A human pluripotent stem cell model of FSHD-affected skeletal muscles

Leslie Caron[1]; Devaki Kher[1]; Kian Leong Lee[2]; Giulia Ferri[3]; Davide Gabellini[3]; Heather Main[1]; Uli Schmidt[1]

1. Genea Biocells, Sydney, Australia
2. Cancer Science Institute of Singapore, National University of Singapore
3. San Raffaele Scientific Institute, Milano, Italy

Background

Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common forms of muscular dystrophy (MD) affecting up to 1:7000 people. This genetic disorder is characterized by a progressive weakness and atrophy of facial, shoulder, and upper arm muscles, eventually affecting the trunk and lower extremities. Currently, our knowledge of the pathogenesis of the disease is very limited and to date, no treatment specifically targets FSHD. Cellular models for FSHD are critical not only for understanding the molecular mechanism of the disease but can also have a significant impact on the development of new therapies. Human embryonic stem cells (hESCs) potentially represent a renewable source of skeletal muscle cells (SkMCs) and provide an alternative to invasive biopsies from affected patients. To generate a cellular model for FSHD, we have developed a robust and efficient monolayer system to differentiate hESC into mature SkMCs, achieving 70% differentiation efficiency after 28 days without cell sorting or genetic manipulation. Here we show characterisation of FSHD specific phenotypes, in SkMCs derived from 3 hESCs carrying the chromosomal deletion causing FSHD type 1, compared to unaffected and Becker Muscular Dystrophy affected lines.

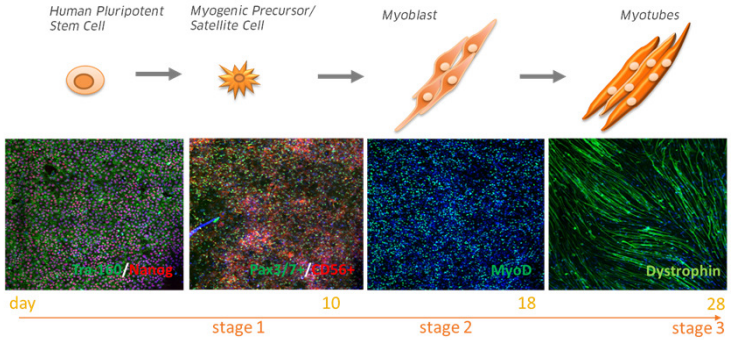

Figure 1. Differentiation of hESC into skeletal muscle cells. Briefly, in our novel protocol, hESCs are first induced to form Pax3,7⁺/CD56⁺ satellite cells (stage1), followed by MyoD⁺ then Desmin⁺ myoblasts (stage 2) and finally mature and multi-nucleated myotubes, which display contractile ability and express the muscle specific markers myogenin, MF20, SkMHC and dystrophin. Each transition is controlled by unique combinations of growth factors and small molecules.

Results

Using our established protocol, 3 FSHD-affected hESC lines were differentiated into SkMHCs and compared to non-affected hESC lines for their differentiation capacities and phenotype.

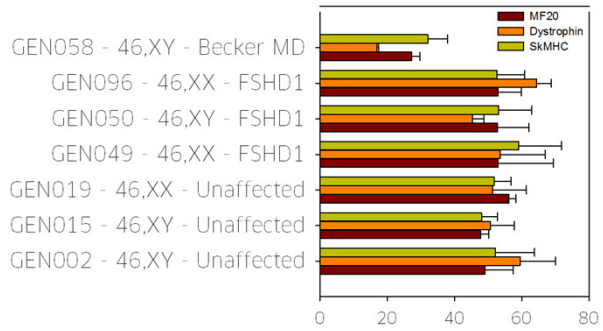

Figure 2. % of skeletal muscle markers + cells after immunostaining.
FSHD-affected cell lines differentiate into SkMCs with the same efficiency as unaffected cell lines and express the same level of Skeletal muscle markers MF20, Dystrophin and SkMHC.

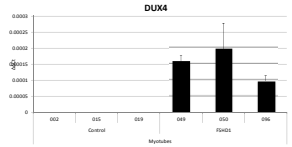

Figure 3. Dux4 expression. To confirm the disease status of our FSHD hESC lines and validate our cellular model, the expression of one of the FSHD candidate gene, Dux4, was analysed by q-PCR. Results showed that while Dux4 is not detected in unaffected myotubes, it is expressed in SkMCs derived from the 3 FSHD hESCs.

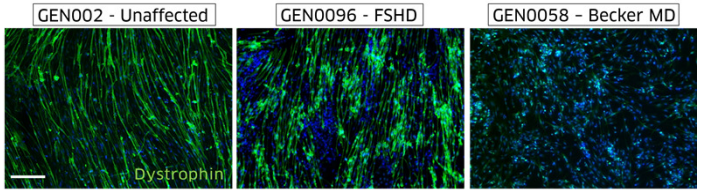

Figure 4. Immunostaining of myotubes derived hESC. Unaffected and FSHD affected SkMCs form a well-organized network of myotubes compared to Becker Muscular Dystrophy SkMCs which had impaired differentiation with a disorganized network of short myotubes. As expected, Becker-SkMCs also express a lower level of dystrophin.

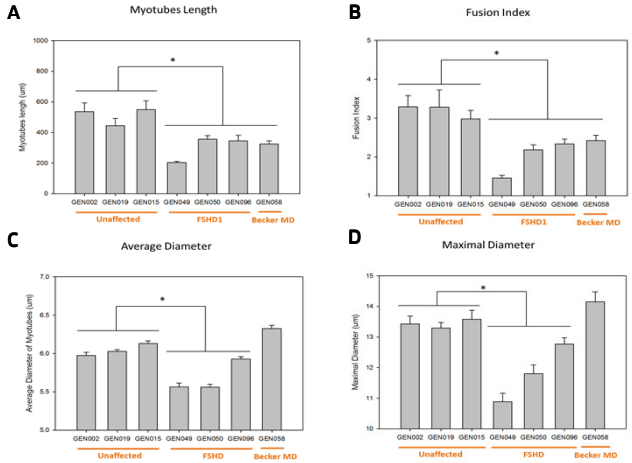

Figure 4. Detailed characterization of the myotubes (using hight-content analysis).
Becker MD and FSHD affected myotubes are shorter that unaffected myotubes (A) and have a lower fusion index (B). Furthermore, FSHD-myotubes appear to be thinner than unaffected myotubes with a smaller average diameter (C)and maximal diameter (D), while on these measures Becker MD myotubes are comparable to control myotubes (C-D).

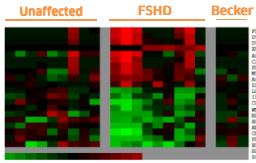

Figure 5: Microarrays have been performed to verify skeletal muscle genes expression and identify disease specific genetic dysregulation. The analysis is currently underway though preliminary data confirmed the presence of skeletal muscle specific genes in all samples and suggest unaffected/FSHD/Becker MD specific changes.

Conclusion

We have demonstrated, for the first time, FSHD specific phenotypes in human pluripotent cell derived skeletal muscles. These morphological phenotypes can be measured in a high content fashion offering great potential . This unique cellular model will be a useful resource for FSHD and other muscular dystrophy research, will help to better understand the disease mechanism and ultimately assist in the development of effective treatments.