Conclusion

PARP and cell death

Caspases may be indispensable for typical apoptotic morphology but they are not the sole determinants of life and death decisions in programmed cell death (PCD). Evidences are accumulating that PCD can occur in complete absence of caspases (Blagosklonny, 2000). One such form of caspase independent cell death is termed as paraptosis (Sperandio *et al.*, 2000). Paraptosis also appears to occur during development of the nervous system, as well as in some cases of neurodegenerative disorders (Stoka *et al.*, 2007). Paraptosis has been described to be mediated by several proteins or factors; Poly(ADP-ribose) polymerase (PARP) being one of them.

PARP-1 mediated cell death is also recently named as 'parthanatos', after poly(ADP-ribose) (PAR) polymer, which is a product of Poly (ADP ribose) polymerase (PARP)-1 activation and *thanatos*, which is the Greek personification of death and mortality (Harraz *et al.*, 2008). PARP is a DNA damage sensor enzyme located in nucleus. PARP enzymes catalyze the attachment of ADP Ribose units from NAD^+ to nuclear proteins following DNA damage by toxic stimuli.

A role for PARP in cell death has previously been demonstrated. PARP inhibitors prevented MNNG induced cell death in HeLa cells (Cipriani *et al.*, 2005). PARP knockout mice are resistant to the development of diabetes induced by the beta-cell toxin streptozocin; PARP-/- mice maintained intracellular NAD^+ levels and resisted streptozocin induced death (Burkart *et al.*, 1999). Over the past decade, a large body of evidence demonstrates that activation of PARP-1 significantly increases during brain ischemia and plays a pivotal role in ischemia induced neuronal injury (Chiarugi, 2005). PARP-1 deficient neurons are protected against cell death caused by NMDA treatment and oxygen & glucose deprivation *in vitro*. Moreover, PARP-1 knockout mice are resistant to neuronal injury *in vivo*, following middle cerebral artery occlusion (Eliasson *et al.*, 1997). PARP-1dependent neuronal death is mediated by a key factor, AIF (Yu *et al.*, 2002). The biological importance of PARP-1 and AIF in focal brain ischemia was further elucidated by various studies (Hong *et al.*, 2004; Komjati *et al.*, 2004; Plesnila *et al.*, 2004).

D. discoideum is known to possess nine PARP genes and does not have caspase gene thus, it provides a better model system to dissect out the role of PARP in paraptosis.

The aim of this study was to identify and establish the kinetic events during oxidative stress induced cell death in *D. discoideum. D. discoideum* that lacks caspases has been used as a model system in this study to explore the role of PARP in paraptotic cell death mechanisms. The information on the role of PARP in *D. discoideum* cell death and development is limited. Therefore, present study describes the events during oxidative stress induced PARP mediated cell death in *D. discoideum* when the action of activated PARP is intercepted by PARP inhibitor benzamide as well as by PARP antisense. This is the first report where the involvement of PARP and the downstream events during oxidative stress induced cell death and development in *D. discoideum,* an ancient eukaryote, are established.

Our results also emphasize that a cell death stimulus like oxidative stress leads to PARP activation causing depletion in the cellular NAD^+ and ATP levels. Subsequent to PARP activation mitochondrial membrane potential (MMP) change was observed which in turn was followed by release of AIF from mitochondria. Released AIF, upon translocation to nucleus caused large scale DNA fragmentation, a hallmark feature of paraptosis. Hence, this study reinforces the earlier observation that PARP-1 activation is required for the translocation of AIF from the mitochondria to nucleus (Yu *et al.*, 2002). PARP-1 chemical inhibitors, PARP-1 genetic ablation, AIF knockdown, or neutralizing AIF using anti-AIF antibodies prevent AIF translocation to the nucleus and inhibit alkylating DNA damage mediated cell death in a variety of experimental paradigms (Lorenzo and Susin, 2007; Moubarak *et al.*, 2007; Xu *et al.*, 2006; Yu *et al.*, 2002). This indicates a pivotal role of mitochondrial AIF release and nuclear translocation in parthanatos. Elucidation of this paraptotic pathway is of key importance, both in understanding the mechanism of PARP mediated cell death and also in identifying potential drug targets.

PARG in cell death

PARP activity is regulated by a cytosolic enzyme poly(ADP-ribose) glycohydrolase (PARG). It was shown that the half life of PAR decreases to less than 1 min after MNNG induced DNA damage (Alvarez-Gonzalez and Althaus, 1989) due to the fact the PARG displays a higher affinity for PAR (Hatakeyama *et al.*, 1986). Thus, PAR catabolism in the nucleus could be regulated, at least in part, by a nuclear-cytosolic shuttling of PARG. PARG shuttling to and from the nucleus is probably a very efficient and rapid process that would make it very difficult for the actual detection of PARG translocation. Supporting this notion, Winstall *et al.*, (1999) demonstrated that nuclear PAR (induced after DNA damage) was undetectable in cells overexpressing PARG despite the fact that overexpressed PARG was observed exclusively in the cytosol. Since poly(ADP-ribosyl)ation of nuclear proteins is an ongoing process within cells, PARG probably constantly shuttles between the nucleus and cytosol depending upon PARP-1 activity. We have demonstrated that oxidative stress induced cell death is partially rescued by PARG inhibition. Our results suggest that PARG could influence PARP-mediated cell death and PARG inhibition is protective in function during oxidative stress induced cell death. It is therefore conceivable that PAR signaling, together with the decrement in NAD^+ and/or ATP, could initiate PARG shuttling and cause its accumulation in the nucleus, but other mechanisms may also be responsible. The elucidation of the mechanisms by which PARP-1 activation signals translocation of PARG would be of paramount importance. Further PARG knockout in *D. discoideum* would enlighten us with the significance of PARP and PARG evolving simultaneously in multicellular organisms.

Proteases in paraptosis and necrosis

Our study also highlights that necrosis occurs in a programmed fashion where proteases cause MMP changes followed by plasma membrane rupture and early loss of plasma membrane integrity. Furthermore, our results suggest that Calpains and Cathepsin D which are instrumental in dismantling the cell during paraptotic cell death

act downstream to PARP. Thus PARP, AIF, Calpains and Cathepsin D are the key players in *D. discoideum* paraptotic cell death acting in a sequential manner.

PARP and development

Another aspect of this study included the role of PARP in development of *D. discoideum.*. Oxidative stress (HA or cumene H_2O_2) showed dose dependent delay on *D. discoideum* growth and development which could also be partially intercepted by benzamide. Studies on long term effects of PARP inhibition on *D. discoideum* development under oxidative stress demonstrated that second generation cells showed normal development signifying that PARP inhibition has no deleterious effect on *D. discoideum* development. However, constitutive PARP down-regulation resulted in blocked development while no effect was observed on growth. Stage specific PARP down-regulation arrested development at slug stage. These results suggest that presence of PARP is essential for complex differentiation and its function may be linked to multicellularity. Involvement of PARP in multicellularity is highly plausible but has to be confirmed by *parp* overexpression and *parp* knockout in *D. discoideum.*

In conclusion, we put forth that *D. discoideum* exhibits paraptosis which is mediated by PARP. PARP and AIF are the major players governing *D. discoideum* cell death kinetics during paraptosis and necrosis induced by oxidative stress. Therefore, PARP-1, PAR polymer and AIF could be potential targets for therapy of neurologic disorders (Wang *et al.*, 2009). This model could also be exploited to screen the compounds/drugs that can intercept or activate necrotic and paraptotic types of cell death or diseases involving PARP.

Foremost outcome of this research is that PARP also plays a key role during development and could be linked to multicellularity; thereby indicating the evolutionary significance of PARP. However, further investigation is required to elucidate the pivotal role of PARP during development. In a nutshell, these results provide new clues on the role of PARP in paraptosis and poly(ADP-ribosyl)ation affecting the complex signaling during cell death and development.

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