Mouse models and genetics of myogenic stem cells

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Facioscapulohumeral muscular dystrophy (FSHD) is neuromuscular disease caused by an unusual deletion on telomeric part of chromosome 4. It is associated with epigenetic alterations in the subtelomeric heterochromatin of the D4Z4 macrosatellite repeat. Each repeat unit encodes double homeodomain protein *DUX4*, a gene that is normally silent in most tissues. Misexpression of the DUX4 in muscle is believed to cause FSHD. Expression of DUX4 has been reported in pluripotent cells and testis. We and others reported that overexpression of DUX4 is toxic for the cells.

To be able functionally to study the effect of DUX4 on the cells we developed novel genetic tool, which enables rapid generation of isogenetically modified cells with conditional transgene expression. Using this system, we showed that DUX4 expression in myoblast recapitulates key features of the FSHD molecular phenotype, including repression of MyoD and its target genes, diminished myogenic differentiation, repression of glutathione redox pathway components, and sensitivity to oxidative stress.

To test the idea that DUX4 may be involved in germ lineage developing, we interrogated the effect of DUX4 expression at different stages during *in vitro* differentiation of mouse ES cells. We found that expression of very low levels of DUX4 is incompatible with pluripotency: DUX4-expressing ES cells downregulate pluripotency markers and rapidly differentiate. Profound analyses revealed that DUX4 induced aneurectodermal program. Embryoid bodies exposed to a pulse of DUX4 expression displayed severely inhibited mesodermal differentiation, but acquired neurogenic potential.

To generate an animal model for FSHD, we introduced an inducible transgene encoding *DUX4* and 3' genomic DNA into an euchromatic region of the mouse X chromosome. Without induction, DUX4 RNA was expressed at low levels in many tissues and animals displayed a variety of unexpected dominant leaky phenotypes, including skin and testes pathologies, and malespecific lethality. We show that these leaky phenotypes are not due to a leaky promoter, but rather to cis element(s) in sequences 3' of the *DUX4* ORF.