Identification of a novel human nuclear envelope LAP1 isoform, LAP1C

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The eukaryotic nucleus is a complex organelle enclosed by a double membrane denoted as nuclear envelope (NE). The NE separates the cytoplasm from the nucleus and is composed by the inner nuclear membrane (INM), the outer nuclear membrane, the nuclear lamina and nuclear pore complexes. The INM contains integral proteins and most of them interact with lamins (the major component of the nuclear lamina) and/or chromatin. One of the first lamin associated proteins identified was lamina-associated polypeptide 1 (LAP1) which is a type II transmembrane protein of the INM (codified by the TOR1AIP1 gene) whose function is poorly understood but is known that it binds directly to lamins and indirectly to chromosomes. Moreover, LAP1 was found to interact with torsinA, which is a central protein of DYT1 dystonia (a human neurological movement disorder) and also with emerin that is another INM associated with human muscular dystrophies. Recent studies have indicated that in fact LAP1 is functionally associated to torsinA in neurons and with emerin in skeletal muscle.

To date of this study the only known human LAP1 isoform was LAP1B. We identified a novel human LAP1 isoform that we named LAP1C. This new isoform is N-terminal truncated, with a molecular weight of approximately 55 KDa contrasting with 68 KDa of LAP1B (Fig. 1A). The existence of this novel isoform was validated by several techniques including LAP1 knockdown, northern blot and liquid chromatography-mass spectrometry. The characterization of novel isoform was achieved by transient expression the HA-LAP1C in human neuronal cell line, SH-SY5Y cells. The results show that the exogenous expressed band corresponding to HA-LAP1C co-migrates with the endogenous LAP1C (Fig. 1B). Moreover, the immunolocalization studies confirmed that HA-LAP1C is mainly found in nuclear envelope and also in some points inside the nucleus (Fig. 1C). Additional phosphorylation assays determined that both isoforms (LAP1B and LAP1C) are post-translationally modified by protein phosphorylation in five residues and two of these were found to be dephosphorylated by Protein Phosphatase 1 (Fig. 1D).

In summary, the results presented are of paramount importance since they open new avenues for the study of DYT1 dystonia and related nuclear envelope-based diseases like muscular dystrophies, where LAP1 (LAP1B and C) and their phosphorylation represent a key regulatory mechanism across the nuclear membrane.

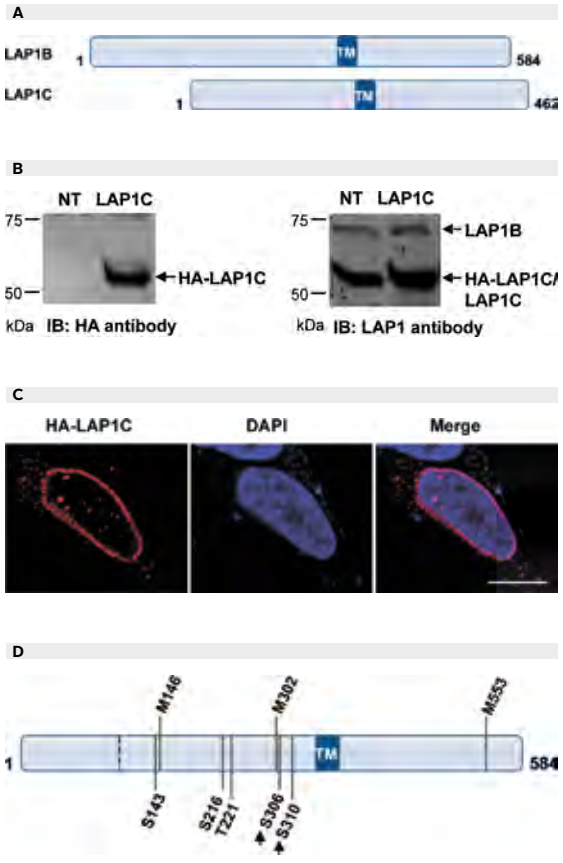

FIGURE 1
Expression, localization and post-translational modifications of LAP1C. A- A schematic representation of the differences of the two LAP1 isoforms (LAP1B and LAP1C) are presented. B- SH-SY5Y cells were transfected with HA-LAP1C and further analyzed by immunoblotting analysis using a HA antibody or using a LAP1 antibody. C- Immunolocalization of HA-LAP1C using a HA antibody and the Alexa Fluor 594-conjugated secondary antibody (red). DNA was stained with DAPI. The images were acquired using the LSM 510 confocal microscope (Zeiss). D- LAP1 isoforms are phosphorylated on Ser142, Ser216, Thr221, Ser306 and Ser310 residues being the last two dephosphorylated by Protein Phosphatase 1 (indicated by arrows). Methionine residues 146, 302 and 553 residues were found to be oxidized. The residues numeration is relative to the LAP1B sequence and the translation initiation site of LAP1C is indicated by a dashed line. TM, transmembranar domain; NT, non-transfected; IB, immunoblotting. Scale bar, 10 µm.