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Optimized methods to investigate the human lysosomal proteome by targeted proteomics

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Introduction

Lysosomes are the main lytic organelles of almost all mammalian cells. They fulfil crucial roles for the degradation and recycling of intra- and extracellular macromolecules and play essential roles in metabolic signaling. These functions are facilitated by >340 lysosome-related proteins, whose loss of function can result in a variety of disorders. Furthermore, a general impairment of lysosomal function, e.g., through external factors, has been described for several conditions. Increases in lysosomal protein levels also have been linked to pathologic conditions, with various types of cancer being most prominent. Therefore, it is not surprising that alterations in lysosomal genes/ transcripts/proteins were suggested as potential biomarkers, including currently ~250 proteins in 245 conditions (1).

Data-independent acquisition (DIA) is an ideal method to allow for an unbiased investigation of the low abundance lysosomal proteins in complex samples. Here, we used dia-PASEF to analyze the lysosomal proteome from four different human cell lines as well as lysosome-enriched fractions from HEK-293T cells.

Methods

Four human cell lines (HEK, HeLa, HUH-7, SHSY-5Y) were grown in adherent cultures and harvested by scraping in ice cold PBS, followed by centrifugation and lysis in 1% SDS, 0.1 M HEPES. Lysosomes were enriched by addition of super-paramagnetic nanoparticles (SPIONs) to the cell culture medium of HEK-293T cells, followed by pulse-chase periods of 24 h each. Subsequently, cells were harvested using a scraper, dounced, and lysosomes enriched using a magnetic stand in combination with LS-columns (both Miltenyi). Proteins were precipitated by acetone, resuspended using 0.1% RapiGest, and digested overnight using trypsin. After precipitation of RapiGest using TFA, peptides were desalted and analyzed by LC-MS/MS employing a nanoElute coupled to a timsTOF HT (both Bruker Daltonics). Peptides were separated on a 25 cm reversed-phase C_{18} column (PepSep, Bruker Daltonics) using a 25-min gradient (32-min total run time). For dia-PASEF a method consisting of eleven TIMS ramps with 2 mass ranges (25 Da window size) in each 75 ms scan was applied. Data were processed with Spectronaut 17 (Biognosys), applying directDIA+ using either a lysosomal protein library (297 proteins) or a full human proteome fasta file.

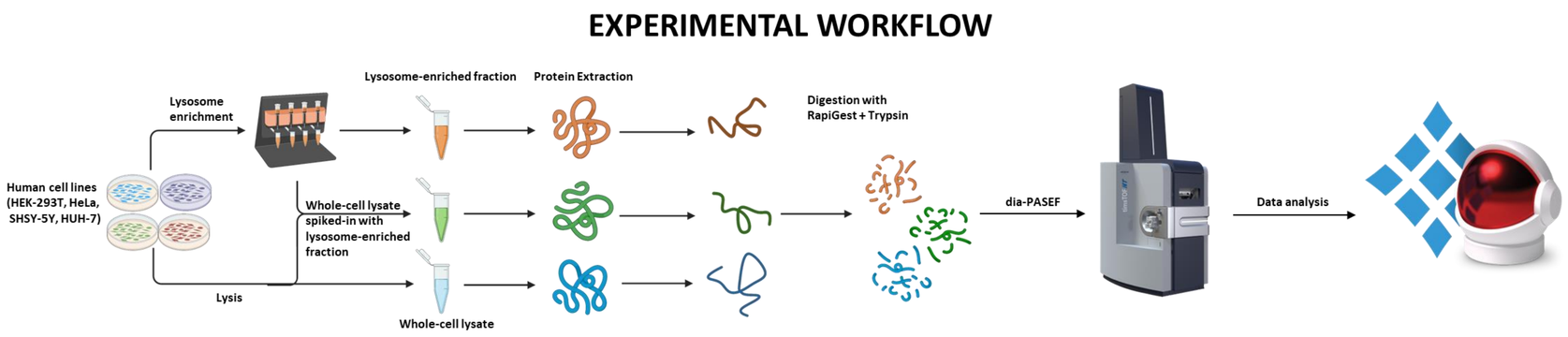

Fig. 1: Experimental workflow to investigate the human lysosomal proteome by targeted proteomics using dia-PASEF data acquisition.

Results

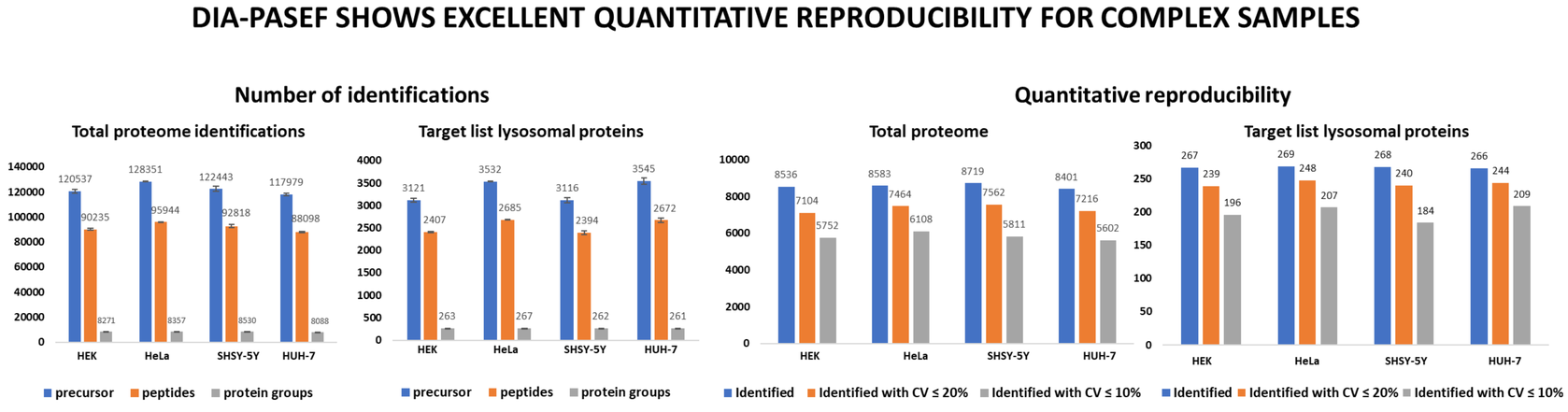

Fig. 2: Using dia-PASEF we identified in total 9,069 proteins / 111,969 peptides from the analysis of all four human cell lines using a 25-min LC-gradient, with 6,686 proteins being present in all of them. With respect to a set of proteins which were previously connected to lysosomes or lysosomal function (2), 271 proteins and 3,264 peptides were identified. The highest number of total and lysosomal proteins was identified for HeLa.

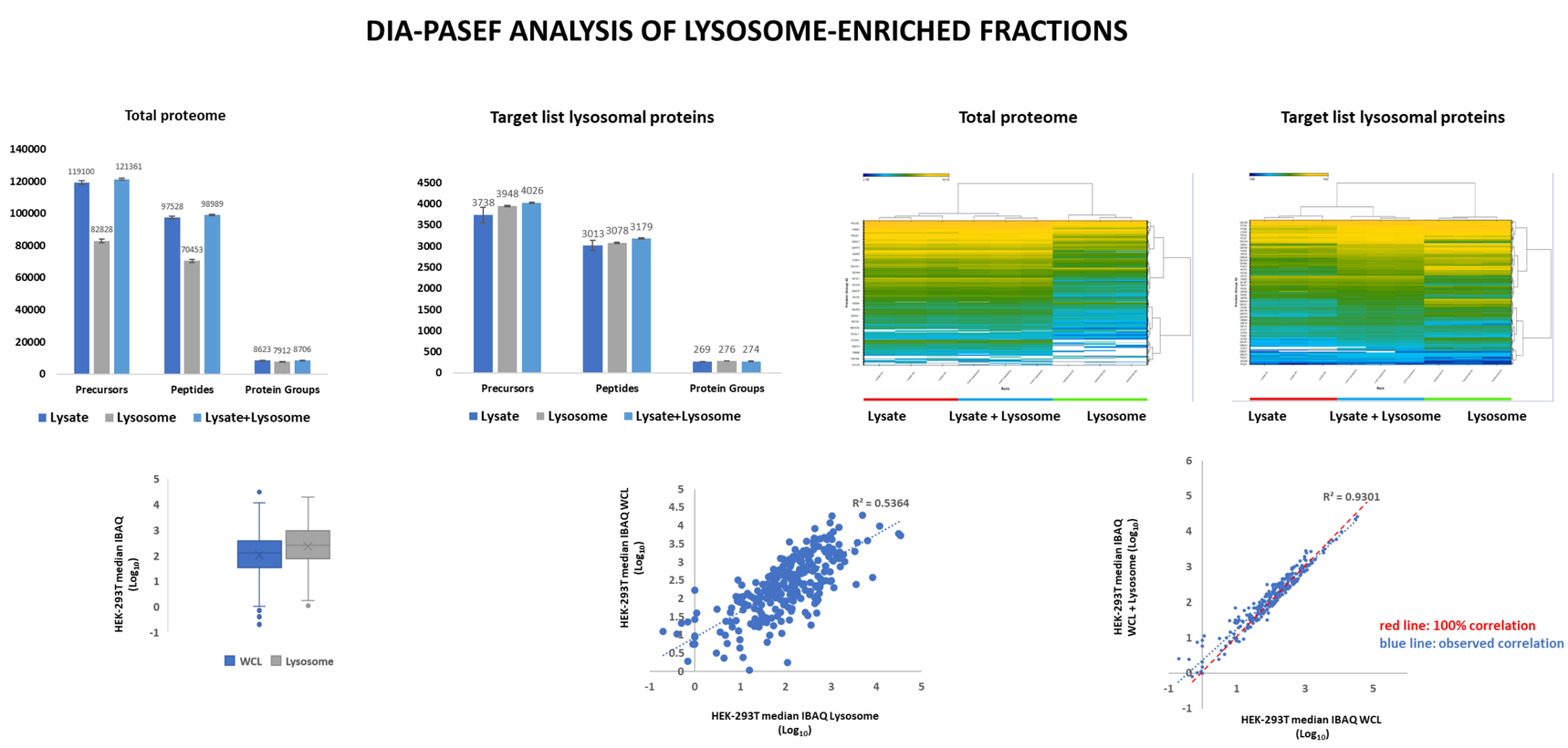

Fig. 3: Lysosome-enriched fractions (LEF) were generated from HEK-293T cells and analyzed by dia-PASEF. Furthermore, a combined sample was generated by spiking LEF into whole cell lysate (WCL), mimicking upregulation of all lysosomal proteins. More than 8,600 protein groups were identified in WCL and WCL + LEF samples with a 60-minute gradient using library-free directDIA+ data processing in Spectronaut 17. The number of identifications is significantly lower in the lysosome-enriched sample, but more than 7,900 protein groups can still be identified. Target data processing identified 276 protein groups in the lysosome-enriched sample. Only minor differences are detectable compared to the non-enriched lysate sample. With a focus on known lysosomal proteins, comparison of LEF to WCL revealed higher median IBAQ values for lysosomal proteins, confirming lysosomal enrichment, and in turn increased relative abundance of these proteins. Correlation of individual IBAQ values for LEF and WCL shows that changes in iBAQ values are not similar for all proteins, implying an effect of sample complexity on signal intensity, or differential loss of certain proteins during lysosome enrichment. The comparison of iBAQ values for WCL+LEF to WCL shows that not all lysosomal proteins are found to be upregulated in the spike-in experiment.

Conclusion

- The presented study focuses on detecting lysosomal proteins in mammalian cell lines using the dia-PASEF approach. Without any specific method development and optimization, we were able to identify more than 90% of the target lysosomal assay consisting of 297 proteins with good reproducibility in just 32-min run time / 25-min gradient length.
- Analysis of lysosome-enriched fractions and WCL spike-in experiments revealed a heterogenous behavior with respect to the observed protein regulation values. In other types of sample material, such as clinical samples, lysosomal proteins are low in abundance, possibly further interfering with the characterization of the lysosomal proteome.
- As this will most likely result in similar effects, the development of a PRM assay was initialized. For timsTOF instruments, PRM is further enhanced by ion mobility parameters for individual peptides, in accordance with the parallel accumulation serial fragmentation (PASEF) method (prm-PASEF). Once appropriate prm-PASEF panels are developed for the full lysosomal proteome, identifying differentially regulated proteins could be accomplished by using such assays. This will enable high throughput analyses of a large cohort of samples.

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3. Mosen, P., Sanner, A., Singh, S., Winter, D. Targeted quantification of the lysosomal proteome in complex samples. Proteomes (2021).