficacy. Relaix therefore generated a rat model (R-DMDdel52) where the deletion mimics those found in a significant portion of patients, followed by a rigorous characterisation of the phenotype. Overall, Relaix observed a severe and progressive disease, in keeping with what is seen in patients affected by DMD, and death around one year of age associated with loss of muscle mass. R-DMDdel52 rats display dystrophin-deficiency, associated with histopathological changes seen in severely affected DMD muscle, including increased fibre size variability with the presence of many very small and hypertrophic fibres, increased centronucleation, increased fibrosis and necrosis. Altogether, these results suggest that R-DMDdel52 is an interesting preclinical model for DMD.

Carey-Fineman-Ziter Syndrome: Bonneman

This syndrome was originally described in two siblings with bilateral facial weakness and Robin sequence (mandibular hypoplasia, hypoglossia, cleft palate), as well as mild proximal weakness with small muscles, delayed motor milestones, scoliosis, and normal intelligence. As part of an investigation into genetic causes for Moebius syndrome biallelic mutations in the gene *myomaker/TMEM8C* were identified in the original family as well as in four additional families with a total of 8 patients [6]. The clinical phenotype was fairly consistent with prominent congenital bifacial weakness but without prominent ophthalmoplegia, prominent axial weakness and milder involvement of extremity muscles. Muscle biopsy analysis was consistent with fiber type disproportion with small type I fibers and unusually large type II fibers. Myomaker has been shown to be critical for myoblast fusion, while germline inactivation in the mouse causes prenatal lethality with muscles consisting of unfused single nucleated myogenic cells. Notably, none of the human genotypes leads to a predicted

complete loss of myomaker, consistent with the notion that a complete loss of myomaker function would also not be compatible with postnatal human life. Myomaker is required on both cells involved in the fusion process, while the more recently discovered pore forming peptide myomixer/myomerger/minion is only required on one of the two fusing cells. While the knock out mouse model is histologically very similar to the myomaker knock out situation, no human equivalent condition has yet been described. Given that myomaker would also be predicted to be required for satellite cell fusion into the myofiber, Carey-Fineman-Ziter Syndrome can be conceptualized as a disorder of muscle satellite cells, with presumed pathogenic mechanisms in both development as well as in later skeletal muscle maintenance. Hypomorphic mouse models will be needed to investigate these important questions in detail. The selective involvement early on of facial and axial muscles is striking and raises additional interesting developmental questions.

Understanding satellite cell regenerative decline with aging: Muñoz-Cánoves

The regenerative function of satellite cells declines with aging. Results from Muñoz-Cánoves's group have shown that quiescent satellite cells have a constitutive autophagic activity that allows them to maintain the correct intracellular balance between synthesis and degradation of proteins and organelles [38]. This basal autophagy is essential to preserve satellite cell homeostasis and proliferative capacity to generate new muscle fibers upon tissue injury. Muñoz-Cánoves observed that autophagy is decreased in satellite cells of aged mice, affecting their internal cleansing process. As a result, they observed that the intracellular accumulation of damaged proteins and organelles such as mitochondria could not be eliminated correctly, triggering a switch from quiescence to a senescence-like state. Muñoz-Cánoves found that pharmacological or genetic restoration of autophagy in aged satellite cells allowed them to recover their regenerative capacity and escape senescence. Conversely, genetic inhibition of autophagy in quiescent young satellite cells caused senescence entry [39]. Since damaged mitochondria are one of the main sources of reactive oxygen species (ROS), the decrease in mitophagy activity associated with age led to an increase in oxidative stress. Treatment with antioxidants led to autophagy/mitophagy recovery *in vivo*, which in turn prevented the intracellular accumulation of

damaged components and entry into senescence, ultimately restoring the regenerative capacity of aged satellite cells. Of note, Muñoz-Cánoves found that a caloric restriction regime in old mice increased satellite cell regenerative functions, at least in part by promoting autophagy (unpublished results). In conclusion, they have shown that autophagy is essential to maintain "stemness". Their more recent results indicate that autophagic activity is also impeded in satellite cells of mdx dystrophic mice, correlating with increased cellular senescence (unpublished results). Therefore, enhancing the regenerative capacity of satellite cells through the pharmacological or dietary activation of autophagy, or reduction of senescence, opens the possibility to future regenerative therapies in the context of muscle regeneration problems associated with aging or muscular dystrophies.

Novel mouse models to study dystrophin in control and disease context: Helge Amthor

Dmd^{mdx} is a frequently used mouse model for DMD and dystrophin-restoring therapies.

Amthor made a strong case that current translational research is methodologically limited by antibody-mediated signal amplification to visualize dystrophin. Amthor shared unpublished data on *Dmd^{EGFP}* carrying a C-terminal EGFP-tag and a *Dmd^{EGFP-mdx}* reporter mouse line carrying additionally in *cis* the *mdx-23* mutation, which were generated in collaboration with the laboratory of Markus Schuelke in Berlin. These novel models facilitate the study of dystrophin expression/restoration not only at sarcolemmal level, but also in neuromuscular junction, myotendinous junction and muscle stem cells, which are central components of skeletal muscle and required for its excitation, force transmission and regeneration. Helge Amthor argued that dystrophin restoration at critical sites of expression is required for therapies to be effective. However, current standards of dystrophin quantification in preclinical models and those from clinical research studies may only be partially addressing this issue. Novel methods are required to improve imaging of dystrophin restoration not only at the sarcolemma but also other key sites listed to better predict benefits of a therapy.

Single cell atlas of aged and dystrophic muscle: Giordani

Lorenzo Giordani attended the workshop as a special young scientist delegate. He highlighted that skeletal muscle repair requires the activation of resident stem/progenitor cells (satellite cells) that can both self-renew and generate differentiated progeny. To correctly execute the regenerative program, satellite cells interact with their microenvironment and require signals from neighboring cells. However, in pathologic contexts such as degenerative myopathies (e.g. DMD) the efficiency of this process progressively declines leading ultimately to incomplete repair, fat infiltration and fibrotic scarring. Understanding the cellular mechanisms behind regeneration impairment is pivotal to the development of novel strategies aimed at slowing down and counteract disease progression. Standard bulk-scale methods had been proven unfit to address these questions since are unable to correctly render the heterogeneity within the populations involved in tissue repair. As a result, defective disease-specific subpopulations remain most of the time undetected. Hence the need to apply a single-cell approach to identify those dysfunctional subsets.

Giordani presented a two-step strategy to study the different resident populations in skeletal muscles and identify those potentially involved in the regeneration impairment. First single-cell transcriptomics and mass cytometry (CyTOF) analyses have been used to attain a clearer understanding of the cellular composition of adult skeletal muscle, leading to the identification of muscle resident tenocyte-like cells (SCX+) as well as a non-multipotent myogenic subset of mesenchymal helper cells. Then, using different algorithms (SPADE, Statistical Scaffold) Giordani overlaid the initial single cell blueprint on both the dystrophic (MDX^{4cv}) and the aged condition. This revealed functional similarities and highlight recurring patterns. Lastly, as proof of concept he showed some preliminary data on CyTOF on human biopsies. The data presented demonstrate that this approach could be successfully applied to any tissue. In particular, in pathologic conditions, it could serve to precisely identify dysfunctional subfractions [40].

This two-step approach could be used to study the specific role of satellite cells and other skeletal muscle population in the pathology of muscular dystrophy. Presenting single cell

technologies (such as mass cytometry) to clinicians could be instrumental to build a collaborative network to successfully apply this approach to different specific muscular dystrophies.

6. Funder's and Patient perspective

Funder's perspective: Stevenson

Alison Stevenson, Senior Grants Manager at Muscular Dystrophy UK, spoke about the charity's work for people with neuromuscular conditions, its research strategy and funding opportunities.

Muscular Dystrophy UK is the UK's largest charity for people with muscular dystrophy and related neuromuscular conditions, and has an annual income of over £7million. Approximately 70,000 people in the UK are affected by muscular dystrophy or a related neuromuscular condition.

The charity provides information, advocacy and other support to enable individuals affected by these conditions to live as independently as possible, because for people living with a muscle-wasting condition every day counts. Muscular Dystrophy UK's recent strategy outlines four priority areas of working for the coming three years: Fast Track to treatments, mental health matters, advances in technology, sport, leisure and exercise.

The charity also funds research into the causes and potential treatments for these conditions, from basic to preclinical to clinical studies. Funding for research projects is primarily for UK researchers and is awarded through a rigorous, competitive process involving peer review and assessment by the charity's Medical Research Committee and Lay Research Panel. Lay summaries must be provided by applicants and are scored by the Lay Research Panel members, making this a crucial part of the selection process.

Alison finished by reflecting on the questions raised by members of the Lay Research Panel around stem cells in muscular dystrophy. Their comments focussed on the need and desire for a potential treatment, and questions were raised about how this would work. For example, what would a stem cell treatment look like, what would the source of stem cells be, how often would a treatment be administered and how would a balance be reached between the need for immune suppression and the risk of infection? Other considerations were: who would be eligible (treatments should be inclusive of children) and how to ensure a quick route to market. Alison acknowledged that much research is currently at a basic level, rather than developing stem cells as potential treatments, but noted it would be important to bear these comments and questions in mind for the workshop discussions and for future research.

Patient's perspective: Davenport

Richard Davenport explored some personal reflections on the effects of muscular dystrophy on his life, pain, rehab and the many changes it brings. He suggested that self-image, our perceptions of ourselves and how we fit into the world, can be really influential in managing the condition but also challenged by those changes. So much so, that bigger physical changes leave our self-image with a gap in meaning and a certain amount of reinvention and resocialisation takes place to fill it. Because life with muscular dystrophy is relentless change and adaption, regular focused counselling, from diagnosis, could greatly assist patients in proactively managing these sometimes existential challenges.

Aside from the individual-focused changes and challenges, Richard also talked about how access becomes critical but the prevalent attitude to access is one of ineffective apology or flat nonchalance. These sorts of "social model" issues become a daily grind as much as the physical struggles.

7. Final remarks

This workshop has shed light on stem cell involvement in the pathogenesis of muscular dystrophies and congenital myopathies, but we need to gain a greater understanding of:

- The involvement of satellite cells in experimental models and humans, particularly the importance of rigorously evaluating all *in vitro* data in the context of what actually occurs *in vivo*.
- The fate and biology of satellite cells during ageing and in disease. For old muscles, a large body of data indicate no major intrinsic problem in myogenic capacity of satellite cells, especially *in vivo*: this is controversial. However, major age-related alterations to the inflammatory cell response are well documented, with adverse effects on the kinetics of regeneration after experimental injury of old muscles. Furthermore, there appears to be minimal incidence of necrosis/ regeneration in healthy old human and mouse muscles (and thus no ongoing myogenesis and regenerating host myofibres to fuse with donor myoblasts in any proposed stem cell therapy).
- The role of satellite cells in development of muscle pathology in DMD, Limb Girdle Muscular Dystrophy and other muscular dystrophies. Much data indicate no major intrinsic myogenic stem (satellite) cell problem *in vivo* for DMD and LGMD2B. The controversy over stem cell deficits in DMD may be due to differences in *in vivo* and *in vitro* environments.
- The impact of metabolism on the muscle repair programme. The work presented here showed that reducing organismal energy levels promotes satellite cell function in mouse models of DMD, whereas other previous work has documented the benefits of high fat diets in supporting muscle fibre repair.
- The evidence of defective satellite cells as primary cause of EMARDD and CFZ Syndrome.
- The efficacy of therapeutic strategies to correct satellite cell dysfunction in muscular dystrophies. For this to be effective, any intrinsic problem with satellite cells in dystrophic muscles *in vivo* must be fully understood. Major problems are well-documented for stem cell transplantation therapy for DMD. For DMD, we may need to target the extracellular matrix (fibrosis) to improve regeneration. Preventing necrosis in DMD may be another effective approach. The possibility of correcting specific intrinsic

defects in satellite cells in either DMD or some of the congenital muscular dystrophies are future possibilities.

Participants:

Helge Amthor (Paris, France), Carmen Birchmeier (Berlin, Germany), Paolo Bonaldo (Padova, Italy), Carsten Bönnemann (Bethesda, USA), Gillian Butler Browne (Paris, France), Dhananjay Chaturvedi (Bangalore, India), Richard Davenport (Portsmouth, UK), Ana Ferreira (Paris, France), Denis Furling (Paris, France), Lorenzo Giordani (Paris, France), Miranda Grounds (Perth, Australia), Heinz Jungbluth (London, UK), Pura Manoz Canoves (Barcelona and Madrid, Spain), Prasant Mishra (Dallas, USA), Jennifer Morgan (London, UK), Francesco Muntoni (London, UK), George Padberg (Nijmegen, The Netherlands), Carmen Paradas (Seville, Spain), Terry Partridge (Washington, USA), Ketan Patel (Reading, UK), Frederic Relaix (Paris, France), Markus Rüegg (Basel, Switzerland), Alison Stevenson (London, UK), Maaïke Van Putten (Leiden, The Netherlands), Alasdair Wood (Monash, Australia), Peter Zammit (London, UK).

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References

- [1] Morgan JE, Zammit PS. Direct effects of the pathogenic mutation on satellite cell function in muscular dystrophy. *Experimental Cell Research*. 2010;316:3100-8.
- [2] Dumont NA, Wang YX, von Maltzahn J, Pasut A, Bentzinger CF, Brun CE, et al. Dystrophin expression in muscle stem cells regulates their polarity and asymmetric division. *Nat Med*. 2015;21:1455-63.
- [3] Boldrin L, Zammit PS, Morgan JE. Satellite cells from dystrophic muscle retain regenerative capacity. *Stem Cell Res*. 2015;14:20-9.
- [4] Ross J, Benn A, Jonuschies J, Boldrin L, Muntoni F, Hewitt JE, et al. Defects in Glycosylation Impair Satellite Stem Cell Function and Niche Composition in the Muscles of the Dystrophic Large(myd) Mouse. *Stem cells*. 2012.
- [5] Urciuolo A, Quarta M, Morbidoni V, Gattazzo F, Molon S, Grumati P, et al. Collagen VI regulates satellite cell self-renewal and muscle regeneration. *Nat Commun*. 2013;4:1964.
- [6] Di Gioia SA, Connors S, Matsunami N, Cannavino J, Rose MF, Gilette NM, et al. A defect in myoblast fusion underlies Carey-Fineman-Ziter syndrome. *Nat Commun*. 2017;8:16077.
- [7] Logan CV, Lucke B, Pottinger C, Abdelhamed ZA, Parry DA, Szymanska K, et al. Mutations in MEGF10, a regulator of satellite cell myogenesis, cause early onset myopathy, areflexia, respiratory distress and dysphagia (EMARDD). *Nat Genet*. 2011;43:1189-92.
- [8] Grounds MD. Obstacles and challenges for tissue engineering and regenerative medicine: Australian nuances. *Clin Exp Pharmacol Physiol*. 2018;45:390-400.
- [9] Grounds MD. The need to more precisely define aspects of skeletal muscle regeneration. *Int J Biochem Cell Biol*. 2014;56:56-65.
- [10] Bigot A, Klein AF, Gasnier E, Jacquemin V, Ravassard P, Butler-Browne G, et al. Large CTG repeats trigger p16-dependent premature senescence in myotonic dystrophy type 1 muscle precursor cells. *Am J Pathol*. 2009;174:1435-42.
- [11] Lemmers RJ, van der Vliet PJ, Klooster R, Sacconi S, Camano P, Dauwerse JG, et al. A unifying genetic model for facioscapulohumeral muscular dystrophy. *Science*. 2010;329:1650-3.
- [12] Dixit M, Ansseau E, Tassin A, Winokur S, Shi R, Qian H, et al. DUX4, a candidate gene of facioscapulohumeral muscular dystrophy, encodes a transcriptional activator of PITX1. *Proc Natl Acad Sci U S A*. 2007;104:18157-62.
- [13] Banerji CRS, Panamarova M, Hebaishi H, White RB, Relaix F, Severini S, et al. PAX7 target genes are globally repressed in facioscapulohumeral muscular dystrophy skeletal muscle. *Nat Commun*. 2017;8:2152.
- [14] Banerji CRS, Zammit PS. PAX7 target gene repression is a superior FSHD biomarker than DUX4 target gene activation, associating with pathological severity and identifying FSHD at the single-cell level. *Hum Mol Genet*. 2019.
- [15] Brais B, Bouchard JP, Xie YG, Rochefort DL, Chretien N, Tome FM, et al. Short GCG expansions in the PABP2 gene cause oculopharyngeal muscular dystrophy. *Nat Genet*. 1998;18:164-7.

- [16] Servian-Morilla E, Takeuchi H, Lee TV, Clarimon J, Mavillard F, Area-Gomez E, et al. A POGlut1 mutation causes a muscular dystrophy with reduced Notch signaling and satellite cell loss. *EMBO Mol Med*. 2016;8:1289-309.
- [17] Goddeeris MM, Wu B, Venzke D, Yoshida-Moriguchi T, Saito F, Matsumura K, et al. LARGE glycans on dystroglycan function as a tunable matrix scaffold to prevent dystrophy. *Nature*. 2013;503:136-40.
- [18] Boyden SE, Mahoney LJ, Kawahara G, Myers JA, Mitsuhashi S, Estrella EA, et al. Mutations in the satellite cell gene MEGF10 cause a recessive congenital myopathy with minicores. *Neurogenetics*. 2012;13:115-24.
- [19] Castets P, Bertrand AT, Beuvin M, Ferry A, Le Grand F, Castets M, et al. Satellite cell loss and impaired muscle regeneration in selenoprotein N deficiency. *Hum Mol Genet*. 2011;20:694-704.
- [20] Lawlor MW, Alexander MS, Viola MG, Meng H, Joubert R, Gupta V, et al. Myotubularin-deficient myoblasts display increased apoptosis, delayed proliferation, and poor cell engraftment. *Am J Pathol*. 2012;181:961-8.
- [21] Heydemann A, Ceco E, Lim JE, Hadhazy M, Ryder P, Moran JL, et al. Latent TGF-beta-binding protein 4 modifies muscular dystrophy in mice. *J Clin Invest*. 2009;119:3703-12.
- [22] Fukada S, Morikawa D, Yamamoto Y, Yoshida T, Sumie N, Yamaguchi M, et al. Genetic background affects properties of satellite cells and mdx phenotypes. *Am J Pathol*. 2010;176:2414-24.
- [23] van Putten M, Putker K, Overzier M, Adamzek WA, Pasteuning-Vuhman S, Plomp JJ, et al. Natural disease history of the D2-mdx mouse model for Duchenne muscular dystrophy. *FASEB J*. 2019:fj201802488R.
- [24] Pasteuning-Vuhman S, Putker K, Tanganyika-de Winter CL, Boertje-van der Meulen JW, van Vliet L, Overzier M, et al. Natural disease history of mouse models for limb girdle muscular dystrophy types 2D and 2F. *PLoS One*. 2017;12:e0182704.
- [25] Boldrin L, Neal A, Zammit PS, Muntoni F, Morgan JE. Donor Satellite Cell Engraftment is Significantly Augmented When the Host Niche is Preserved and Endogenous Satellite Cells are Incapacitated. *Stem cells*. 2012.
- [26] Siles L, Ninfali C, Cortes M, Darling DS, Postigo A. ZEB1 protects skeletal muscle from damage and is required for its regeneration. *Nat Commun*. 2019;10:1364.
- [27] Sztal TE, Sonntag C, Hall TE, Currie PD. Epistatic dissection of laminin-receptor interactions in dystrophic zebrafish muscle. *Hum Mol Genet*. 2012;21:4718-31.
- [28] Gurevich DB, Nguyen PD, Siegel AL, Ehrlich OV, Sonntag C, Phan JM, et al. Asymmetric division of clonal muscle stem cells coordinates muscle regeneration in vivo. *Science*. 2016;353:aad9969.
- [29] Wood AJ, Cohen N, Joshi V, Li M, Costin A, Hersey L, et al. RGD inhibition of itgb1 ameliorates laminin-a2 deficient zebrafish fibre pathology. *Hum Mol Genet*. 2018.
- [30] Irwin WA, Bergamin N, Sabatelli P, Reggiani C, Megighian A, Merlini L, et al. Mitochondrial dysfunction and apoptosis in myopathic mice with collagen VI deficiency. *Nat Genet*. 2003;35:367-71.
- [31] Grumati P, Coletto L, Sabatelli P, Cescon M, Angelin A, Bertaggia E, et al. Autophagy is defective in collagen VI muscular dystrophies, and its reactivation rescues myofiber degeneration. *Nat Med*. 2010;16:1313-20.
- [32] McKee KK, Crosson SC, Meinen S, Reinhard JR, Ruegg MA, Yurchenco PD. Chimeric protein repair of laminin polymerization ameliorates muscular dystrophy phenotype. *J Clin Invest*. 2017;127:1075-89.

- [33] Reinhard JR, Lin S, McKee KK, Meinen S, Crosson SC, Sury M, et al. Linker proteins restore basement membrane and correct LAMA2-related muscular dystrophy in mice. *Sci Transl Med*. 2017;9.
- [34] Bjornson CR, Cheung TH, Liu L, Tripathi PV, Steeper KM, Rando TA. Notch signaling is necessary to maintain quiescence in adult muscle stem cells. *Stem Cells*. 2012;30:232-42.
- [35] Brohl D, Vasyutina E, Czajkowski MT, Griger J, Rassek C, Rahn HP, et al. Colonization of the satellite cell niche by skeletal muscle progenitor cells depends on Notch signals. *Dev Cell*. 2012;23:469-81.
- [36] Mourikis P, Sambasivan R, Castel D, Rocheteau P, Bizzarro V, Tajbakhsh S. A critical requirement for notch signaling in maintenance of the quiescent skeletal muscle stem cell state. *Stem Cells*. 2012;30:243-52.
- [37] Lahmann I, Brohl D, Zyrianova T, Isomura A, Czajkowski MT, Kapoor V, et al. Oscillations of MyoD and Hes1 proteins regulate the maintenance of activated muscle stem cells. *Genes Dev*. 2019;33:524-35.
- [38] Garcia-Prat L, Martinez-Vicente M, Perdiguero E, Ortet L, Rodriguez-Ubreva J, Rebollo E, et al. Autophagy maintains stemness by preventing senescence. *Nature*. 2016;529:37-42.
- [39] Sousa-Victor P, Gutarra S, Garcia-Prat L, Rodriguez-Ubreva J, Ortet L, Ruiz-Bonilla V, et al. Geriatric muscle stem cells switch reversible quiescence into senescence. *Nature*. 2014;506:316-21.
- [40] Giordani L, He GJ, Negroni E, Sakai H, Law JYC, Siu MM, et al. High-Dimensional Single-Cell Cartography Reveals Novel Skeletal Muscle-Resident Cell Populations. *Mol Cell*. 2019;74:609-21 e6.