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[Autophagy 5:1, 61-74; 1 January 2009]; ©2009 Landes Bioscience

Research Paper

PARP-1 is involved in autophagy induced by DNA damage

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Key words: autophagy, DNA damage, PARP-1, energy depletion, Beclin 1, ATG-5, mTOR, doxorubicin

Autophagy is a lysosome-dependent degradative pathway frequently activated in tumor cells treated with chemotherapy or radiation. PARP-1 has been implicated in different pathways leading to cell death and its inhibition potentiates chemotherapy-induced cell death. Whether PARP-1 participates in the cell's decision to commit to autophagy following DNA damage is still not known. To address this issue PARP-1 wild-type and deficient cells have been treated with a dose of doxorubicin that induces autophagy. Electron microscopy examination and GFP-LC3 transfection revealed autophagic vesicles and increased expression of genes involved in autophagy (*bnip-3, cathepsin b and l and beclin-1*) in wild-type cells treated with doxo but not in *parp-1*$^{-/-}$ cells or cells treated with a PARP inhibitor. Mechanistically the lack of autophagic features in PARP-1 deficient/PARP inhibited cells is attributed to prevention of ATP and NAD$^+$ depletion and to the activation of the key autophagy regulator mTOR. Pharmacological or genetical inhibition of autophagy results in increased cell death, suggesting a protective role of autophagy induced by doxorubicin. These results suggest that autophagy might be cytoprotective during the response to DNA damage and suggest that PARP-1 activation is involved in the cell's decision to undergo autophagy.

Introduction

Autophagy is a dynamic process of protein degradation, which is typically observed during nutrient deprivation. Whether autophagy in cancer cells causes death or protects cells is controversial. In multiple studies, autophagy has been inhibited pharmacologically or genetically, resulting in contrasting outcomes—survival or death—depending on the specific context. Autophagic cell death is characterized by massive degradation of cellular contents, including essential organelles such as mitochondria, by means of complicated intracellular membrane/vesicle reorganization and lysosomal activity.[5,10,19] It is involved in development and stress responses and has been observed in multiple neurodegenerative diseases.[5,10,19] Because the mechanism is not well defined, some autophagic cell death events might have been attributed to apoptosis. Moreover, these two modes of cell death frequently occur in parallel. For example, deprivation of neural growth factor induced simultaneous autophagic and apoptotic cell death in primary sympathetic neurons.[9] However, caspases are not required for autophagic cell death,[5,19] and Bcl-2 and Bcl-X_L do not inhibit autophagic cell death in a mammary epithelial morphogenesis model.[5] Furthermore, like apoptosis, autophagic cell death is involved in preventing tumorigenesis: autophagic activity was found to be suppressed in malignant tumors;[10] some autophagic regulators, such as Beclin 1,[1,26] and death-associated protein kinase (DAPk),[12,29] are putative tumor suppressors.

The nuclear enzyme PARP-1 converts β-nicotinamide adenindinucleotide (NAD$^+$) into polymers of poly(ADP ribose)(PAR), which participate in regulating nuclear homeostasis.[27] Many different cell insults infringing DNA damage have been shown to be able to activate PARP. However, once hyperactivated by genotoxic stress, PARP-1 causes NAD$^+$ and ATP depletion, eventually leading to irreversible cellular energy failure and cell death.[11] The pathophysiological significance of PARP-1 hyperactivation is well exemplified by the remarkable therapeutic efficacy of PARP-1 inhibitors in experimental models of disorders characterized by DNA damage such as ischemia, diabetes, shock, inflammation and cancer.[11] Recently, several studies have broadened the role of poly(ADP-ribosyl)ation in cell killing showing that PARP-1 activation also occurs during apoptosis, and inhibition of PAR formation impairs activation of the apoptotic machinery prompting a cascade of events leading to PAR-dependent mitochondrial dysfunction and rapid release of apoptosis-inducing factor (AIF).[36] Despite their pathogenetic relevance, the involvement of PARP-1 in autophagy and the regulatory mechanisms underlying energetic derangement during PARP-1 hyperactivation are still elusive. To address this issue, we used *parp-1*$^{+/+}$ and *parp-1*$^{-/-}$ immortalized mouse embryonic fibroblasts cells exposed to the PARP-1 activator and clinically relevant drug doxorubicin.

In the present study we show that DNA damage induced with a moderately high dose of doxo elicits a cell response with morphological and biochemical characteristics of autophagy. PARP-1 deficient or inhibited cells display a decreased autophagic response. The

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Submitted: 09/16/08; Revised: 10/22/08; Accepted: 10/27/08

Previously published online as an *Autophagy* E-publication:
http://www.landesbioscience.com/journals/autophagy/article/7272