Lamins are intermediate filament proteins constituting the major structural component of the nuclear lamina, a fibrillar meshwork that lines the inner nuclear membrane in eukaryotic cells. The nuclear lamina plays various cellular functions, providing skeletal support for the nuclear envelope, mediating the attachment of the nuclear envelope to interphase chromatin, and allowing the proper organization and anchoring of the nuclear pore complexes. Vertebrates synthesize a variety of lamins, namely lamin A, B and C, which are encoded by different genes or generated by differential RNA splicing. Recently, gene duplication and protein overexpression of lamin B1 (LMNB1) have been reported in pedigrees with autosomal dominant leukodystrophy (ADLD). However, how the overexpression of LMNB1 affects nuclear ultrastructure remains unexplored. To investigate the morphological changes associated with the overexpression of LMNB1 we transiently transfected mammalian cells with bicistronic expression vectors containing cDNA for both the LMNB1 protein and the enhanced green fluorescent protein (EGFP) reporter or the EGFP reporter alone. Coupling in vivo confocal fluorescent microscopy with transmission electron microscopy (TEM) and electron tomography (ET), we were able to selectively focus our ultrastructural investigation only on EGFP-positive transfected cells. The over-expressed LMNB1 was located to the nuclear lamina, as revealed by confocal fluorescent microscopy. TEM and ET observations on the LMNB1 over-expressing cells revealed the presence of membrane structures forming extensive arrays of stacked cisternae aligned with the nuclear envelope, or laying inside the nucleoplasm. These cisternae clearly expressed LMNB1 within their stacks, as revealed by immuno EM. Moreover, in the nuclear envelope of those cells overexpressing LMNB1 the nuclear pores complexes were clustered together. To minimize the artifacts due to chemical fixation and room temperature dehydration, the transfected cells, once analyzed by confocal fluorescent microscopy, were processed by high pressure freezing and freeze substitution. Respect to conventional EM preparation, nuclear membranes appeared smoother; and the stacked cisternae were separated by a larger nucleoplasmic space. These morphological data clearly demonstrate that LMNB1 overexpression deeply alters the structural organization of the cell nucleus.

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Fig. 1: Fluorescence images of nuclei from LMNB1-EGFP transfected HEK293 cells growing on a grid carbon-printed on an ACLAR disk. The nuclei were counterstained with Hoechst-33342.

Fig. 2: Fluorescence images of nuclei from LMNB1-EGFP transfected HEK293 cells. Higher magnification of a region of interest from Fig. 1 showing the over-expressed LMNB1 located at the nuclear lamina (double arrowhead), in the nucleoplasm (arrow), but also in the cytoplasm (arrowhead). The nuclei were counterstained with Hoechst-33342.

Fig. 3: Representative TEM image of HEK293 cells overexpressing LMNB1 showing an array of stacked membranes aligned with the nuclear envelope (arrowheads). Abbreviations: cyt, cytoplasm; n, nucleus.

Fig. 4: Representative TEM images of HEK293 cells overexpressing LMNB1 showing isolated cisternae present inside the cytoplasm close to the membranes stack (arrowheads). Abbreviations: cyt, cytoplasm; n, nucleus.