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Role of mitochondria in apoptotic and necroptotic cell death in the developing brain

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ABSTRACT

Hypoxic–ischemic encephalopathy induces secondary brain injury characterized by delayed energy failure. Currently, therapeutic hypothermia is the sole treatment available after severe intrapartum asphyxia in babies and acts to attenuate secondary loss of high energy phosphates improving both short- and long-term outcome. In order to develop the next generation of neuroprotective therapies, we urgently need to understand the underlying molecular mechanisms leading to cell death. Hypoxia–ischemia creates a toxic intracellular environment including accumulation of reactive oxygen/nitrosative species and intracellular calcium after the insult, inducing mitochondrial impairment. More specifically mitochondrial respiration is suppressed and calcium signaling is dysregulated. At a certain threshold, Bax-dependent mitochondrial permeabilization will occur leading to activation of caspase-dependent and apoptosis-inducing factor-dependent apoptotic cell death. In addition, hypoxia–ischemia induces inflammation, which leads to the release of TNF-α, TRAIL, TWEAK, FasL and Toll-like receptor agonists that will activate death receptors on neurons and oligodendroglia. Death receptors trigger apoptotic death via caspase-8 and necroptotic cell death through formation of the necrosome (composed of RIP1, RIP3 and MLKL), both of which converge at the mitochondria.

1. Introduction

The causes of neonatal brain damage in response to hypoxic–ischemic insult are multifactorial. In the developing brain, lack of oxygen availability results in an initial depletion of high energy phosphates, in particular ATP and phospho-creatine. These levels return transiently to baseline but are followed by a second more prolonged depletion of cellular energy reserves accompanied by progression of brain injury [1,2]. These disturbances in energy metabolism trigger a number of pathophysiological responses but there is a common convergence at the level of the mitochondria. This range of injurious events includes the release of excitatory amino acids activating glutamate receptors (NMDA, AMPA), activation of nitric oxide synthase on neurons and oligodendroglial precursors, leading to increased intracellular Ca^{2+} and accumulation of reactive oxygen species (ROS) through release of nitric oxide (NO) [1,3].

2. Effect of calcium on mitochondria

Activation of NMDA and AMPA receptors after HI (hypoxia–ischemia), in response to excitotoxic amino acid release, results in cellular uptake of calcium. Indeed, we have found increased deposits of intracellular calcium in the endoplasmic reticulum, cytosol, nucleus and more significantly in the mitochondrial matrix of neurons [4]. Not only does this influx activate a number of intracellular signaling pathways, it is taken up by mitochondria causing mitochondrial swelling, impairment of respiration, increased production of reactive oxygen species and may ultimately trigger mitochondrial permeabilization (MP) and cell death [1,5,6] (Fig. 1).

3. Mitochondrial permeabilization and apoptosis

Mitochondria determine cell fate in many different ways. They can induce cell death due to their ability to release proapoptotic proteins, which occurs following MP. MP can occur either through selective

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; Apaf-1, apoptotic protease activating factor 1; CAD, caspase-activation DNase; cyt c, cytochrome c; Cy, cyclophilin; Drp-1, dynamin-related protein 1; ENDO, endonuclease; AIF, apoptosis-inducing factor; ATP, adenosine triphosphate; MOMP, mitochondrial outer membrane permeabilization; MP, mitochondrial permeabilization; NMDA, N-methyl-D-aspartate; HI, hypoxia–ischemia; LPS, lipopolysaccharide; MLKL, mixed lineage kinase domain-like protein; NO, nitric oxide; RIP, receptor-interacting serine/threonine-protein kinase 1; ROS, reactive oxygen species; TNF, tumor necrosis factor; TLR, Toll-like receptor; TRADD, tumor necrosis factor receptor type 1-associated DEATH domain; TRIF, TIR-domain-containing adapter-inducing interferon-β; TRAIL, TNF-related apoptosis-inducing ligand; TWEAK, tumor necrosis factor (ligand) superfamily, member 12

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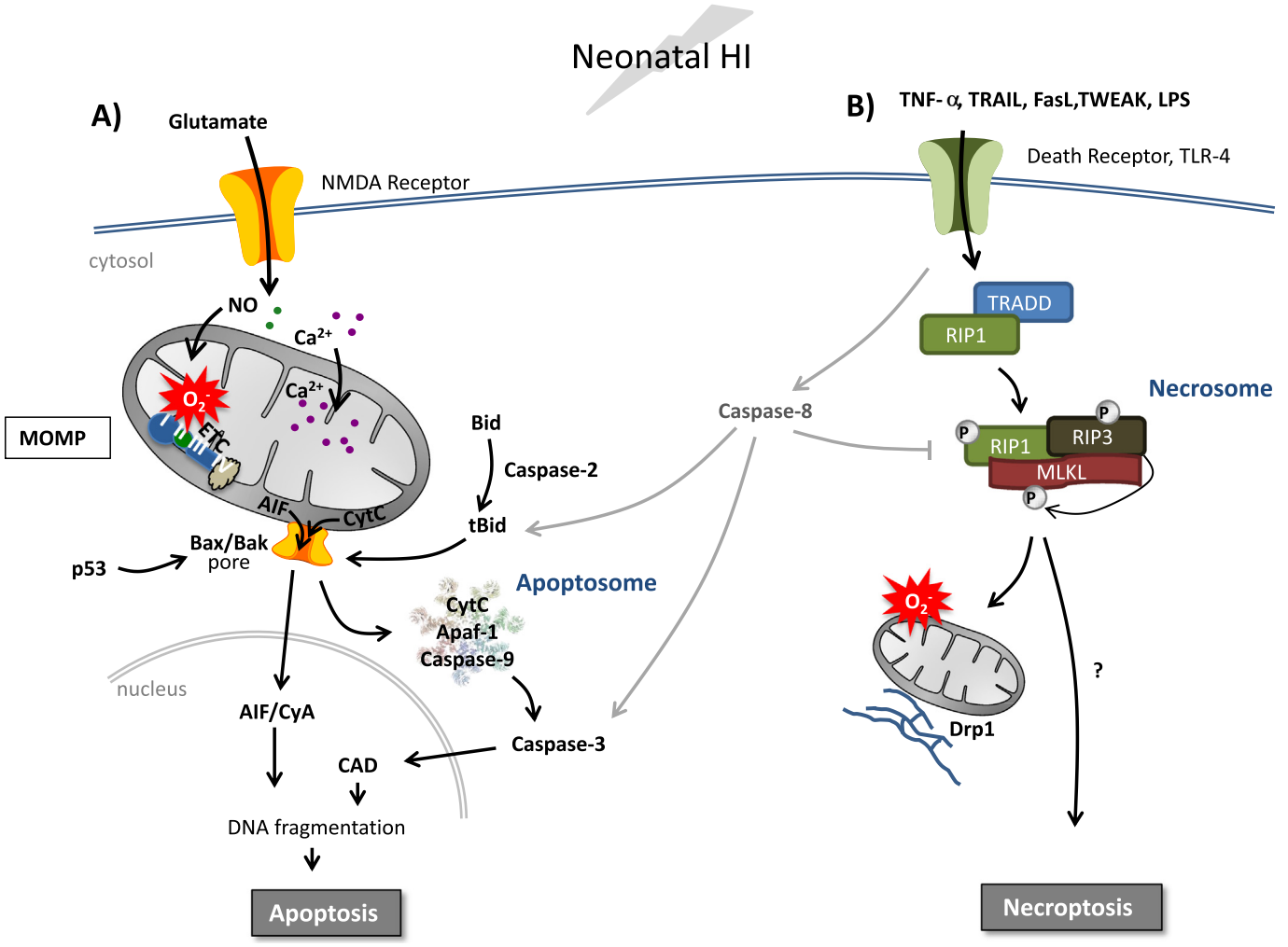

Fig. 1. Interweaving apoptosis and necroptosis pathways after neonatal HI insult. A) Neonatal HI induces mitochondrial accumulation of calcium, increased production of reactive oxygen species, and suppression of mitochondrial respiration that culminates in MOMP. Changes in Bcl-2 family proteins induce Bax-dependent MOMP leading to the release of cytochrome c (cyt c) and apoptosis-inducing factor (AIF). Cyt c induces apoptosome formation leading to caspase-3 activation, caspase-activated DNase (CAD) and DNA degradation. AIF forms a complex with cyclophilin A (CyA) which translocates to the nucleus and induces chromatinolysis and apoptotic cell death. B) Concomitantly, inflammatory microglia and astroglia will release tumor necrosis factor-α (TNF-α) or other ligands (FasL, TWEAK, TRAIL and lipopolysaccharide, LPS) leading to the activation of death receptors, which in turn can induce both apoptosis and necroptosis depending on the availability of caspases. Recruitment of TRADD (or other adaptor proteins) and RIP1 will lead to caspase-8 activation and cleavage of Bid leading to apoptotic cell death. Alternatively, under conditions when caspase-8 is inhibited, TRADD facilitates the interaction and activation of RIP1 and RIP3. RIP3 phosphorylates and recruits MLKL to the necrosome which can then be targeted to both plasma and mitochondria-associated endoplasmic reticulum membranes triggering increased reactive oxygen species, fission and necroptosis. Alternative non-mitochondrial mechanisms may also play a role in the induction of necroptosis.

opening of the outer mitochondrial membrane, mitochondrial outer membrane permeabilization (MOMP), or be the result of opening of the mitochondrial permeability transition pore, which permeabilizes both the outer and inner mitochondrial membranes [7]. MOMP appears predominantly to induce apoptosis whereas mitochondrial permeability transition pore opening results in mitochondrial swelling and tends to lead to necrotic cell death. Importantly, cyclophilin D has been shown to be implicated in mitochondrial permeability transition pore opening in the adult brain after ischemia [8], whereas Bax-dependent MOMP seems to be the predominant mechanism in the neonatal brain following HI [9]. Mitochondria can also be involved in necroptosis (see below).

Mitochondrial permeabilization results in the release of key proapoptotic proteins cytochrome c, apoptosis inducing factor (AIF), endonuclease (endo) G and Smac/Diablo from the mitochondria to the cytosol [3,5,10–12]. Each protein has different downstream targets, but all contribute to cell death. Following translocation to the cytosol, cytochrome c binds to Apaf-1 forming an apoptosome which binds to procaspase-9 leading to caspase-3 activation [13]. Smac/Diablo also enhances the activity of caspases, whilst AIF, which is caspase-independent, interacts with cyclophilin A. This complex then translocates to the nucleus and is associated with DNA fragmentation which has been shown to occur following neonatal HI [10]. High expression of proapoptotic proteins such as caspase-3, Bax and Bcl-2 during development strongly suggests that apoptosis is more prominent in the immature brain compared with the adult [5,11,12].

3.1. Apoptosis and neonatal HI brain injury

The induction of MOMP in the immature brain after HI depends on Bcl-2 family proteins; Bax translocates from the cytosol to the mitochondria and in association with Bak forms pores in the outer membrane resulting in the subsequent release of proapoptotic proteins. In the immature brain, Bax and its associated proteins are highly expressed, with further upregulation of expression occurring following neonatal HI [9,14,15]. Pharmacological inhibition of Bax-dependent mitochondrial permeabilization prior to neonatal HI attenuates the severity of brain injury [16] highlighting that, in the immature brain, Bax-dependent MOMP is a critical event leading to execution of cell death.

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