Gene edited human muscle stem cells as Advanced Therapies

Prof. Dr. Simone Spuler

Frontiers in Translational Medicine _ Scientific and Structural Challenges _ 23 April 2021
Muscle Research Unit
https://www.mdc-berlin.de/spuler

Patient care

2500 patients in Charité muscle outpatient clinic

Diagnosis and follow-up

Supportive care

Clinical trials

We want muscular dystrophies to become treatable

Translational Research

Human muscle stem cells

ATMP—new therapies

Muscular dystrophy

Gene editing

MYOPAX

SUMUS
Muscular dystrophies

50 different **monogenic progressive** disorders

Incidence 30/100.000

No treatment
Stefano Biessi,1,2 Antonio Filareto,3 and Thomas A. Rando4,5,6

Muscular dystrophies are a heterogeneous group of genetic diseases, characterized by progressive degeneration of skeletal and cardiac muscle. Despite the intense investigation of different therapeutic options, a definitive treatment has not been developed for this debilitating class of pathologies. Cell-based therapies in muscular dystrophies have been pursued experimentally for the last three decades.
It is the right time!

The Nobel Prize in Chemistry 2020

Development of a method for genome editing

Emmanuelle Charpentier
Max Planck Unit for the Science of Pathogens, Berlin, Germany

Jennifer A. Doudna
Howard Hughes Medical Center
University of California, Berkeley, USA
SATELLITE CELL OF SKELETAL MUSCLE FIBERS

ALEXANDER MAURO. From The Rockefeller Institute

In the course of an electron microscopic study of the peripheral region of the skeletal muscle fiber of the frog, the presence of certain cells, intimately associated with the muscle fiber, have been observed which we have chosen to call satellite cells. Since these cells have not been reported previously and indeed might be of interest to students of muscle histology and furthermore, as we shall suggest, might be pertinent to the vexing problem of skeletal muscle regeneration, a brief communication describing this finding is warranted prior to a more detailed study.

is that the peripheral muscle nuclei proper occur much more frequently than the satellite cells. It is interesting that upon alerting other investigators to these findings, similar cells have been found in electron micrographs of two other muscles of the frog, namely sartorius (2) and tibialis anterior, and of the sartorius and tongue muscle of the white rat (4). (Though the direct evidence is restricted to these two vertebrates, it seems reasonable to hazard a guess that skeletal muscle fibers in vertebrates in general contain satellite cells.)
Conversion of mdx myofibres from dystrophin-negative to -positive by injection of normal myoblasts

T. A. Partridge*, J. E. Morgan*, G. R. Coulton*, E. P. Hoffman† & L. M. Kunkel†

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† Division of Genetics, Children's Hospital, Pediatrics, Harvard Medical School and Howard Hughes Medical Institute, Boston, Massachusetts 02115, USA

An important corollary to the recent advances in our understanding
Failure of transplantation of myoblasts for therapeutic purposes

1995

MYOBLAST TRANSFER IN THE TREATMENT OF DUCHENNE’S MUSCULAR DYSTROPHY

## Cells with possible myogenic potential

<table>
<thead>
<tr>
<th>Cell</th>
<th>Characteristics</th>
<th>Systemic delivery</th>
<th>+</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Satellite cell</td>
<td>Under basal lamina, selfrenewal, Pax7+, CD56+, MyoD-</td>
<td>No</td>
<td>Regeneration and SC pool</td>
<td>No in vivo trials with human SCs, low numbers</td>
</tr>
<tr>
<td>MuSC</td>
<td>Integrin α7+, CD34+</td>
<td>?</td>
<td>Regeneration and SC pool</td>
<td>As Sat cells</td>
</tr>
<tr>
<td>SM Precursors</td>
<td>β1-integrin+, CXCR4+, CD45-, Sca1+, Mac1-</td>
<td>?</td>
<td>++ Regenerative potential, SC replenishing</td>
<td>As Sat cells</td>
</tr>
<tr>
<td>Myoblasts</td>
<td>After Sat cell activation, MyoD+, Desmin+, Myf5+</td>
<td>No</td>
<td>Easily isolated and expanded, many human trials</td>
<td>Not efficient in regeneration. Limited in vitro expansion</td>
</tr>
<tr>
<td>Mesangioblasts</td>
<td>Blood vessel wall, Flk1+, CD34+, Sca1+, vWF-</td>
<td>Yes</td>
<td>Easy to expand, human trial in progress</td>
<td>In vitro myogenesis requires myoblasts</td>
</tr>
<tr>
<td>Pericytes/ MDSCs</td>
<td>Periphery of blood vessel, NG2, proteoglycan, ALP, PDGFRβ, CD56-</td>
<td>Yes</td>
<td>Easy to expand</td>
<td>Variable, limited regenerative potential</td>
</tr>
<tr>
<td>SP cells</td>
<td>Sca1+, ABCG2+transporter, CD45-, CD43-, Pax7-, c-kit-</td>
<td>?</td>
<td>Can be isolated from different tissues</td>
<td>Must be cocultured with myoblasts</td>
</tr>
<tr>
<td>CD133+</td>
<td>Blood or muscle tissue</td>
<td>Yes</td>
<td>Muscle regeneration better than myoblasts</td>
<td>Efficient myogenesis needs myoblasts or Wnt7a+ cells</td>
</tr>
<tr>
<td>Embryonic stem cells</td>
<td>Derived from blastocyst</td>
<td>Yes</td>
<td>Pluripotency</td>
<td>Ethics, immune response, tumorigenic</td>
</tr>
<tr>
<td>IPS (Nobel prize 2012)</td>
<td>Can be obtained from many tissues. Oct3/4+, Sox-, c-Myc+, klf4+, Nanong+</td>
<td>Yes</td>
<td>Pluripotency</td>
<td>Genetic manipulation tumorigenicity, risk of viral infection</td>
</tr>
<tr>
<td>MSCs</td>
<td>Many tissues, CD34-, CD45-, CD73+, CD90+, CD105+, CD117+</td>
<td>Yes</td>
<td>Readily available, autologous</td>
<td>Delivery unclear, limited long-term therapeutic contribution</td>
</tr>
<tr>
<td>PW1-interstitial cells</td>
<td>Muscle interstitial cells. PW1+</td>
<td>?</td>
<td>Contribute to muscle regeneration, SC and interstitial population</td>
<td>No information on human cells</td>
</tr>
</tbody>
</table>
2011: No satellite cells  ❗ No muscle regeneration!

- Relaix F, Zammit PS. Satellite cells are essential for skeletal muscle regeneration: the cell on the edge returns centre stage. Development 139: 2845-2856; 2012
Characterization of satellite cells

Yin H et al. Physiol Rev 2013;93:23-67
Muscle regeneration depends on satellite cells
Pax7+ satellite cells enrich within the human myofiber fragment

- Human skeletal muscle biopsy specimen
- $5^3\text{mm}$

- Manual dissection into fiber fragments
- **No** enzymatic digest

Human muscle fiber fragment 7 days in culture
Enrichment of PAX7 positive cells within the fragment

Pax7
NCAM

Pax7
Desmin
10d
Myoblasts form colonies outside the fiber fragments and fuse to myotubes.
Proof: *Ex vivo* expanded human satellite cells regenerate muscle *in vivo*

Marg et al., *Nat Commun*, 2019

NSG mice

18 Gy

PAX7  Laminin  hu Lam A/C  Laminin  Hoechst
Our innovation: new isolation and cultivation technique for human muscle stem cells

- Muscle biopsy
- Manual dissection
- Hypothermia 5°C, 7 days
- Native oligoclonal cell colonies
- Long-term storage

- 100% myogenic cells
- High regenerative potential

- Marg et al., *Nature Communications*, 2019

Spuler; BIH Lecture; 23. April 2021
Advanced Therapy Medicinal Products (ATMP): young and growing market

### ATMPS IN THE EU MARKET

<table>
<thead>
<tr>
<th>ATMP classification</th>
<th>Product</th>
<th>Market approval EMA</th>
<th>Market approval FDA</th>
<th>Orphan design</th>
</tr>
</thead>
<tbody>
<tr>
<td>GMTP</td>
<td>Glybera®</td>
<td>2012-2017*</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Strimvelis®</td>
<td>2016</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kymriah®</td>
<td>2018</td>
<td>2018</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Yescarta®</td>
<td>2018</td>
<td>2017</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Imllytic®</td>
<td>2015</td>
<td>2015</td>
<td>X/-</td>
</tr>
<tr>
<td></td>
<td>Luxturna®</td>
<td>2018</td>
<td>2017</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Zyneglo®</td>
<td>2019</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>TEP (autologous)</td>
<td>Holoclar®</td>
<td>2015</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MACI®</td>
<td>2013-2014*</td>
<td>2016</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ChondroCelect®</td>
<td>2009-2016*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spherox®</td>
<td>2017</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sCTMP</td>
<td>Zalmoxis®</td>
<td>2016</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Provenge®</td>
<td>2013-2015*</td>
<td>2010</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alosifel®</td>
<td>2018</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

• *First approved ATMPs were not successful* on the EU market:
  - Difficulties in national pricing negotiations, competing products
• **Newer ATMPs** are primarily targeting **rare diseases** (orphan designation).

### FORECAST OF WORLDWIDE REVENUE FROM REGENERATIVE MEDICINE NEXT YEARS (IN BILLION €)


>1000 ATMPS CURRENTLY IN CLINICAL STUDIES
Our concept for developing treatments for muscular dystrophy
1st in human clinical trial using primary human muscle stem cells as ATMP

Indication: Epispadias

POC: yes, uncontrolled design
Kajbafzadeh, 2008, 2011

Preclinic: Supported by BIH/SPARK

Trial: Financed by BMBF (from 5.2021)

Timeframe: 2022-2025
Our concept for developing treatments for muscular dystrophy
„Gene therapy“ I: Exon-Skipping and Stopcodon-readthrough

X = Mutation

Promotor

Exon

Exon

Exon

Exon

DNA

splicing

mRNAs

Proteins
„Gene therapy“ II: Additional cDNA copy
„Gene therapy“ II: Additional cDNA copy

Sarepta Therapeutics: Muscular dystrophy trial for SGCB mutations
“Gene therapy“ III: Precise correction of mutation
Family with sarcoglycanopathy

Age at onset in both: 7-8
Wheelchair bound: 13-15 years of age
No cardiac involvement so far
Both mutations were previously reported

Fendri et al., 2006 *Neuromusc Disorders*

**p.Ala53Thr, missense**

Piccolo et al., 1995 *Nat Genet*

**Aberrant splicing**
95% pure myoblasts were obtained from the LGMD2D patient.
SGCA c.157G>A induces co-skipping of exons 2 and 3
Generation and characterization of patient iPSC

OCT4  Nanog  SOX2  SSEA4

+Ehochst

Ectoderm
Beginning rosette formation, beginning stratification

Mesoderm
Immature mesenchyme

Endoderm
Cuboidal vacuolated epithelial cells, lining cyst-like space
Genome editing with CRISPR/Cas systems

Kim et al., 2014 Nat Rev Gen
Adenine Base Editing (ABE) for precise A>G nucleotide conversions

Gaudelli et al., 2017 Nature
SGCA c.157G>A mutation is an ideal candidate for ABE
ABE repairs the SGCA c.157G>A mutation in patient iPSC
Highly efficient repair of SGCA c.157G>A in PHSats (primary human myoblasts)

No editing of predicted off-target sites
SGCA c.157G>rep express sarcoglycan, give rise to human myofibers *in vivo* and make new muscle stem cells 

NSG mice

16 Gy
Are we there?
Are we there?

No:
- Plasmid based
- Venus reporter
- No GMP
Transfection of primary muscle human stem cells with mRNA-GFP

Stadelmann, unpublished
Transfection of primary muscle human stem cells with ABE-mRNA plus sgRNA

Stadelmann, unpublished
Summary:
Translational Workflow for gene corrected primary human muscle stem cells

Patients

<table>
<thead>
<tr>
<th>Gender</th>
<th>Carrier</th>
<th>Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>♂</td>
<td>♂</td>
<td>♂</td>
</tr>
<tr>
<td>♀</td>
<td>♀</td>
<td>♀</td>
</tr>
</tbody>
</table>

Generation of primary human muscle stem cells

- Muscle biopsy
- Manual dissection
- Hypothermia 5°C, 7 days
- Native oligoclonal cell colonies
- Long-term storage

Translational impact

- Quantification of correction
- Off-target analysis
- Viability and functionality of corrected cells
- In vivo regenerative capacity

CRISPR/Cas9-derived genetic correction
What is next: *in vivo* editing

Coop: Ralf Kühn, MDC
Muscle Research Unit
https://www.mdc-berlin.de/spuler

We want muscular dystrophies to become treatable

Patient care
2500 patients in Charité muscle outpatient clinic
Diagnosis and follow-up
Supportive care
Clinical trials

Translational Research
Human muscle stem cells
ATMP—new therapies
Muscular dystrophy
Gene editing

Charité
Max-Delbrück-Centrum
Deutsches Krebsforschungszentrum
SGK Stiftung Gisela Krebs
Berlin Institute of Health
SPARK-BIH
Else Kröner Fresenius Stiftung
SUMUS
Thank you