

HEALTH AND HUMAN SCIENCES

Optineurin and the Pathogenesis of Parkinson's Disease

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Parkinson's disease (PD) is a neurodegenerative disease characterized by a resting tremor and slowed movement. These motor deficits result from cell death in a brain region called the substantia nigra (SN). Additionally, insoluble clusters of toxic proteins, termed Lewy Bodies (LB), develop in the brain. Lewy Body accumulation is reported to increase in scope and severity as PD progresses and, notably, does not affect the SN until halfway through disease progression. Therefore, according to the hypothesis that LB overload ultimately contributes to cell death, clinical intervention of PD does not occur until cell death in the SN is imminent.

Autophagy is the cellular process responsible for degrading dysfunctional proteins, which is compromised in PD. Since the failure of this critical pathway precedes LB development, elucidating the mechanisms by which autophagy is impaired could allow for earlier attempts targeted at attenuating LB proliferation.

Optineurin (OPTN, green) is a protein critical for autophagy, where it acts as a cargo adapter by linking substrates for degradation to the site of autophagic breakdown. Despite mutations in the OPTN gene being linked to glaucoma and ALS, the protein has received minimal attention. Given the overlap between disease pathology and the cellular role of OPTN, I sought to examine OPTN in vivo (in a living organism).

Rotenone is a naturally occurring pesticide that has been extensively characterized for its ability to recapitulate PD pathology; in our study, rats received injections for 24 hours, 5 days, or until the onset of behavioral deficits (typically approximately 7 days later, which was associated with overt neurodegeneration). We then performed quantitative immunofluorescence on rat tissues in order to evaluate changes to OPTN's distributional conformation within cells. Additionally, we quantified OPTN colocalization with a marker for autophagic vacuoles (LC3, red).

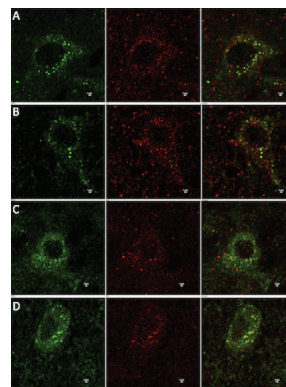
Immediately following rotenone administration, we saw significant alterations to OPTN distribution in cells. This change favored the formation of bright circular dots, termed puncta, by 24% relative to control animals. In subsequent stages, the percent of OPTN existing as puncta fell to a 1% increase at 5 days and a

30% decrease in end-stage animals (relative to control). Increased puncta formation relative to the control average is consistent with the notion that OPTN expression is upregulated for its ability to bind cargo.

Interestingly, and despite an OPTN conformation that favored cargo transport, colocalization with autophagic vessels was significantly diminished relative to control average. We observed a 32% decrease at 24 hours and a 37% decrease at 5 days. Although there are many possible explanations for decreased colocalization, one likelihood is that OPTN and LC3 migrate to different cargo.

In summary, we have provided the first evidence that OPTN is enriched in clinically relevant PD regions and that distribution is altered in preclinical environmental PD models. Significant impairment to OPTN–LC3 colocalization also may be indicative of the impaired autophagy observed in PD. Ultimately, understanding how PD deficiencies manifest themselves in ways beyond motor dysfunction could allow clinicians to recognize more quickly cases in order to incorporate appropriate intervention strategies prior to neurodegeneration.

Research advisor Jason Cannon writes: "Aberrant autophagy has been linked to the pathogenesis of PD. Yet, where in the brain and at which disease stages that autophagy disruptions occur is unknown. Joey's work has begun to make some important advances in these areas. In animal models of PD, Joey has helped to show that key proteins involved in autophagy are altered in expression and cellular localization prior to cell death, and also that brain regions involved in both motor and nonmotor symptoms of PD are affected. His data has implications in understanding the pathogenesis of Parkinson's disease and also, potentially, in the identification of new therapeutic pathways."



A-D) Representative images of OPTN (green) and LC3 (red) expression in control, 24 hour-, 5 day-, and end-stage animals, respectively; significant increases to the fraction of cellular OPTN in punctate conformation and in cellular LC3 expression are observed at 24 hours; $n = 120-170$ cells analyzed/group; significance is $p < 0.05$ for all groups.