UMONS Université de Mons

Faculté de Médecine et de Pharmacie

Muscle Disease

Biology and Biomarkers

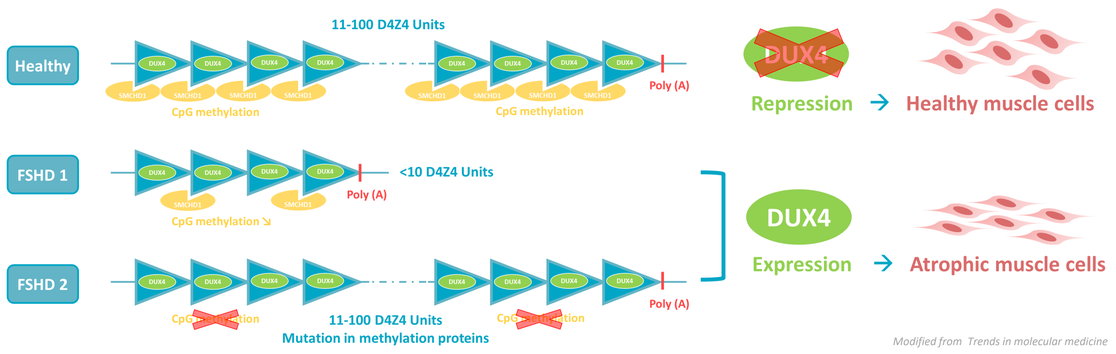
Development of peptides for specific skeletal muscle-targeted drug delivery

Aline Derenne[1], Carole Kouakou[2], Alexandra Belayew[3], Anne-Emilie Declèves[1], Alexandra Tassin[4], Carmen Burtea[2] and Frédérique Coppée[1]

[1] Laboratory of Metabolic and Molecular Biochemistry, Research Institute for Health Sciences and Technology, University of Mons, Belgium [2]Department of General, Organic and Biomedical Chemistry, NMR and Molecular Imaging Laboratory, University of Mons, Belgium
[3] Dean department, University of Mons, Belgium [4]Laboratory of Respiratory Physiology, Pathophysiology & Rehabilitation University of Mons, Belgium

FSHD

Facioscapulohumeral muscular dystrophy



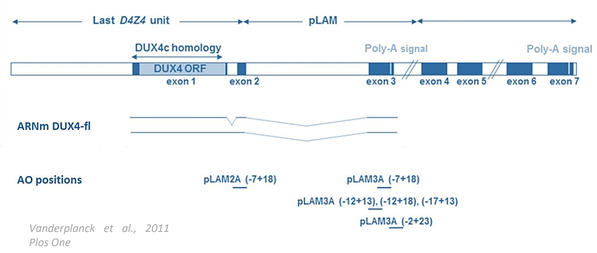
Modified from Trends in molecular medicine

FSHD
- The most frequent hereditary muscle dystrophy in adults
- Asymmetric muscle weakness and atrophy progressing into a rostrocaudal axis
- Caused by a genetic/epigenetic disorder associated with a chromatin opening at 4q35
  → **DUX4 gene misexpression in skeletal muscle cells**

DUX4
- Encodes a transcription factor
- Its misexpression → large gene deregulation cascade
  → muscle wasting, inflammation and oxidative stress

Therapeutic strategy

Antisens oligonucleotides



Vanderplanck et al., 2011 Plos One

FSHD = **No curative therapy !**

↳ **BUT** Prof. Belayew/Dr. Coppée's team (Belgium) (Coll. Prof. Wilton (Australia))
→ Development of Antisens agents (ASO)
= 2'OMePS and vPMO against DUX4 mRNA
➔ DUX4 expression and Atrophic myotubes *in vitro*

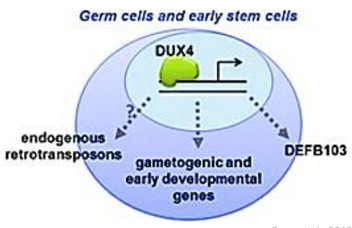
FDA → approbation of ASO drugs for 2 other type of muscular dystrophies (Duchenne/Spinal)

ASO in FSHD ➔ potential clinical trials ??

Targeted therapy

Major issue for ASOs clinical trials :
Muscle cells uptake should be
- **Efficient** → ↘ renal clearance
- **Specific** → **!!** DUX4 Physiological and essential roles in embryonic development and germ lines

→ Development of a **targeted therapy** to bring ASO only into muscle tissue and facilitate their entry

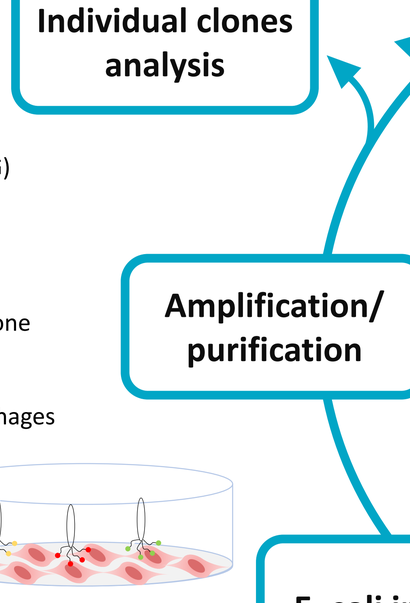


Geng et al., 2012 Dev. Cell

Muscle-targeting

Methods

Whole Cell Panning

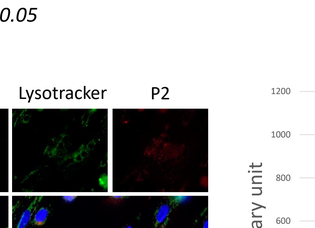


Negative selection :
On collagen
On human hepatocytes (HepaRG)

Positive selection:
On human myotubes
(FSHD/familial control derived muscle cells from UMMS Wellstone Center, Boston)

Elution → extracellular bound phages
Lysis → Internalized phages

3 rounds of selection

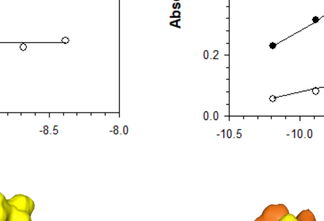


Target protein Panning

Negative selection :
On Bovine serum albumin

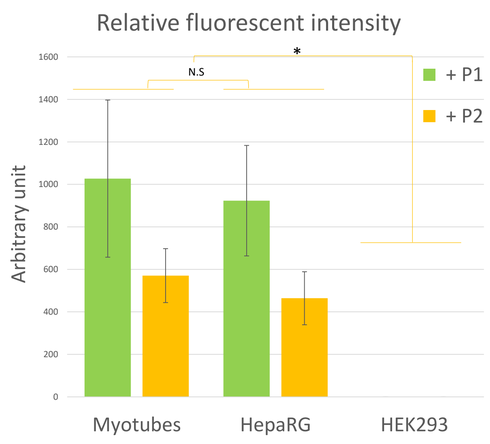
Positive selection:
On a muscle specific membrane protein fragment (Dec1-AD protein)

3 rounds of selection

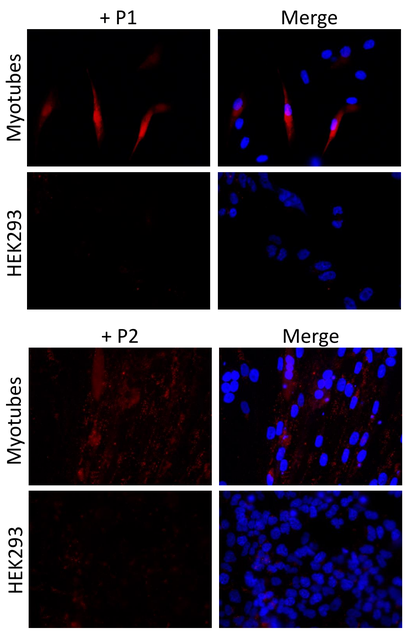


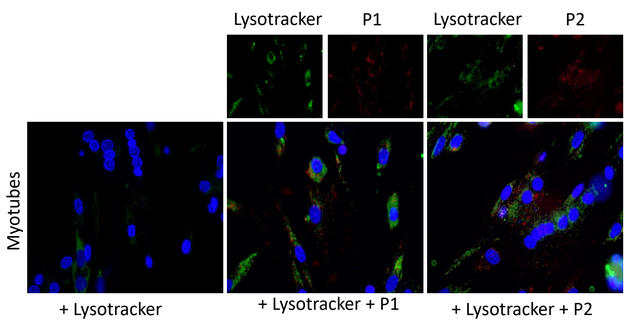
Results

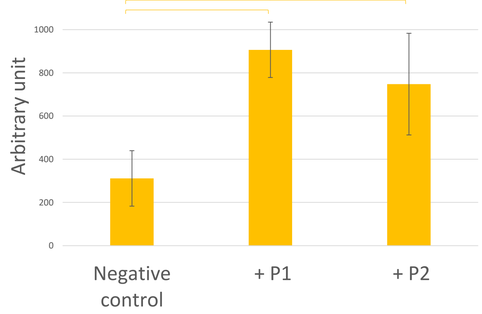
Whole Cell Panning



Endocytosis assay. *Cells were incubated 2hrs with selected peptides (conjugated with rhodamine B). After washings, living cells were observed with fluorescent microscope and endocytosis was evaluated by measurement of peptides relative fluorescent intensity. *Anova, p<0.05*

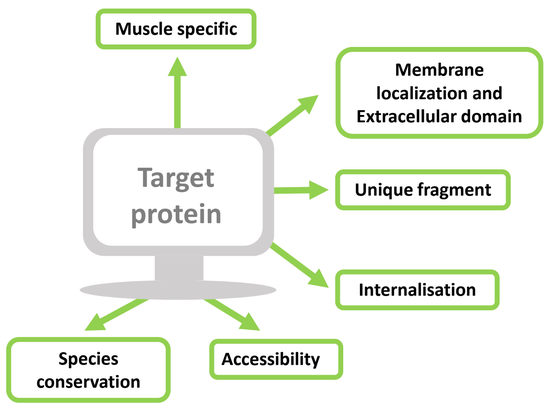






Endocytosis pathway assay. *Cells were incubated 2hrs with selected peptides (conjugated with rhodamine B) and Lysotracker® (fluorescent dye for labeling and tracking acidic organelles in live cells) in media. After washings, cells were observed with fluorescent microscope and we could observe a significant increase in Lysotracker fluorescence and a partial colocalization with peptides, suggesting their internalization by endocytosis. *T-test, p<0.05*

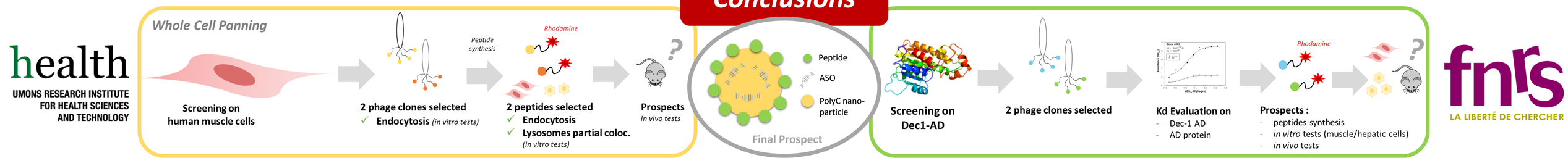
Target protein Panning



Unique protein fragment
➔ Chemical synthesis
= Dec1-AD

(A) *Determination of the dissociation constant (Kd) for most promising phage clones. Concentration-response curves calculated for each peptides on Dec1-AD protein fragment (black dots) or BSA (black circles). Clone 49R3 is the most promising with a Kd≈1,33x10^{-10} M to Dec1-AD and a negligible one for BSA. Association constant (Ka) = 1/Kd.* ***(B) H****ierarchical flexible* ***pep****tide* ***Dock****ing approach through fast generation and ensemble docking of peptide conformation, referrers to* ***HPepDock*** *(Huanglab 2019). Dec1-AD in orange and peptide in yellow.*

Conclusions



Acknowledgments: Aline Derenne held a fellowship from F.R.S-F.N.R.S (Belgium). We thanks FNRS Belgium (CDR n°26119623) for the funding of this study