IJAE
Vol. 116, n. 1 (Supplement): 172, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Morphological analysis of genetic modulation of PINK1 on mitochondrial alterations, autophagy and cell death

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Mutations in the PTEN-induced putative kinase1 (PINK1) represent the second most common cause of autosomal recessive Parkinson's disease. The PINK1 protein has a mitochondrial localization and interacts with a variety of proteins, including the pro-autophagy protein beclin1 and the ubiquitin-ligase parkin. In particular, PINK1 is able to recruit parkin to the surface of dysfunctional mitochondria, to promote the ubiquitination of several mitochondrial proteins and the subsequent activation of the mitophagy cascade. Aim of this study was to use a dopaminergic cell model and transmission electron microscopy to characterize whether the modulation of PINK1 expression: (i) modifies the number and morphology of mitochondria and of autophagy organelles (autophagosomes); (ii) alters the recruitment of beclin1, parkin and ubiquitin to the mitochondria; (iii) affects cell survival. We used PC12 cells transfected either with the empty vector (pcDNA), or vectors expressing wild type PINK1 (PINK1wt), a pathogenic mutant (PINK1^{W437X}), shRNA against rat PINK1 (shPINK1) or scramble (shSCR). Samples were analyzed both in baseline conditions and following methamphetamine (METH) treatment to provide a neurotoxic, autophagy-dependent stimulation. We showed that, especially upon METH exposure, the modulation of PINK1 levels dramatically affected the morphology and clearance of mitochondria. In fact, the number of abnormal mitochondria was reduced in PINK1wt, while it was significantly increased upon shPINK1 and also, to a lesser extent, in PINK1^{W437X} cells. In keeping with this, mitochondrial ubiquitin clusters and mitochondrial levels of parkin and beclin1 were increased in PINK1wt cells while they were reduced both in PINK1 silenced and PINK1^{W437X} cells. Interestingly, the number of autophagic vacuoles was unaffected by PINK1 modulation in baseline conditions, and was significantly reduced only in cells lacking functional PINK1 and upon METH exposure. All these effects were significantly associated with a modulation of apoptotic cell death. Our data provide robust sub-cellular evidence that PINK1 counteracts neurodegeneration by simultaneously recruiting beclin1, parkin and ubiquitin and thus enhancing the clearance of damaged mitochondria. In the absence of functional PINK1 and upon autophagy stress, we observed a failure of the autophagy system at large, with marked accumulation of dysfunctional mitochondria and dramatic increase in apoptotic cell death.

Keywords: PINK1, Parkinson's disease, methamphetamine, mitophagy, apoptosis