Screening for muscle regenerative drugs using Pax7+ muscle progenitors derived by differentiation of human embryonic stem cells.

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1) Summary

Muscle wasting disorders that include muscular dystrophy, cachexia, disuse atrophy, aging and many others have different etiology but all feature excessive loss of muscle mass due to the systematic depletion of satellite stem cell population and decrease in muscle regenerative potential.

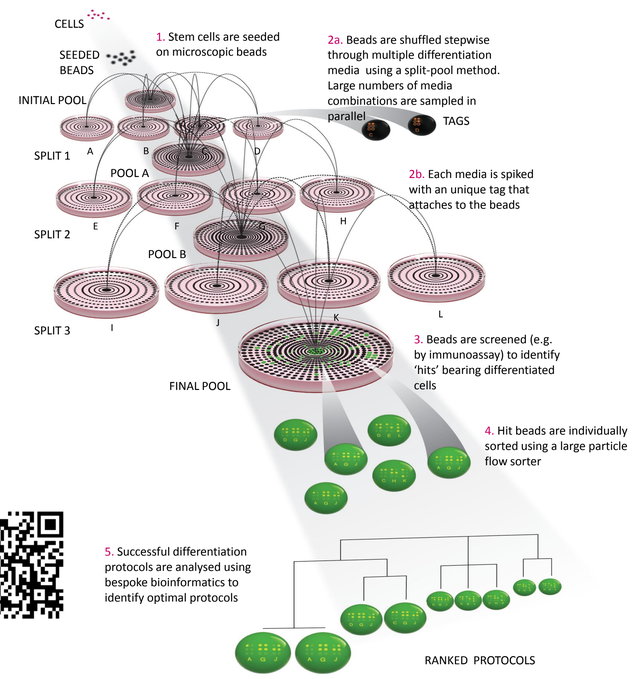
- Enhancing muscle regeneration by treatment with small molecules drugs that stimulate myofiber regeneration can be beneficial in treating muscle-wasting diseases regardless of their cause. Here we describe a development of high content screening platform for muscle regenerative drugs using Pax7+ myogenic progenitors derived from human embryonic stem cells (hES).
- Novel serum-free differentiation protocols for generation of Pax7+ myogenic progenitors from hES cells were identified using a bead-based combinatorial technology, termed Combicult®.
- A high content screening system based on the expression of mature skeletal muscle markers was developed and optimized. Screening of chemical libraries including FDA approved drugs revealed a number of hit compounds that promote myogenesis *in vitro*. Target analysis revealed a number of putative drug targets and lead compounds for further development.

2) Project Overview

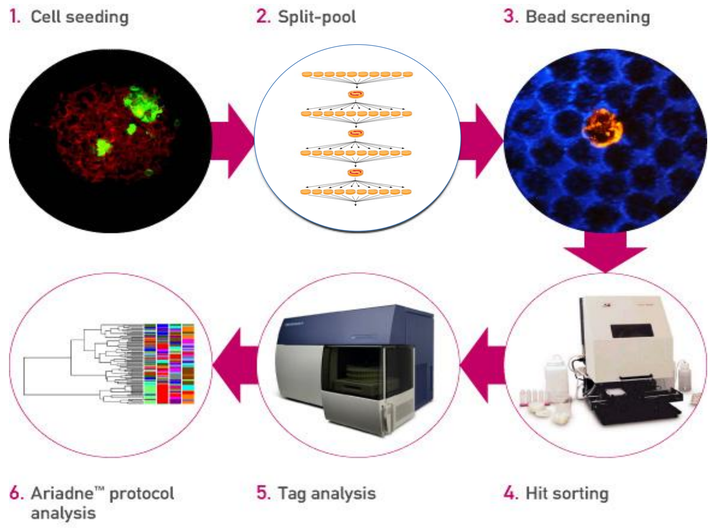
- ➢ The new myogenic protocols were discovered using a high throughput CombiCult® screen:
  - Human ES cells were grown on microcarriers and shuffled randomly through 35 culture conditions, with concomitant labelling of the beads using nanomaterial tags.
  - 4,840 distinct protocols were sampled in a single experiment.
  - Following screening, beads bearing cells positive for myogenic specific markers were identified and their cell culture history were deduced from tags deconvolution, 10 unique protocols were identified.
  - Candidate protocols were ranked using a bespoke bioinformatics program Ariadne®.
  - Top ranking protocols were further validated in monolayer cultures and tested in myogenesis assays.
- ➢ ProScreen Assay development; Optimization of cell seeding, screening media and ICC for high content screening and identification of positive control
  - Pilot screens and Z-factor calculation

3) Combicult® Platform

Combinatorial cell culture (CombiCult®) is a bead-based, combinatorial technology specifically developed for discovery of novel stem cell differentiation protocols.



4) CombiCult® Workflow



5) CombiCult® Experiment for Muscle Differentiation

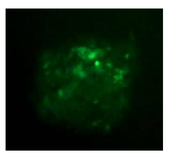
35 media compositions were tested in 4,840 combinations over a 4-step, split pool experiment – bead aliquots were analysed on day 7, day 11 and day 14

Pluripotent hESC

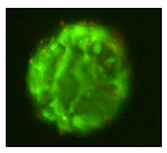
Matrix size: 8x11x11x5 =4 840

Examples of 'hit' beads picked for tag analysis:

Pax7 +ve , day 7



MyHC +ve, day 17



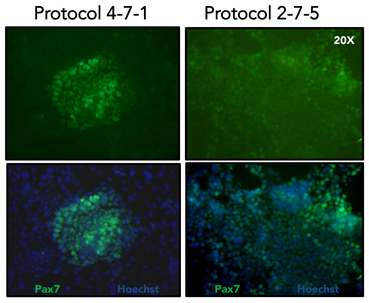
The culture history of each hit bead was deduced from analysis of tags. The significance of each protocol was determined using Plasticell's Ariadne™ software. CombiCult® protocols 020705, 040411 and 060905 produce Pax7+ progenitors from hESCs

Diagram shows overlay of all positive protocols leading to Pax7+ differentiation. Three top protocols suggested by Ariadne™ marked in pink, blue and green.

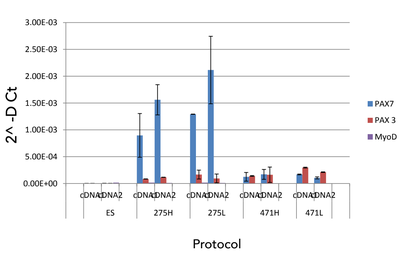
6) Validation of Protocols for Pax7+ Progenitor Cells

Selected CombiCult® derived protocols for differentiation of hES cells into Pax7+ myogenic progenitors were validated in monolayer cultures. Pax7 expression was tested by ICC and qPCR.

Protocol 4-7-1 Protocol 2-7-5

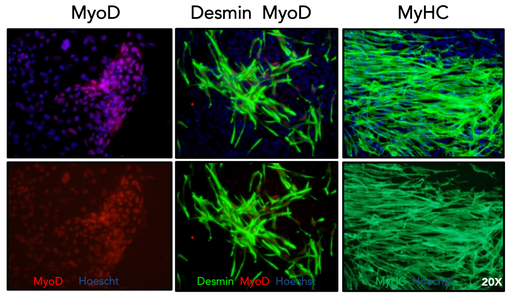


Expression of muscle satellite cell markers

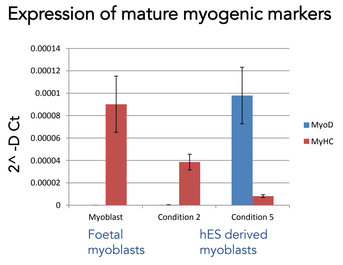


Pax7+ muscle progenitors derived by 020705 protocol were further differentiated into Desmin+ MyoD+ MyHC+ muscle cells in myogenic media containing TGFb inhibitor SB431542.

MyoD Desmin MyoD MyHC

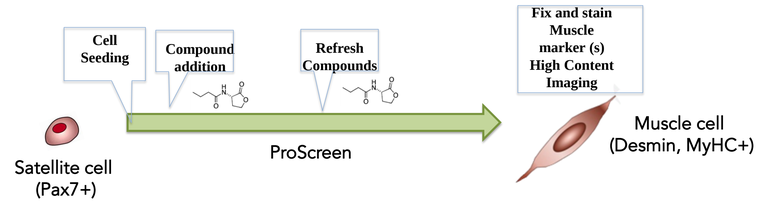


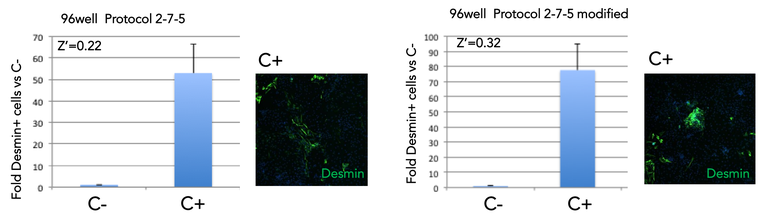
Expression of mature myogenic markers



7) ProScreen Assay Development

hES derived Pax7+ progenitor cells were used to develop high content screening assay based on the expression of skeletal muscle differentiation markers.





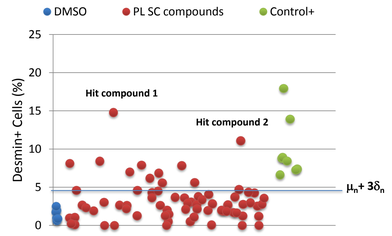
To reduce spontaneous differentiation we tested several combinations of serum-free media. Screening media and seeding density were optimized to produce acceptable Z-factor value.

8) Screening of Annotated Libraries

A. Plasticell Stem Cell compound library (PL SC):

70 compounds (screened in triplicates)
Primary hits: 9 compounds (12% hit rate)

The cut-off for assigning hits was set at 3 standard deviations above the mean value of the DMSO controls.

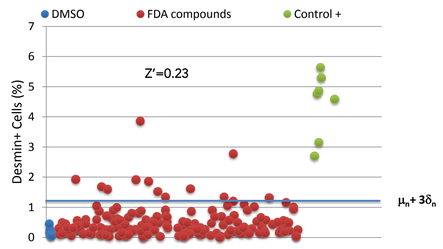


Hit compound 1:

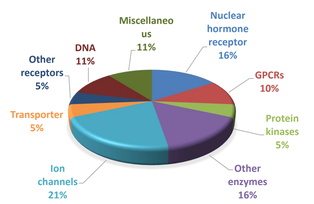
Hit compound 2:

B. FDA compound library:

785 compounds (screened in duplicates)
Primary hits: 35 compounds (4.5% hit rate)

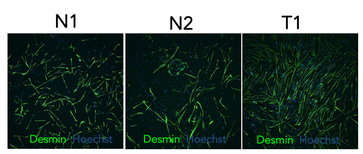


Drug targets by molecular class: Common targets for FDA screens hits



C. GSK compound library:

GSK compound library: 598 compounds (screened in duplicates)
Primary hits: 22 compounds (3.7% hit rate)



Primary targets from GSK SCTB screen hits

N1	nuclear receptors
N1	
N2	
E1a	Enzyme, cytoplasm
E1b	
Ch1	Ion receptors, membrane
Ch2	
T1	Protein kinases
L2	
M1	
M2	

9) CONCLUSIONS

- Plasticell's screening technology, CombiCult®, was used to screen near 5000 of media combinations in order to discover optimal serum-free, feeder-free protocols for the differentiation of hES cells into Pax7 positive muscle progenitors.
- Validation of the 10 top ranking protocols identified 3 novel media combinations that were highly efficient in the induction of hES into pax7+ muscle progenitor cells.
- High content screening assay using hES derived Pax7+ progenitor cells was developed based on the expression of skeletal muscle differentiation markers.
- High throughput phenotypic screening of several highly annotated chemical libraries including FDA approved drug library revealed several functional groups of small molecules that promote myogenesis in vitro. The regenerative properties of these small molecule drugs will be further tested in animal models of neuromuscular disorders for future therapeutic applications.

ACKNOWLEDGEMENT:
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