CYTOO
PHYSIOLOGICAL ASSAYS FOR HCS

DEVELOPMENT OF A ROBUST ASSAY FOR MUSCLE WASTING DRUG DISCOVERY

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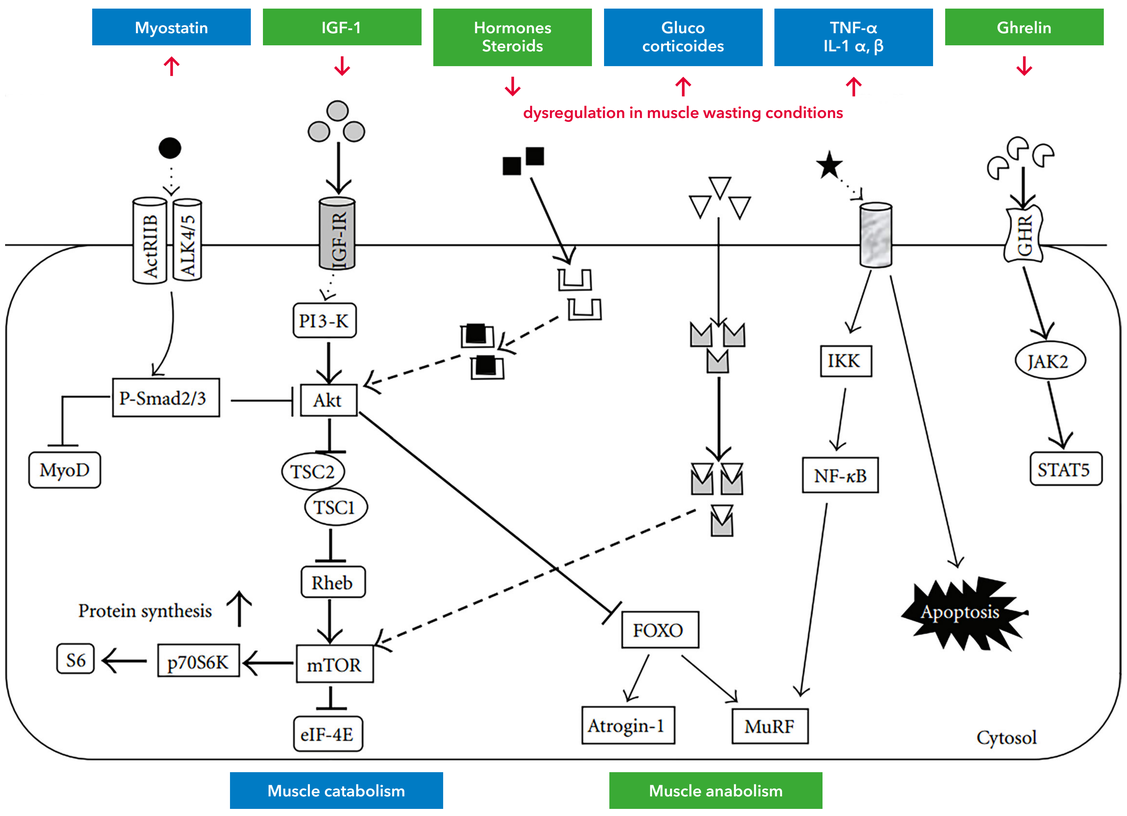
ABSTRACT

Muscle wasting results from a large panel of dysregulations in muscle physiology. It is present systemically in the elderly (sarcopenia); it can be due to acute or chronic illness (cachexia), or appear in various muscular diseases such as dystrophies. During the last 15 years, extensive research has led to a better understanding of the signaling pathways implicated in the loss of muscle mass (atrophy) and offered promising drug targets. However, to date, the muscle is the last undrugged organ. This is mostly due to the fact that animal models poorly phenocopy the human diseases because of genetic and physiological differences but most importantly because muscle is also the last organ for which no relevant *in vitro* model has been established.

In this context, we have developed and characterized the first *in vitro* fully automated human myotube model called MyoScreenΦ™. It relies on a tight control of the microenvironment that guides the differentiation of human primary myoblasts. Myotubes formed on micropatterns present a high level of maturation together with a highly standardized morphology. Thanks to the development of a dedicated image analysis, we demonstrated that MyoScreenΦ™ model responds to reference compounds inducing both atrophy and hypertrophy for which effects can be quantified robustly and reproducibly by High Content Analysis (Z'Factor > 0.5). To further establish a model for muscle wasting correction, we assessed if atrophy can be rescued by different molecules.

MOLECULAR MECHANISMS LEADING TO MUSCLE WASTING

Molecular pathways involved in muscle homeostasis



Red arrows indicate upregulation and downregulation of signling pathways leading to muscle waste. *Adapted from Sakuma & Yamaguchi, 2012*

In muscle wasting situations such as sarcopenia and cachexia, several signaling pathways lead to the modification of the genes expression profile which ultimately increases autophagy and myofibril degradation. In addition, downregulation of PI3K/Akt/mTOR pathway results in a reduction of protein synthesis and an accelerated protein degradation. Altogether, dysregulation of these signaling pathways all lead to an imbalance between anabolism and catabolism, with protein loss ultimately influencing the most muscle atrophy.

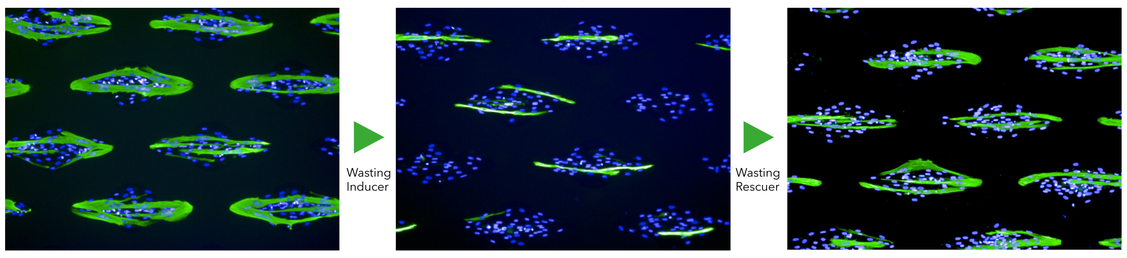
STRATEGY FOR THE DEVELOPMENT OF AN *IN VITRO* MUSCLE WASTING MODEL

Overview

MyoScreenΦ™ model
Healthy myotubes formation

Wasting modeling
Myotube induced-atrophy

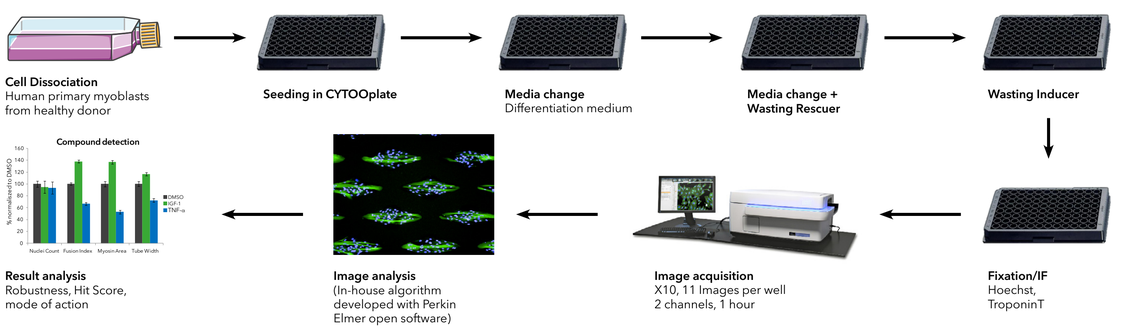
Wasting Rescue
Healthy myotube characteristics



Muscle loss can be triggered through different pathways. Difficult access to human primary myoblasts from patients with muscle wasting conditions represents a major bottleneck for the establishment of relevant *in vitro* models compatible with screening. To overcome this problem, our strategy is based on reproducing muscle loss on our healthy MyoScreenΦ™ model.

Using this approach, muscle loss can be triggered by different signaling pathways implicated in muscle wasting. Once atrophy is mimicked, compounds rescuing the induced phenotype can be screened.

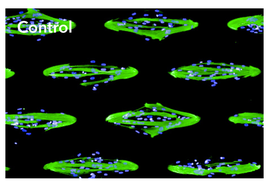
Experimental set up

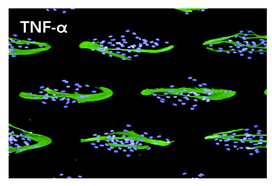


A fully automated process with dedicated image analysis was developed to streamline the screening and identification of compounds implicated in muscle wasting rescue.

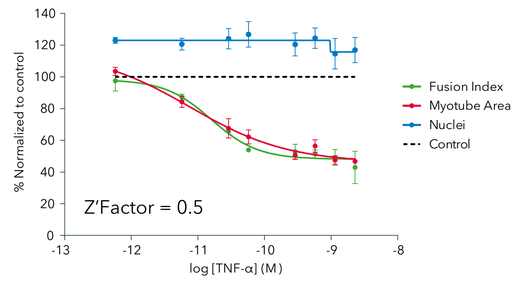
MYOSCREENΦ™ RESPONDS TO DIFFERENT MUSCLE WASTING STIMULI

Pathway	Wasting Inducer	Myotube Area (Control=100%)
Glucocorticoid	Dexamethasone	40%
Smad2/3	Myostatin	70%
	TGF-β	40%
NF -κB	IL 1-β	65%
	TNF-α	45%





TNF-α dose response

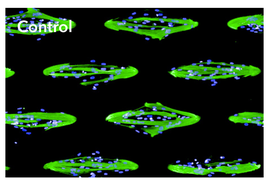


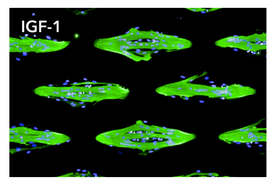
Dose responses have been conducted with 5 compounds covering 3 different signaling pathways implicated in muscle wasting leading to the identification of concentration inducing strong atrophy without toxicity. Z'Factor > 0,3 have been obtained for all atrophy inducers.

RESCUE ASSAY: MYOSCREENΦ™ ALLOWS A ROBUST RESCUE OF INDUCED MUSCLE WASTING

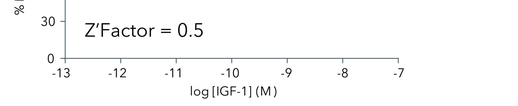
Characterization of potential wasting rescuer

Pathway	Wasting Rescuer	Myotube Area (Control=100%)
Akt/mTOR	IGF-1	140%
Transcriptionnal regulation	TrichostatinA	150%
Smad2/3	Follistatin	100%



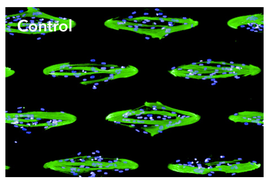


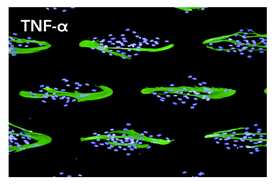
IGF-1 dose response

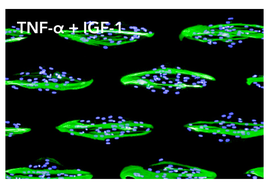


In absence of wasting inducers, dose responses of IGF-1 and TrichostatinA induce a dose dependent induction of hypertrophy without toxicity. Follistatin did not affect muscle phenotype.

Dose dependent detection of wasting rescue

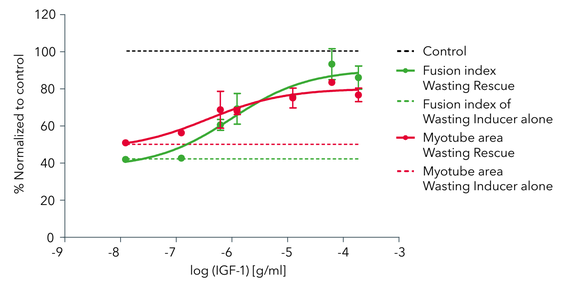






Dose responses of the three potential rescuers have been performed with a fixed dose of wasting inducers allowing a dose dependent detection of wasting rescue.

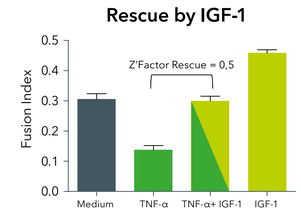
TNF-α + IGF-1 dose response



Compatibility with drug screening

Wasting modeling	Myotube Area (Control=100%)	Wasting Rescuer	Myotube Area (Control=100%)	Z'Factor
Dexamethasone	40%	IGF-1	150%	0,45
Myostatin	70%		120%	0,6
IL 1-β	65%		110%	0,52
TNF-α	45%		85%	0,34
Dexamethasone	40%	Trichostatin A	110%	0,43
Myostatin	70%		120%	0,7
IL 1-β	65%		100%	0,32
TNF-α	45%		90%	0,4
Myostatin	70%	Follistatin	90%	0,2

Rescue by IGF-1



Robust rescue of three signaling pathways involved in muscle wasting has been obtained using IGF-1 and TrichostatinA while follistatin specifically rescues myostatin-induced atrophy. Average Z'Factor were calculated from 20 wells for each conditions in three independent experiments

CONCLUSION

Altogether, our results showed that we have developed the first powerful *in vitro* human striated muscle assay compatible with High Content Screening that can be applied for the automated detection of molecules that rescue induced muscle wasting. Such model offers therefore new avenues for large scale screening campaigns to discover novel cures for several muscle wasting disorders.

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