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Histology and Histopathology
From Cell Biology to Tissue Engineering

Molecular and morphological analysis of the golgi apparatus of dopaminergic neurons in human substantia nigra: new citopathological findings in Parkinson's disease

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Parkinson's disease (PD) is the most common movement disorder and the second chronic progressive neurodegenerative disease after Alzheimer's disease. Fragmentation of the Golgi ribbon is a common feature of many neurodegenerative diseases, including PD. However little is known about the causes of this alteration. Most of the cytopathological findings of this disease have been obtained from cellular and animal experimental models but studies in human tissue are scarce. Our previous studies, using cellular models of Parkinson´s disease, demonstrate that fragmentation is an early event, previous to □-synuclein aggregation and cytoskeletal alterations, which is directly related to the alteration of the homeostasis of a limited number of Golgi regulatory proteins. The purpose of this study was to analyze the Golgi complex of dopaminergic neurons in the substantia nigra of PD necropsies using morphological and biochemical techniques.

Human nigra substantia pars compacta (SNpc) samples: The human postmortem control and PD samples used were obtained from the Neurological Brain Bank of Navarra, Banc de Teixits Neurològics (Serveis Cientificotècnics, Hospital Clínic, Universitat de Barcelona) and Fundación CIEN□□Instituto de Salud Carlos III). *Immunofluorescence:* 5 µm sections of paraffin-embedded, formalin-fixed susbtantia nigra samples were treated with 0.5% citrate buffer to maximize antibody penetration into the tissue before to the immunofluorescence tecnique. Autofluorescence was blocked with Sudan black. *Western blotting analysis:* Frozen samples of substantia nigra were lysated, resolved on NuPAGE gels, transferred onto PVDF membranes and immunolabelled. Proteins were detected by using ImageQuant LAS 4000. *Ultrastructural analisis:* Frozen and formalin-fixed samples were postfixed with glutarldehyde/osmium tetratoxide, dehydrated and embedded in Epon 812. For ultrastructural immunolocation samples were postfixed with 2% paraformaldehyde and 0.2% glutaraldehyde and processed for cryoimmunoelectron microscopy. *Statistical analysis:* Mann-Whitney U test was used to compare means between PD and control necropsies. P-values < 0.05 were considered significant.

At light microscopy, the Golgi apparatus of dopaminergic neurons from PD samples appeared fragmented and distributed throughout the cytoplasm. Electron microscopy demonstrated that the Golgi ribbon is altered but the typical stacked appearance of this organelle is not loss. The biochemical analysis showed a reduction of the levels of GTPase Rab 2 and Golgi structural protein GRASP65. In contrast, we found an increase of Rab1, the SNARE protein syntaxin 5 and regulatory protein golgin 84. The level of other proteins analyzed were not altered (tubulin, giantin, Rab8, GM130). We also observed main alterations in the distribution of Rab1 and the SNARE protein syntaxin 5, which is accumulated mainly out of the cell in extracellular inclusions.

We demonstrate for the first time that the expression of Golgi structural and transport regulatory proteins is altered in human samples of PD which may explain the fragmentation of this organelle. These alterations may disturb the trafficking of many proteins that are essential for neuronal function. New experiments in human samples will be need to give a complete view of the cytopathology of PD.