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SESSION VII
NEW FINDINGS AND TECHNIQUES IN NEUROSCIENCE

IMAGING THE LYSOSOME IN CELLULAR SYSTEMS

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Lysosomal storage disorders (LSDs) are a group of rare diseases characterized by a genetic-derived lysosomal metabolism error leading to an excess of substrate accumulation. Within the complex spectrum of symptoms, the LSDs strongly affect the central nervous system (CNS). However, currently available treatments include enzymatic replacement therapies directed to peripherical symptoms only, mainly for the inability of the proteins to pass the blood-brain barrier. In the last years, in fact, innovative therapies are formulated to deliver the drug to the CNS, leading to the emerging need of comprehensive reliable testing systems.
We set up an *in vitro* platform using peripheral cells, i.e. primary human fibroblasts from patients affected by two model pathologies (alpha-mannosidosis, aMAN; Niemann-Pick A, NP-A) and healthy subjects. In the second phase of the study, we translated the readouts on neuronal cells, isolating primary neuronal/astrocytes cultures from cerebral cortex of WT mice and transgenic animal models of the two diseases. Lysosomal defects were analyzed by morphological and functional tools, and validation of the readouts was performed assessing the restoration of the lysosomal features by treatment with approved enzyme replacement therapies. LAMP1 staining was set up for the analysis by confocal microscopy. Using the voxel-based IMARIS image analysis software, we reconstructed the volume of each single lysosome in the cell, quantifying the average lysosome volume per cell, also tracking the 3D intracellular distribution. We described how the mutations lead to an increase in number and volume of lysosomes, and the change in distribution, with the lysosomal net accumulating around the nucleus. We than implemented the morphological analysis with functional data using LysoTrackerRed and DQ-BSA staining. The number of LysoTrackerRed stained lysosomes significantly increased in mutated cells, describing an acidification of the lysosomal content. Moreover, the accumulation of DQ-BSA resulted increased as well, demonstrating that mutant lysosomes are not able to properly degrade the BSA. For each readout, scaling to High-Content Screening methodology was considered, to increase the statistical and translational power of the platform. In conclusion, we used two model pathologies to set up an imaging-based *in vitro* platform aimed to recapitulate the morphological and functional lysosomal dysfunction in a dish, efficiently respond to replacement therapies.

PROTEASOME INHIBITORS: *IN VITRO* MORPHO-FUNCTIONAL CHANGES

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Chemotherapy has significantly increased patient survival rates, but it has also led to a rise in chemotherapy-induced peripheral neuropathy (CIPN), which can severely impact quality of life and may lead to treatment discontinuation. Among chemotherapy drugs, 20S proteasome inhibitors like bortezomib (BTZ) and carfilzomib (CFZ), approved by the U.S. Food and Drug Administration for treating multiple myeloma and some other liquid tumors, have several limitations, including CIPN development. This study aims to investigate the effects of BTZ and CFZ on the cytoskeleton and mitochondria in primary cultures of dorsal root ganglion (DRG) sensory neurons isolated from adult mice. Neurons were treated with BTZ (10 nM) and CFZ (60 nM) for 10 and 24 h. Mitochondrial activity and functionality were assessed using the Seahorse Assay, mitochondrial membrane potential and mitochondrial trafficking measurements, while mitochondrial morphology was analysed using Mitochondrial Network Analysis (MiNA). Then, we examined the drug-induced changes in multiple proteins involved in the cytoskeleton and mitochondrial dynamics using immunoblotting. Our data show that both BTZ and CFZ impact mitochondrial membrane potential at 10 h post-treatment while mitochondrial respiration is affected by both drugs only after 24 h. Regarding mitochondrial trafficking, both BTZ and CFZ appear to affect anterograde transport as early as 12 h post-treatment, but only BTZ causes a significant increase in the number of stationary mitochondria at 24 h. Moreover, BTZ can alter microtubule dynamics by promoting more stable tubulin isoforms, as evidenced by increased MAP2 expression and stable tubulin modifications, such as acetylated and Δ2-tubulin, as early as 10 hours after treatment. Taken together, these results suggest that changes in mitochondrial morphology might represent a common mechanism of cellular toxicity, while the neurotoxic effects of BTZ might be linked to specific cytoskeletal alterations, resulting in significant axonal transport impairment. A deeper understanding of these pathways could help to identify potential therapeutic targets for BTZ-induced CIPN.

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