OSTEOPONTIN INTERACTS WITH PROTEINS INVOLVED IN APOPTOSIS, PROTEOLYSIS AND MICROTUBULE STABILITY - A POSSIBLE NEUROPROTECTIVE ROLE IN PARKINSON'S DISEASE

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Osteopontin (OPN) is a glycosylated phosphoprotein found in brain and peripheral tissues. Its expression in the substantia nigra in Parkinson's disease (PD) is decreased in surviving dopaminergic neurones and is present in activated microglia, suggesting a neuroprotective role. Indeed, OPN protects against MPP+-induced cell death in primary mesencephalic cultures and 6-OHDA-induced cell loss in vivo, while inactivation of OPN aggravates cell death. OPN regulates iNOS transcription, hydrogen peroxide formation and cytokine levels, although the specific protein interactions involved in OPN-mediated neuroprotection are unknown, and are the focus of this study.

OPN-protein interactions were investigated by yeast two hybrid assay, using full length human OPN to screen a human foetal brain cDNA library. Interactions were confirmed using co-immunoprecipitation and glutathione-S-transferase (GST) pull-down assays from rat brain.

A number of proteins involved in apoptosis, protein degradation, and microtubule stability interacted with OPN, including MAP1A and MAP1B which regulate microtubule stability; RNF138, an E3 ubiquitin-ligase; proteasome β1 subunit, a subunit of the 20S proteasome involved in the ubiquitin-dependent cleavage of peptides; BAT3, SGTα and EF1A, proteins implicated in control of apoptosis; DnaJB1, a co-chaperone of Hsp70s and pleiotrophin, a growth factor. Co-immunoprecipitation and GST pull-down assays confirmed the interactions in rat brain. Some of these interactions were inhibited by amino acid substitutions at known OPN protein binding sites, specifically Y165A and D139E.

These findings indicate a role for OPN in regulation of apoptosis, proteolysis and cytoskeleton stability in the brain, suggesting that OPN may act as a multifunctional neuroprotective agent in PD.