[10:40~11:20]

Potential role of onconeural antigen, cdr2 in dopaminergic neuronal cell death: involvement of calpain and proteasome system.

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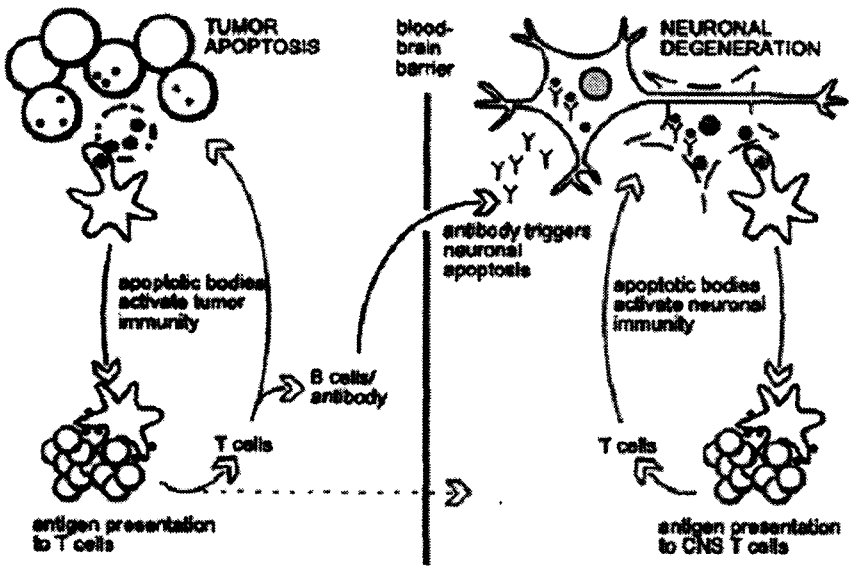
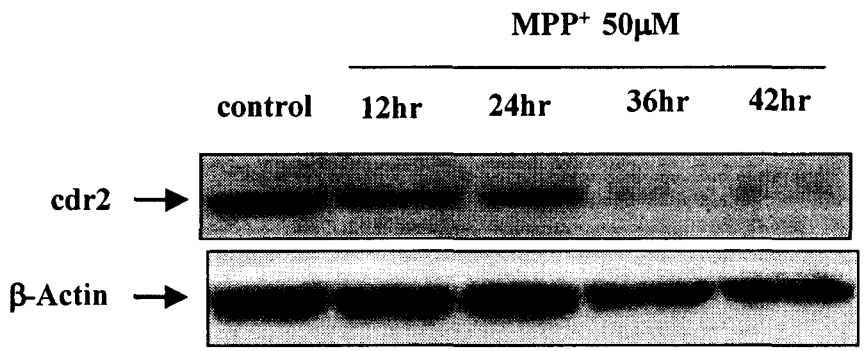
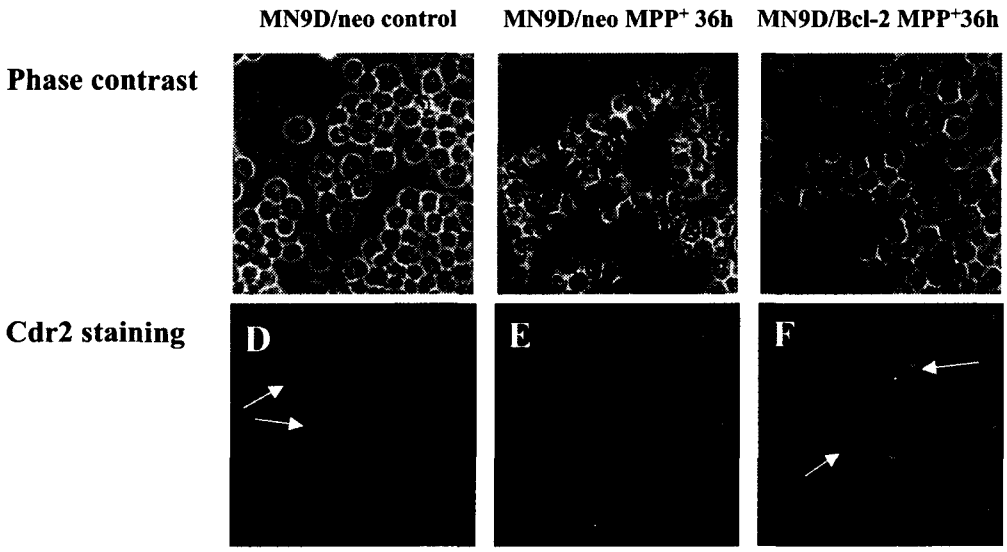
The cdr2 is a cerebellar degeneration-related gene encoding putative paraneoplastic cerebellar degeneration antigens. Cdr2 is originally expressed in the central nervous system. Its ectopic expression in the peripheral nervous system leads to a generation of antibody eventually resulting in neurodegeneration of Perkinje cells in the cerebellum as shown below.



In the preliminary study, levels of cdr2 protein decreased in a time-dependent manner in MPP+-induced MN9D cell death as determined by western blot analysis. As such, discernible decrease in cdr2 protein level was detected 36 hrs following MPP+ treatment.

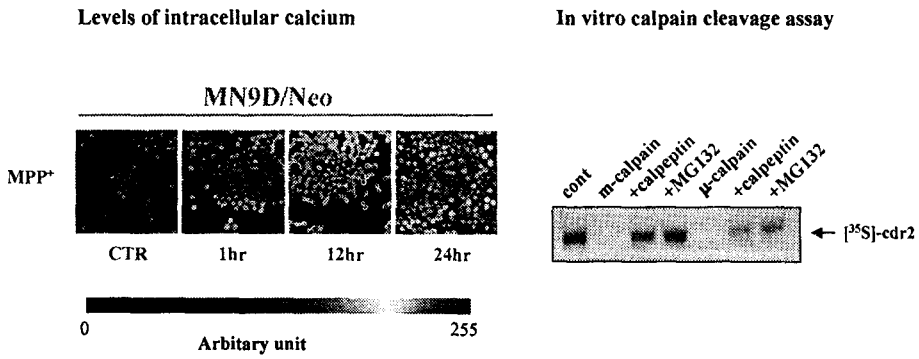


Using immunocytochemistry, it was also confirmed that level of cdr2 protein indeed decreased in individual dopaminergic neurons both in vitro culture and in vivo animal models exposed to MPP+. Overexpression of Bcl-2 completely blocked MPP+-induced down-regulation of cdr2 in MN9D cells.

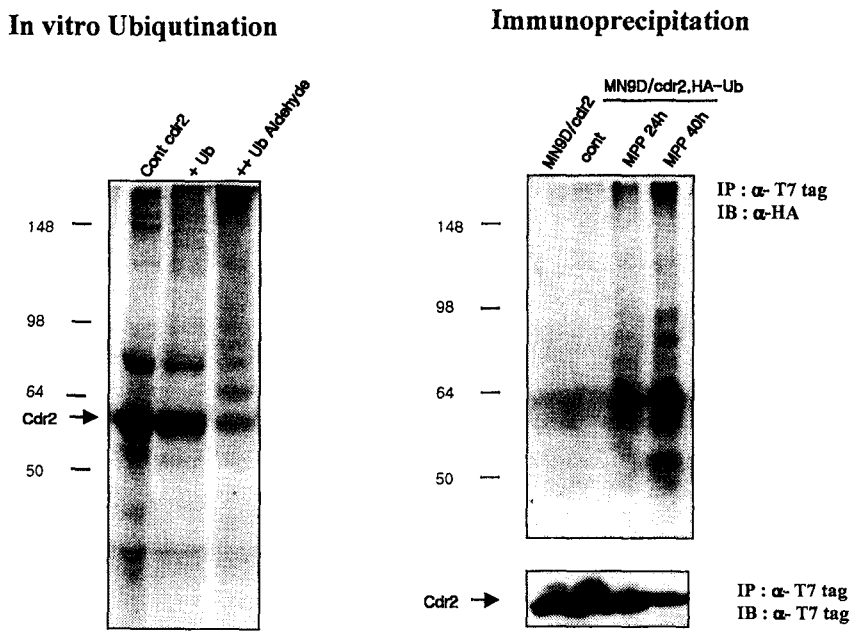


To examine array of protease(s) responsible for the drug-induced degradation of cdr2, MN9D cells were treated with MPP+ in the presence or the absence of various protease inhibitors. Results indicated that cdr2 may be degraded by either calpain and/or ubiquitine proteasome system. This notion was further supported by in vitro calpain and ubiquitination assay.

calapin-mediated cleavage of cdr2

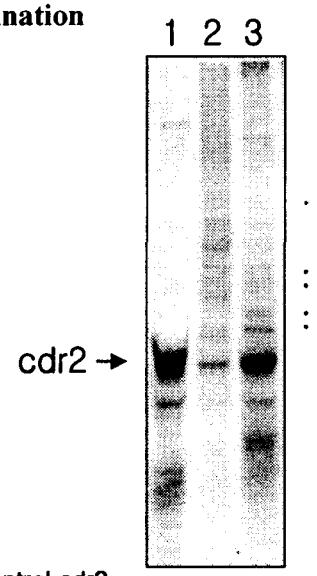


proteasome-mediated cleavage of cdr2



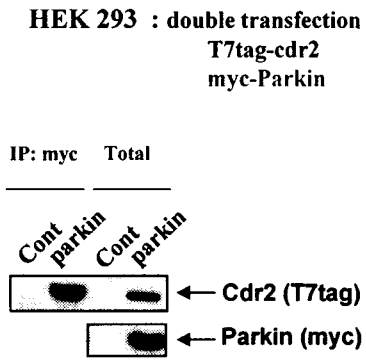
Interestingly, both in vitro ubiquitination assay and co-immunoprecipitation study raised the possibility that parkin binds to cdr2 and acts as one of E3 ligases to degrade cdr2 during MPP+-induced neuronal death In various conditions that preserved levels of cdr2, MPP+-induced loss of dopaminergic neurons were significantly prevented as exmined in both MN9D cells.

In vitro Ubiqutination

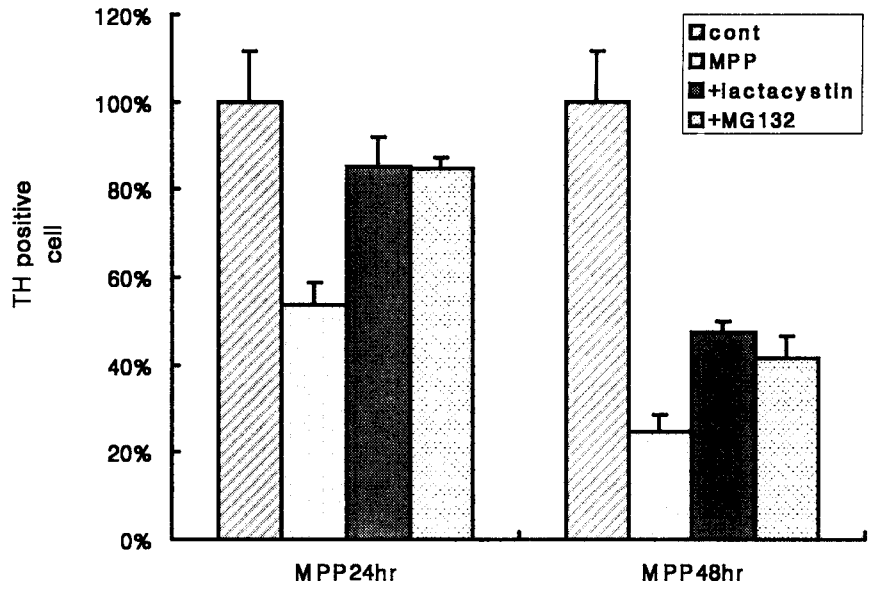


1. Control cdr2
2. Cdr2 + ubiquitination kit
3. Cdr2 + E1, E2, E3(parkin),Ubiquitin, ATP,Ubiquitin Aldehyde

Immunoprecipitation



Double immunocytochemistry using anti-cdr2 and anti-tyrosine hydroxylase also revealed that co-treatment with calpeptin (a calpain inhibitor), MG132 or lactacystin (proteasome inhibitors) restored MPP+-induced decrease in cdr2 protein levelsand subsequently rescued cells from MPP+-induced death in primary cultures of dopaminergic neuronal cells.



Taken together, present data suggest that cdr2 seems to play a critical role for determining cell death and survival in experimental models of Parkinson's disease.