

## **WNT pathway alterations in FSHD**

Sébastien Charron, Kelly Vancutsem, Céline Vanderplanck, Céline Lancelot, Gille Carnac\*, Dalila Laoudj-Chenivresse\*, Alexandra Belayew and Frédérique Coppée.

Laboratory of Molecular Biology, Health Institute, University of Mons, 6, Avenue du Champ de Mars, B-7000 Mons, Belgium ; \*INSERM U1046 Physiologie et Médecine expérimentale Cœur et Muscle, Biosciences Institute, CHU A. de Villeneuve, 34295 Montpellier France

DUX4 is a potent transcription factor that initiates a large gene deregulation cascade in FSHD muscle cells but most of the cellular pathways causing the pathology are still unknown. In addition, the homologous DUX4c protein is also induced in FSHD and probably contributes to the pathology. Different non-muscular symptoms have been reported in patients affected with FSHD which could be related to perturbations of the canonical/ $\beta$ -catenin and/or the non-canonical planar cell polarity (PCP) WNT pathways (1). These pathways play essential roles in muscle development and regeneration.

In the present study, we analyzed whether the WNT pathway was altered in FSHD muscle cells. We first observed activation of a reporter gene for the canonical WNT pathway in FSHD and in DUX4- or DUX4c-overexpressing myoblasts. We then profiled mRNA expression for 84 genes related to the WNT pathway in control and FSHD proliferating or differentiating myoblasts. In healthy individuals, we identified new genes involved at the onset of muscle differentiation. The FSHD myotube present either an atrophic (aFSHD) or a disorganized (dFSHD) phenotype in a proportion that differs from one primary myoblast line to another. The aFSHD myoblasts presented the strongest WNT pathway mRNA deregulations with decreases in mRNAs involved in proliferation and in the non-canonical PCP pathway (CCND1, AP1 complex, etc.). In contrast, the dFSHD myocytes/myotubes presented the strongest WNT pathway mRNA deregulations with increases in mRNAs encoding antagonists or involved in cell proliferation (FRZB, CCND1, etc.) and decreases in mRNAs encoding agonists such as WNT10A. Some of the deregulated genes were confirmed at the protein level. Following myoblast treatment with an siRNA against  $\beta$ -catenin the aFSHD myotubes formed upon differentiation were enlarged (similar to dFSHD) while the dFSHD myotubes were thinner (similar to aFSHD).

In conclusion, we identified WNT pathway perturbations in both FSHD myotubes phenotypes but even if some WNT components were similarly altered (agonists/antagonists, receptors/co-receptors, co-regulator of  $\beta$ -catenin transcriptional activity) we also identified several ones presenting different changes between aFSHD and dFSHD myoblasts and myotubes. Targeting the WNT pathways therefore seems an attractive target in therapeutic strategies for FSHD with the caution that the most important components to target still have to be identified and will most probably differ according to the pathological myotube phenotype.