Investigation of the neuroprotective protein parkin – solubility, ligand binding and ubiquitination

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ABSTRACT

The parkin protein is an E3 ligase which mediates ubiquitination of cellular proteins destined for degradation by the 26S proteasome. Mutations in the parkin gene and subsequently loss of parkin function are associated with a familial form of Parkinson's disease indicating that a functional parkin protein is essential for survival of dopaminergic neurons in substantia nigra. Parkin activity is also inhibited by post-translational modifications whereby lack of parkin activity contributes to the progression of sporadic Parkinson's disease. The aim of this thesis was to investigate the impact of different factors on parkin activity. It was found that the parkin protein is phosphorylated in cells and that the N-terminal part contains several phosphorylation sites which could be phosphorylated in vitro by different kinases. Phosphorylated parkin has retained its ubiquitinating activity. In addition, it was found that the N-terminal part of parkin binds tubulin and microtubuli. Phosphorylation of this part of parkin did not inhibit its microtubuli-binding activity.

Aggregation and thereby inactivation of parkin occur when cells are exposed to oxidative stress, proteasomal inhibition or apoptotic agents. Certain disease-associated mutations in the parkin gene also induce aggregation of the protein at high intracellular concentration in transiently transfected cells. However, these mutant proteins are soluble when expressed at a lower intracellular concentration in stably transfected cells. This indicates that aggregation is not the pathogenic mechanism of these mutants, but that other molecular mechanisms such as ligand binding, intracellular localisation or enzymatic activity are affected by these mutations.