Poly (ADP-Ribose) Polymerase-1 causes mitochondrial damage and neuron death mediated by *Bnip3*, *J Neurosci.* 2014 Nov 26;34(48):15975-87

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Background

Hypoxia/ischemia is one of the major causes of mitochondrial dysfunction and neuronal cell death. So far, it has been reported that the DNA damage repair enzyme Poly (ADP-Ribose) Polymerase-1 (PARP1) gets activated during hypoxia/ischemia, leading to mitochondrial membrane permeability transition

and caspase independent neuronal death mediated by nuclear translocation of the mitochondrial proapoptotic factor AIF. On the other hand, Bnip3, a proapoptotic BH3-only protein of the Bcl-2 family, is also found to be upregulated during hypoxia/ischemia, resulting in mitochondrial permeability transition and neuronal death in a manner similar to that of PARP1. This led the authors to hypothesize that the pathway of neuronal cell death induced by PARP1 and Bnip3 may be linked. Also, it is reported that PARP1 mediated NAD⁺ depletion leads to inhibition of SIRT1,¹ a NAD⁺ dependent class III histone deacetylase enzyme which targets many transcription factors responsible for maintaining mitochondrial integrity including the FoxO (Fork-head box O) family of proteins. Thus, they also hypothesized that PARP1 mediated inhibition of SIRT1 may lead to derepression of FoxO3a resulting in upregulation of Bnip3 under hypoxic conditions.

Study Design

All the experiments were done in primary neuronal culture from 16th day embryonic mouse cortices. Hypoxia was induced by a mixture of 5%CO₂/95%N₂ in a humidified hypoxic chamber at 37°C. 50 μM of MNNG, a DNA alkylator, was used for 30 min to activate PARP1. As negative controls, cells were either treated with the PARP1 inhibitor PJ34 or were taken from *Parp1*^{-/-} and *Bnip3*^{-/-} mice.

The authors used fluorescence microscopy for quantifying intracellular propidium iodide (red) and calcein fluorescence (green) as indicators for dead and live cells respectively and demonstrated that hypoxia induces neuronal cell death which occurs via activation of PARP1 and Bnip3. During normoxic conditions, MNNG mediated activation of PARP1 leads to upregulation of Bnip3 which in turn leads to mitochondrial membrane potential loss as measured by the fluorescent dye JC-1 under confocal microscope. Bnip3 dependent nuclear localization of mitochondrial AIF was also observed under such conditions which could be mitigated by exogenous addition of NAD⁺. Hyperactivation of PARP1 during hypoxia was found to cause a decline in the NAD⁺ levels in the neuronal cells which in turn lead to inhibition of Sirtuin 1 (SIRT1). Immunoprecipitation fol-

lowed by immunoblotting revealed that SIRT1 interacts directly with FoxO3a at a baseline level. Also, during hypoxia induced PARP1 activation, increased FoxO3a acetylation and nuclear translocation of FoxO3a was observed. Further, by chromatin immunoprecipitation followed by real time-PCR, increased binding of FoxO3a was observed at the upstream promoter region of Bnip3 during PARP-1 dependent hypoxia. SIRT1 silencing by lentiviral shRNA treatment during normoxic conditions in the absence of PARP1 activation caused Bnip3 upregulation, enhanced FoxO3a acetylation and increased binding of FoxO3a to the Bnip3 upstream promoter. FoxO3a silencing during hypoxia leads to decreased Bnip3 transcription- validating the role of FoxO3a as a transcription factor in the expression of Bnip3, decreased loss of mitochondrial membrane potential and enhanced neuronal survival.

Implication

The authors have demonstrated that under hypoxic conditions, PARP1 activation leads to NAD⁺ depletion which in turn leads to inhibition of SIRT1 causing hyperacetylation and nuclear translocation of FoxO3a. FoxO3a drives the expression of Bnip3 which leads to mitochondrial membrane potential loss and AIF release ultimately resulting in neuronal death.² Thus, Bnip3 is the mediator in PARP1 induced mitochondrial integrity loss and neuronal cell death during hypoxia. Interestingly, the authors here have shown for the first time that NAD⁺ depletion has a direct effect on inhibition of SIRT-1, which is a master regulator of genes like FoxO3a and Bnip3 responsible for maintaining mitochondrial integrity and function. Thus, by replacing NAD⁺ exogenously, PARP-1 mediated neuronal cell death during hypoxia maybe inhibited.³

The authors, however, have not shown the pathway leading to neuronal death downstream of Bnip3. Also, the role of AIF in Bnip3 mediated neuronal death needs to be explored further.

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Reference

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