Peroxiredoxin 4 is confined to the endoplasmic reticulum in human brain and associated with Lewy body formation in Parkinson's disease and dementia with Lewy bodies

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Parkinson disease (PD) is a progressive neurodegenerative disorder characterised by formation of Lewy bodies within the doperminergic neurons within the *substantia nigra* and depending on the staging of the disease, marked loss of these neurons. Similarly, dementia with Lewy bodies (DLB) involves the formation of Lewy bodies within the cortical neurons resulting in marked synaptic loss and eventually neuronal loss and dementia.

Lewy bodies contain a range of cellular proteins including a high proportion of α-synuclein, a small protein whose suspected role is in synaptic vesicle recycling. There is still debate as to whether Lewy bodies are protective or destructive to neurons. Yeast models expressing alpha synuclein suggest that α-synuclein blocks trafficking from the endoplasmic reticulum to the Golgi apparatus and results in endoplasmic reticulum stress.

Peroxiredoxin IV is the least characterized of the peroxiredoxin family of antioxidant enzymes and is different from other 2-Cys members in that it contains a hydrophobic leader which suggests that peroxiredoxin IV is a secreted protein. Recent pulse chase experiments have indicated that peroxiredoxin IV is in fact confined to the endoplasmic reticulum and possibly involved in protection against endoplasmic reticulum oxidative stress and as a molecular chaperone. This study was conducted to determine the cellular localization of this enzyme in the human brain and whether peroxiredoxin IV is present in the endoplasmic reticulum and associated with the formation of Lewy bodies in PD and DLB.

Human brain proteins from control, PD and DLB tissue were separated using PAGE, transferred to a Polyvinylidene difluoride membrane (PVDF) and then probed with a peroxiredoxin IV antibody to determine the specificity of the antibody and the molecular forms. Light immunohistochemistry using the same antibody was performed using paraffin embedded sections from the same tissue to determine the general distribution of peroxiredoxin IV. Confocal immunofluorecence using the peroxiredoxin IV antibody and specific cellular and organelle markers was used to determine the specific cellular and sub-cellular distribution. In addition, colocalization with α-synuclein was used to determine if peroxiredoxin IV and the endoplasmic reticulum was associated with Lewy body pathology in PD and DLB.

On Western blotting, peroxiredoxin IV stained as two prominent proteins at approximately 27kd and 65kD under reducing conditions. Peroxiredoxin IV is predicted to be 31kD with a 4kD leader sequence indicating the monomer in human brain is the cleaved mature form. This protein functions as a committed dimer and the 65kD form is probably the dimer although it is slightly larger than predicted. Peroxiredoxin IV was abundant in neurones, both light and confocal immunohistochemistry showed prominent granular staining in the neurones with low level staining in oligodendrocytes. Astrocytes did not appear to be labelled and low level staining microglia appeared to be from ingested material. Peroxiredoxin IV co-localised with the ER/Golgi marker SAR1 indicated that peroxiredoxin IV is confined to the ER in human brain supporting cellular pulse chase studies. SAR1 had a slightly larger area of distribution suggesting that peroxiredoxin IV does not translocate to the Golgi. However, peroxiredoxin IV did not co-localise with the lysosomal marker Lamp2 indicating it is not associated cellular degradation *via* lysosomes.

Co-localization of peroxiredoxin IV with the Lewy body marker α-synuclein showed that there is a close association between Lewy body formation and the ER. In pre-Lewy body neurones, α-synuclein and peroxiredoxin IV positive vesicular aggregations were observed to be coalescing between the ER and Golgi supporting recent findings in yeast models that α-synuclein blocks docking of ER vesicles with the Golgi apparatus. A variety of Lewy body forms were observed and all forms were closely associated with the ER as determined by the peroxiredoxin IV staining.

In conclusion these results show for the first time that peroxiredoxin IV is present in human neurones and that it is confined to the ER. These results also suggest that Lewy bodies are seeded from α-synuclein vesicular aggregations unable to dock with the Golgi apparatus giving new insights into the formation of this pathologic structure.