Subcellular Compartmentalization of P-ERKs in the Lewy Body Disease Substantia Nigra

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KEYWORDS: extracellular signal–related kinase (ERK); Lewy body disease; substantia nigra; mitochondria; mitogen activated protein kinase

Extracellular signal–regulated kinases (ERKs) are involved in regulating neuronal survival, differentiation, and plasticity. Recent studies also indicate that ERKs can play a detrimental role in models of oxidative neuronal injury.1,2 Our previous studies showed discrete cytoplasmic accumulations of phosphorylated ERKs (P-ERKs) in Parkinson’s disease and other Lewy body diseases, and in 6-hydroxydopamine–treated neuronal cell cultures.3 As the effects of ERK phosphorylation are critically dependent upon subcellular localization and access to downstream targets, we investigated the subcellular distribution of P-ERKs in Lewy body disease using double-label confocal microscopy. The association of P-ERK granules with a subset of (sometimes enlarged) mitochondria suggests a potential interaction between mitochondrial function and the ERK signaling pathway in degenerating dopaminergic neurons.

METHODS

Substantia nigra sections from a diffuse Lewy body disease patient were stained for P-ERKs as described previously3 and then incubated with organelle-specific antibodies and appropriate Cy3-conjugated secondary antibodies. Negative controls included double labeling of sections substituting nonimmune mouse or rabbit IgG for the primary antibodies. The slides were observed using a Zeiss laser scanning confocal imaging system.

RESULTS AND DISCUSSION

Immunofluorescence staining showed coarse, granular, or vesicular-appearing accumulations of P-ERKs in substantia nigra neurons. The immunoreactivity of the P-ERKs was confined to the cytoplasm and did not colocalize with nuclear stains.3
FIGURE 1. Double labeling of P-ERK (A) with the 60-kDa mitochondrial protein (B). In the overlap image (C), note that regions of colocalization (arrows) often appear as smaller punctate areas within the P-ERK profile. Enlarged mitochondria occasionally appear to be associated centrally within vesicular-appearing P-ERK profiles (star).
P-ERK granules were occasionally colocalized with the early endosome marker Rab5, but not with markers of the lysosome (cathepsin D), 20S proteasome (β subunit), or endoplasmic reticulum (cytochrome P450 reductase). P-ERK immunoreactivity was more commonly co-localized with 60-kDa (Fig. 1) and 110-kDa mitochondrial proteins, and with manganese superoxide dismutase. The areas of colocalization, verified by orthogonal analysis, usually appeared as smaller punctate areas within the P-ERK profile. A second type of association was also observed in which vesicular-appearing P-ERK accumulations appeared to envelop, rather than colocalize with, enlarged mitochondria (Fig. 1, star).

Sustained activation of ERKs is associated with 6-OHDA toxicity in B65 (see Ref. 2) and SH-SY5Y cell lines (C.T. Chu, unpublished data), and MEK inhibitors confer significant protection. P-ERK staining is cytoplasmic, attaining a discrete punctate appearance following commitment to cell death. Moreover, substantia nigra neurons in patients with the full spectrum of Lewy body diseases display unusual, discrete cytoplasmic, but not nuclear, accumulations of P-ERK immunoreactivity. In this study, we found that the P-ERK granules can be associated with an early endosomal marker, but were more commonly associated with mitochondrial markers.

Partial colocalization of a subset of P-ERK granules with an early endosome marker, Rab5, may reflect physiologic recruitment and assembly of Ras-ERK signaling cascades on endosomal surfaces. Recent evidence that ERK1 can phosphorylate Rab5 further suggests the possibility for cross-talk between the Ras-ERK signaling pathway and the endocytic machinery.

Mitochondria are vulnerable to various insults and can undergo enlargement and structural disorganization, associated with decreased membrane potential and reduced ATP production. The association of P-ERK granules with a subset of (sometimes enlarged) mitochondria suggests a potential interaction between mitochondrial function and the ERK signaling pathway in dopaminergic neurons. P-ERKs have been reported to form signaling modules with other MAP kinases in cardiac mitochondria. Alternatively, it is possible that these structures reflect sequestration of damaged mitochondria.

REFERENCES