CDK5-mediated phosphorylation of VRK3 in neuronal apoptosis

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Research question and background

Although extracellular signal-related kinase 1/2 (ERK 1/2) activity is generally associated with cell survival, prolonged ERK activation induced by oxidative stress also mediates neuronal cell death. Because oxidative stress is a common feature of neurodegenerative diseases including Alzheimer's disease (AD) and Parkinson's disease (PD), and ALS, it is worth to study endogenous protective mechanism against oxidative stress-induced neuronal cell death. We show that oxidative stress-induced cyclin-dependent kinase 5 (CDK5) activation stimulates neuroprotective signaling via phosphorylation of vaccinia-related kinase 3 (VRK3) at Ser 108. The binding of vaccinia H1-related (VHR) phosphatase to phosphorylated VRK3 increased its affinity for phospho-ERK and subsequently downregulated ERK activation. Overexpression of VRK3 protected human neuroblastoma SH-SY5Y cells against hydrogen peroxide (H_2O_2)-induced apoptosis.

Methods and tissues used

Brain sample procurement. Formalin-fixed, paraffin-embedded prefrontal cortical brain samples and frozen prefrontal cortical brain samples from adults were procured from the Netherlands Brain Bank (NBB). The cases analyzed in this study were strictly defined according to the Braak and Braak staging, with control cases being brains completely devoid of Lewy bodies and amyloid-beta pathologies (Braak and Braak stage 0-2). The characteristics of these samples are provided in the Table 1.

Brain protein extraction. Snap-frozen post-mortem human brain were grinded and homogenized in Triton lysis buffer (20 mM Tris, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton-X, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4) supplemented with protease inhibitors from Roche. Samples were sonicated and centrifuged at 15,000 rpm for 30 min at 4 °C. The supernatant was removed and the protein concentration determined by Bradford Reagent (Amresco). Samples were denatured for 5 min at 95 °C with SDS sample buffer containing β-mercaptoethanol. The proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes.

Immunohistochemistry analysis of human brain. Four-micrometer-thick sections from the prefrontal cortical brain were cut at regular intervals on glass slides. Paraffin-embedded tissue sections were first deparaffinized in xylene, then rehydrated with decreasing concentrations of ethanol and placed in water. Sections underwent antigen retrieval using citrate buffer (10 mM sodium citrate, pH 6.0), then were washed and blocked with PBS containing 3% BSA and 0.2% Triton X-100 for 1 h at room temperature. Primary antibodies were diluted in blocking solution. After overnight incubations, sections were washed three times with PBS. Secondary antibodies coupled to Alexa fluorophores were diluted in blocking solution. After 1 h incubation in the dark, sections were washed three times with PBS. For counterstaining, sections were further incubated with 2 μg/ml Hoechst for 10 min at room temperature, then washed three times with PBS. Slices were mounted and visualized by fluorescence microscopy.

Results and conclusion

We showed an association between phospho-VRK3 levels and the progression of

human AD and PD. Together our work reveal that endogenous protective mechanism against oxidative stress-induced neuronal cell death.

Table 1 Clinicopathological details of subjects used for western blotting and immunohistochemistry.

Profile and Clinical data of AD and PD patients and control cases for immunohistochemistry

Diagnosis	NBB no	Age(yr)	Sex	Braak	Amyloid	BraakIb	PMD(hours)
Control	11-091	76	M	0	O	0	06:45
	05-074	79	M	1	A	1	06:30
	12-070	79	M	2			05:45
	12-104	79	M	2	A		06:30
	09-300	71	V	1	A		07:10
AD	08-107	77	M	4	C		04:05
	09-185	70	M	4	C		04:00
	12-022	79	M	4	C		04:05
	07-315	71	M	5	B		05:25
	11-002	71	V	5	C		04:15
	05-154	67	V	6	C		06:05
PD	11-117	78	M			4	06:15
	04-108	73	M	1	A	5	05:35
	09-207	67	V	1	B	6	07:40
	09-235	70	M	1	B	5	05:15
	10-322	71	M	3	C	6	05:05
	11-026	77	M	3	O	6	05:50

NBB no, Netherlands Brain Bank number; Braak: braak stage based on amyloid-beta(0-2: non-demented AD, 3-6: mild to severe)
Amyloid, a type of amyloid; BraakIb, braak stage based on lewy body (0-2: non-demented PD, 3-6: mild to severe);
PMD, post-moterm delay; Control, non-demented control; AD, Alzheimer's disease; PD, Parkinson's disease; M, male.

Profile and Clinical data of AD and PD patients and control cases for Western blotting

Diagnosis	NBB no	Age(yr)	Sex	Braak	Amyloid	BraakIb	PMD(hours)
Control	12-070	79	M	2			05:45
	09-300	71	V	1	A		07:10
	12-049	70	V	2	A		07:35
	12-059	78	V	2	A		04:35
AD	08-107	77	M	4	C		04:05
	09-185	70	M	4	C		04:00
	12-022	79	M	4	C		04:05
	07-315	71	M	5	B		05:25
PD	11-117	78	M			4	06:15
	04-108	73	M	1	A	5	05:35
	09-207	67	V	1	B	6	07:40
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