Mimicking Laminopathies with model cell lines

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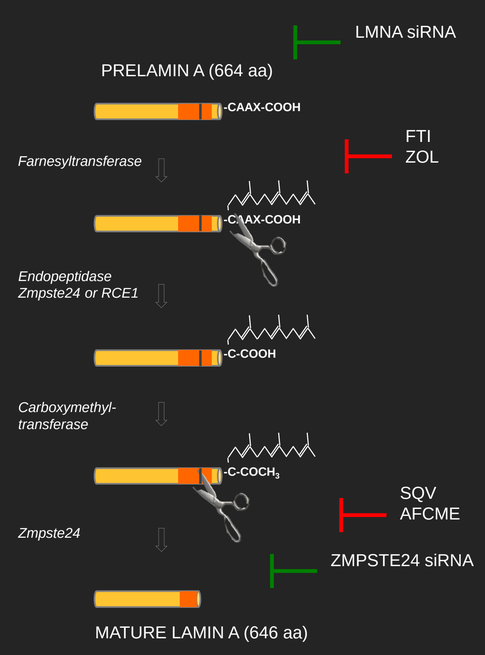
Introduction

The nuclear lamina physically supports the cell nucleus and has a central role in gene regulation. Mutations in the LMNA gene, which encodes A-type lamins, cause laminopathies, including muscular dystrophies, lipodystrophies, cardiomyopathies and the premature aging syndrome Hutchinson-Gilford Progeria (HGPS). In earlier work, we revealed significant phenotypical changes in cells from laminopathy patients: whereas normal cells maintain a rigid, ovoid nuclear shape, laminopathy cells have dysmorphic nuclei, which are highly moldable and prone to rupture. However, the exact contribution of these nuclear aberrancies for disease development remains unclear. Because patient material is scarce and highly variable, we optimized a set of cellular models using either chemical or genetic perturbation strategies that interfere with the maturation process of the lamin A/C protein. Using a combined approach of high content microscopy and molecular techniques we now show increased levels of reactive oxygen species, mitochondrial dysfunction and ER stress in model cells that accumulate specific prelamin A isoforms.

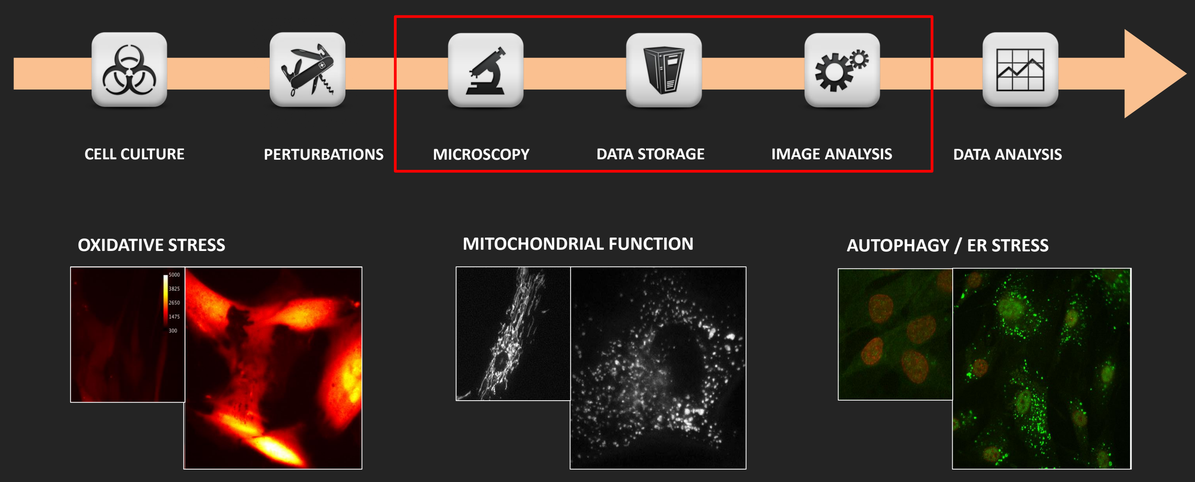
Experimental models

Functional lamin A is produced in a series of post-translational modifications, including farnesylation and proteolytic cleavage. Most laminopathies are associated with mutations that result in formation of **intermediate isoforms** of this pathway. We have used chemical (red) and genetic (green) perturbations to induce accumulation of either non-farnesylated (FTI, ZOL) or farnesylated prelamin A (SAQ, AFCME, ZMPSTE24 siRNA) or to reduce the amount of total lamin A (LMNA siRNA).



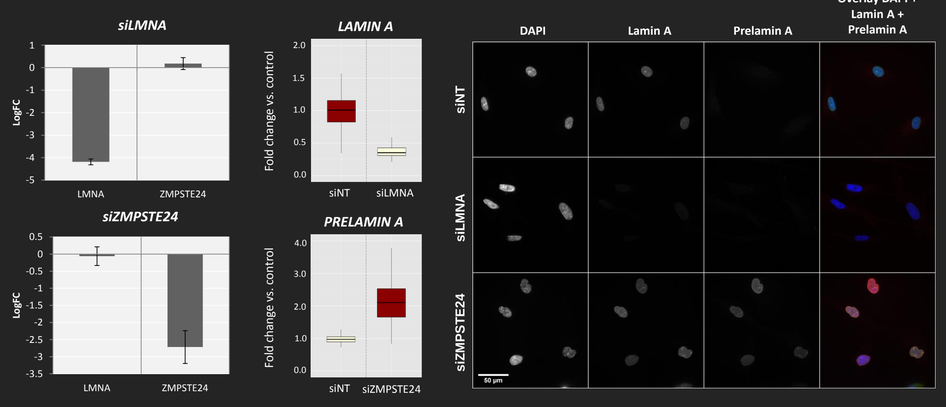
High Content Workflow

A generic pipeline was developed for high content analysis of cellular stress. In brief, the workflow is based on **automated image acquisition and analysis** of 96-well plates containing human fibroblasts, which have been stained with a variety of stress sensors, such as CM-DCFDA (ROS) or TMRM (Mitochondria).

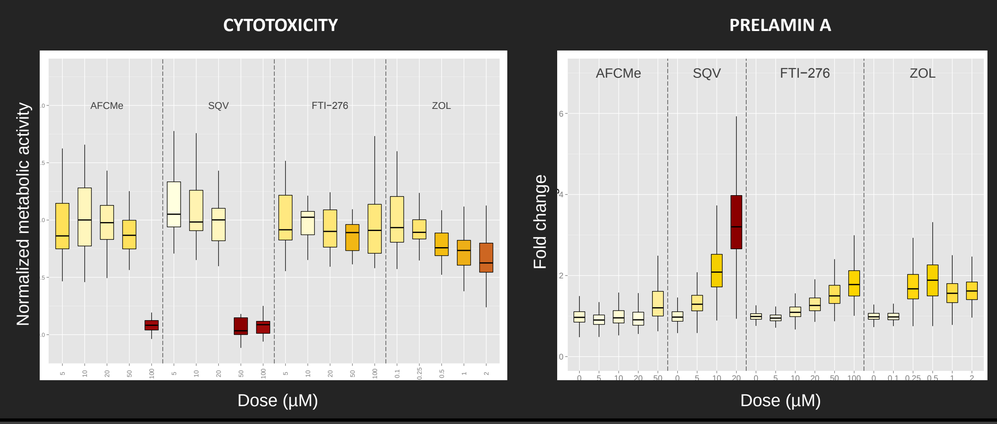


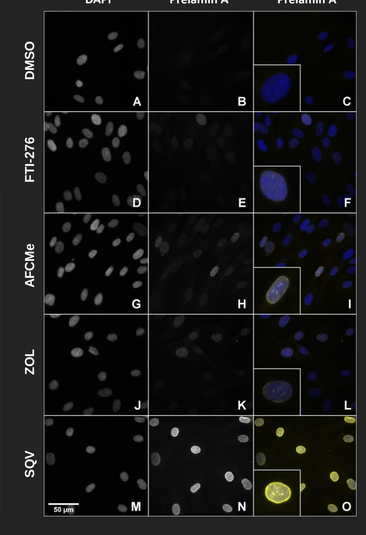
Validation

We validated the efficiency of **genetic perturbations** by quantifying LMNA and ZMPSTE24 **expression levels** using RT-qPCR**.** Immunofluorescence confirmed the **accumulation of prelamin A** in cells treated with ZMPSTE24 siRNA and **reduction of lamin A** in cells treated with LMNA siRNA. Both knock downs resulted in **aberrations of the nuclear lamina**.



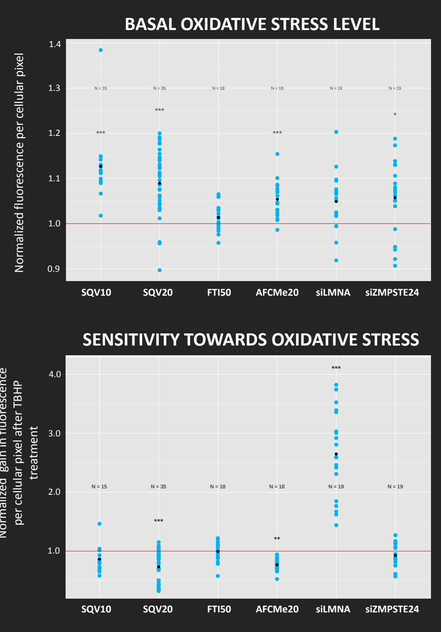
To validate the effect of the **chemical perturbations**, a dose-response analysis was performed in which cytotoxicity and prelamin A accumulation was measured. For every drug, a dose was selected that resulted in moderate accumulation of prelamin A without being toxic on the short term (48h). Clearly, Saquinavir induced the most prominent accumulation of prelamin A.





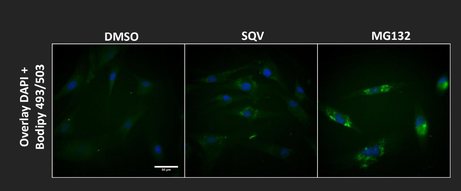
Oxidative Stress

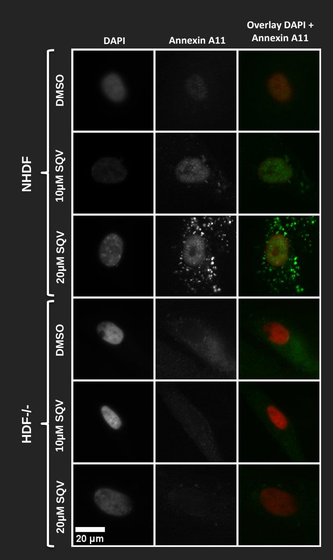
Short-term chemical treatments that lead to accumulation of farnesylated prelamin A increased basal ROS levels significantly, whereas non-farnesylated did not. knock down of ZMPSTE24 confirmed this specific ROS increase. The sensitivity towards exogenously induced ROS (H2O2 treatment) on the other hand was reduced, plausibly because of the already increased basal levels of ROS. Surprisingly, knock down of LMNA increased the sensitivity towards ROS dramatically.



ER stress

ER stress is typically accompanied by accumulation of cytoplasmic lipid droplets (LD). These were visualized by fluorescence microscopy. A potent proteasome inhibitor MG132 was used as positive control. Saquinavir clearly induced the accumulation of lipid droplets.

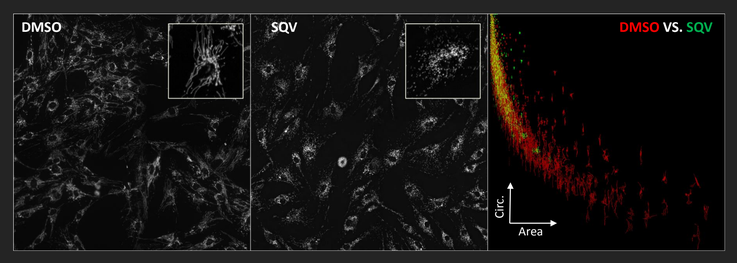




SQV treatment also resulted in the accumulation of the calcium binding protein annexin A11 in LDs and additional cytoplasmic foci. Interestingly Lamin A/C deficient cells (HDF-/-) lack nuclear and cytoplamsic annexin A11 even after SQV treatment.

Mitochondria

Major sources of ROS are dysfunctional mitochondria. Therefore, we measured the mitochondrial potential in the different models using the mitochondrial dye TMRM. Unexpectedly, we found most conditions to result in cells with increased mitochondrial potential (hyperpolarization), especially upon accumulation of farnesylated prelamin A. In addition SQV caused marked mitochondrial fragmentation exemplified by the increased mitochondrial circularity and decreased area.



Conclusion and perspectives

Several characteristics of laminopathies can be mimicked by chemical and genetic perturbations of the nuclear lamina, such as increased levels of ROS. Accumulation of farnesylated prelamin A specifically induces mitochondrial dysfunction and ER stress; two processes that can enhance ROS production and induce oxidative stress. Unfortunately chemical treatments may also trigger off-target effects, presumably due to proteasome inhibition. That is why our follow-up work focuses on generation of faithful model cell lines by means of genome editing.

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