Quantitative proteomics reveals differential effects of ZMPSTE24 and LMNA knockdown on human fibroblasts

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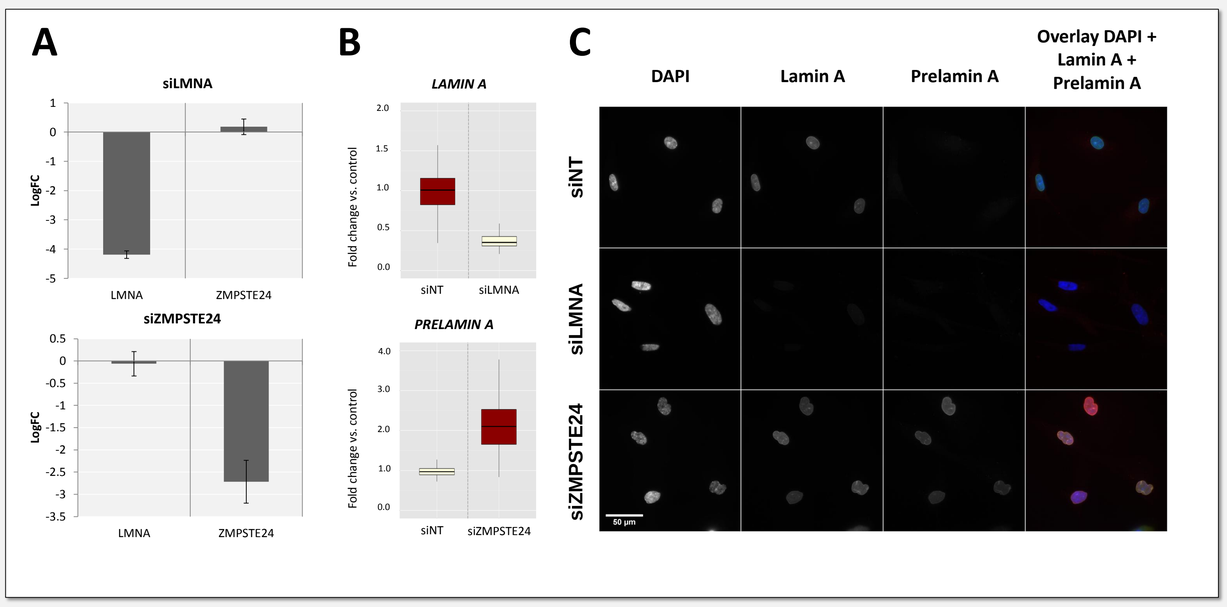
Introduction

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 a spectrum of tissue-specific and systemic diseases collectively called laminopathies. To elucidate the molecular mechanisms underlying this phenotypic diversity, we set out to identify changes in the proteome upon specific lamin perturbations. More specifically, mature lamin A was reduced or farnesylated prelamin A was enriched by knockdown of *LMNA* or *ZMPSTE24*, respectively, in human dermal fibroblasts. To quantitatively compare protein composition, we made use of SILAC-based shotgun proteomics. Gene ontology analysis of the most significant hits revealed that the largest fraction of the differentially produced proteins in LMNA depleted cells were cytoskeletal proteins, more specifically those involved in actin cytoskeleton organization (e.g. ARPC4 and FSCN1). *ZMPSTE24* knockdown on the other hand, mainly altered the levels of mitochondrial proteins, such as TOMM70A, CYC1, STOML2 and ATP5H.

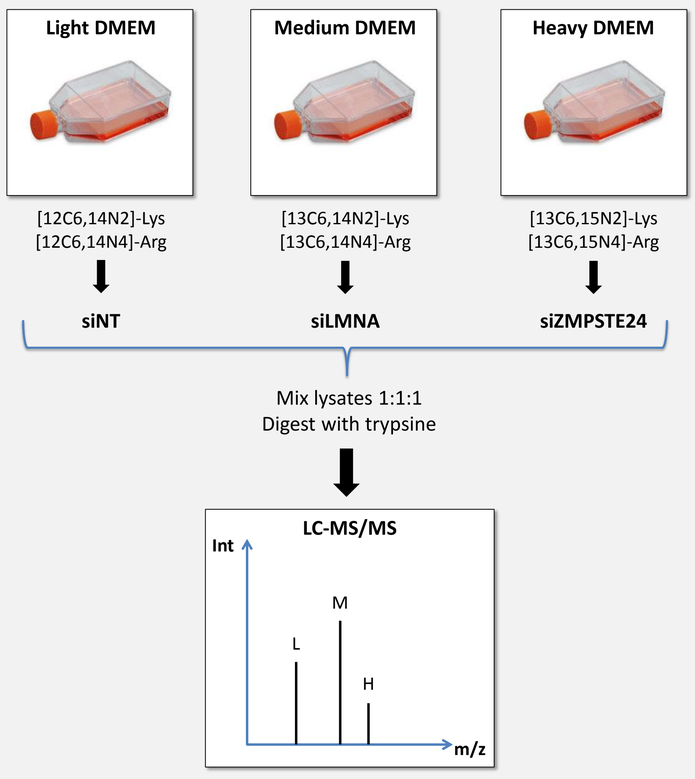
Knockdown of ZMPSTE24 and LMNA

ZMPSTE24 and LMNA expression was reduced by RNAi. Human dermal fibroblasts were transfected with non-targeting siRNA (siNT), LMNA siRNA (siLMNA), or ZMPSTE24 siRNA (siZMPSTE24). After 72h the cells were subcultured and stabilized for 24h, transfection was repeated and 72h later RNA extraction, protein extraction or immunofluorescent staining was performed. We validated the efficiency of genetic perturbations by quantifying LMNA and ZMPSTE24 expression levels using RT-qPCR (A). Immunofluorescence confirmed the accumulation of prelamin A in cells treated with ZMPSTE24 siRNA and reduction of lamin A in cells treated with LMNA siRNA (B and C). Both knockdowns resulted in aberrations of the nuclear lamina (siLMNA = 77% dysmorphic nuclei/ total nuclei, siZMPSTE24 = 50% and siNT = 10%) and ZMPSTE24 knockdown induced cellular senescence (siZMPSTE24 = 31% SABG positive cells/ total cells, siLMNA = 7% and siNT = 10%).



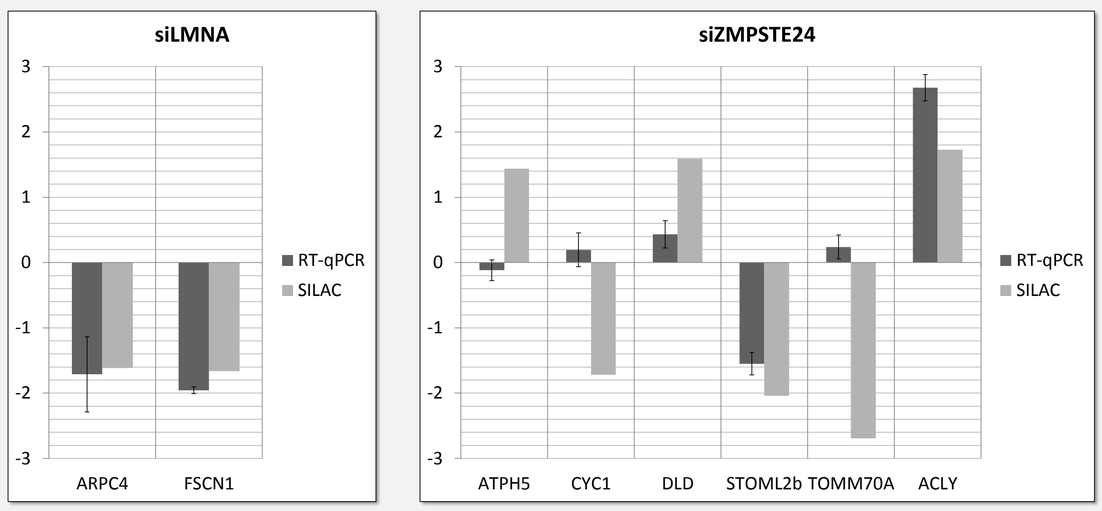
SILAC-based quantitative proteomics

Stable isotope labeling by amino acids in cell culture (SILAC) was used to perform quantitative proteomics. A triple labeling experiment was set up by culturing human dermal fibroblasts in DMEM medium with arginine (Arg) and lysine (Lys) which contained stable ^{13}C and ^{15}N isotopes. Light DMEM contained ^{12}C and ^{14}N isotopes, medium DMEM ^{13}C isotopes, and heavy DMEM ^{13}C and ^{15}N isotopes. After 5 passages, incorporation was tested and knockdowns were conducted. The lysates of the different knockdowns were mixed 1:1:1 and trypsinized. Next the peptides were fractionated with liquid chromatography (LC) and analyzed with tandem mass spectrometry (MS/MS). Peptides were identified and quantified with MaxQuant.



Cytoskeleton and mitochondria

Gene ontology analysis revealed that LMNA knockdown interfered with the expression of cytoskeletal proteins, more specifically those involved in actin cytoskeleton organization, such as ARPC4 and FSCN1. ZMPSTE24 knockdown induced differential expression of mitochondrial proteins, such as TOMM70 A, CYC1, STOML2 and ATP5H or genes which play a role in the citrate cycle, such as DLD and ACLY. Gene expression was quantified with RT-qPCR. The gene expression of ARPC4, FSCN1, STOML2 and ACLY corresponded with protein expression. ATP5H, CYC1, DLD and TOMM70A was not perturbed on transcription level, thus the observed differential protein expression for these genes might be due to an imbalance in protein turnover.



Conclusion and future perspectives

We have shown that LMNA knockdown interfered with the expression of cytoskeletal proteins. ZMPSTE24 knockdown perturbed the expression of mitochondrial proteins and proteins involved in the citrate cycle.

In the near future the top hits need to be validated with western blotting. In addition, cytoskeleton organization should be studied in LMNA knockdown cells and mitochondrial metabolism in ZMPSTE24 knockdown cells. Furthermore patient cells should be included in the experiments.

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