

Vital functions of Apoptosis Inducing Factor (AIF)

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ABSTRACT

In many models of programmed cell death, the mitochondrial protein AIF translocates to the nucleus, where it induces the chromatin condensation and DNA degradation. However, today it is well established that this flavoprotein is bifunctional. In addition to its lethal function in the nucleus of dying cells, AIF plays a vital bioenergetic role in healthy ones by regulating mainly the activity of the mitochondrial respiratory chain complex I. Hypomorphic or deletion mutants of AIF have led to the generation of the first reliable mouse model of complex I deficiency syndrome, which leads to progressive ataxia and blindness due to neuronal degeneration, as well as a dilated cardiomyopathy, skeletal muscle atrophy and metabolic dysfunction. Here, we discuss recent progress in the quest to understand AIF's involvement in cell survival and in the regulation of mitochondrial respiratory chain complex I.

Keywords: *Apoptosis Inducing Factor (AIF), Programmed cell death, Mitochondrial respiratory chain complex I*

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INTRODUCTION

Apoptosis Inducing Factor (AIF), which is confined to mitochondria of normal healthy cells, was initially identified as the first caspase-independent cell death effector (1). Indeed, upon mitochondrial outer membrane permeabilization (MOMP) – a feature of most, if not all, apoptotic pathways (2) – AIF is released from mitochondria with other mitochondrial proteins (3) and translocates first to the cytosol and then to the nucleus, where it participates in chromatin condensation and chromatinolysis (1).

The AIF gene is localized on human chromosome X and encodes a 613 amino acid protein, which carries an N-terminal mitochondrial localization signal (MLS) (4). In

healthy cells, once the precursor protein is imported into mitochondria, the MLS is cleaved by a mitochondrial peptidase and the mature AIF protein is inserted, into the inner membrane, facing the intermembrane space (5). In cells induced to die, upon the integration of the death signal in the mitochondrion and MOMP, the N-terminal membrane-anchored portion of AIF is removed by a cysteine protease (6), thus generating a soluble protein that could be released into the cytosol. The mitochondrio-nuclear translocation and the lethal function of AIF were studied in various paradigms of cell death, leading to the conclusion that AIF is not a general cell death effector and the activation of its apoptotic function is rather signal-dependent and cell type-dependent. However, AIF's involvement in several neuronal cell death pathways has been established (7-14). Several of these studies were

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achieved with the help of the Harlequin (Hq) mutant mouse model, which exhibits an 80% downmodulation in the expression level of AIF (15). Indeed, *in vivo*, excitotoxic studies using kainic acid-induced seizure showed that Hq mice had significantly less hippocampal damage than wild-type littermates (13). In addition, the brain of Hq mouse is resistant to ischemia reperfusion damage (8,12). The protective effect of AIF's downmodulation seems to be tissue-specific as contrary to the brain, the heart of the mutant Hq mouse subjected to ischemia reperfusion progressed towards cardiac insufficiency after surgical constriction of the aorta more rapidly than control hearts (16). Electron microscopic studies localized AIF to condensed chromatin within the nuclei of dying cells (17). When added to purified nuclei *in vitro*, in a cell free system, recombinant AIF protein provoked chromatin condensation (18). Crystal structure analyses and mutagenesis experiments (17,19) revealed the existence of multiple positively charged amino acids that were necessary for its nuclear apoptotic function. Positively charged amino acids mediate AIF's electrostatic interaction with nucleic acids. Recombinant AIF interacts with naked DNA with a preference for single stranded DNA over double stranded DNA (20). Obviously, further experiments will be required to establish the link between AIF's chromatin condensing activity and its capacity to bind naked DNA.

As AIF's lethal activity is not the scope of this minireview, readers are invited to read the following references for further information regarding the signaling pathways regulating AIF's apoptotic function in specific cell death models (21-23).

In addition to the mapping of the apoptotic function of AIF to the c-terminal portion of the protein, very interestingly, crystal structure analyses and mutagenesis experiments revealed a significant homology between an internal, non-apoptotic, segment of AIF and bacterial NADH-

Oxidases (17-19). The finding hinted toward the possibility that AIF could fulfill a nonapoptotic enzymatic function in the healthy cells AIF. Accordingly, AIF is a bifunctional flavoprotein that uses flavin adenine dinucleotide (FAD) as a co-factor for its NADH oxidase non-apoptotic activity (24). Phenotypic characterization of mouse models, obtained either by tissue-specific genetic ablation of AIF gene or the downmodulation of its expression, has been instrumental for the elucidation of AIF's vital non-apoptotic activity and its impact on cell survival.

AIF's mitochondrial activity is vital

The first piece of evidence about AIF's non-apoptotic function came with the characterization of the Harlequin (Hq) mutant mouse model, which was initially noted for its baldness, a late onset progressive ataxia and blindness caused by neuronal degeneration. In addition, signs of severe oxidative stress were observed in various degenerating brain regions of the animal (15). Klein et al (15) found that the phenotype was due to a retroviral insertion in the first intron of AIF gene, which caused an 80% drop in the expression level of AIF. For the first time, the loss of AIF's activity was linked with neurodegeneration and it was shown that AIF was required for the survival of post-mitotic neurons in the aging brain.

All attempts to create AIF null mice, by homologous recombination, were unsuccessful (25,26) and the reason for the lack of success was that AIF is indispensable for cell survival during advanced embryogenesis (27). Conditional and tissue-specific genetic deletion of AIF gene has been used for the assessment of AIF's vital role during mouse development. The specific deletion of AIF in the prospective midbrain and cerebellum revealed that AIF was necessary for cell-type specific neurogenesis in the developing brain (28). In addition, a defective cortical development and reduced neuronal survival was observed in mutant

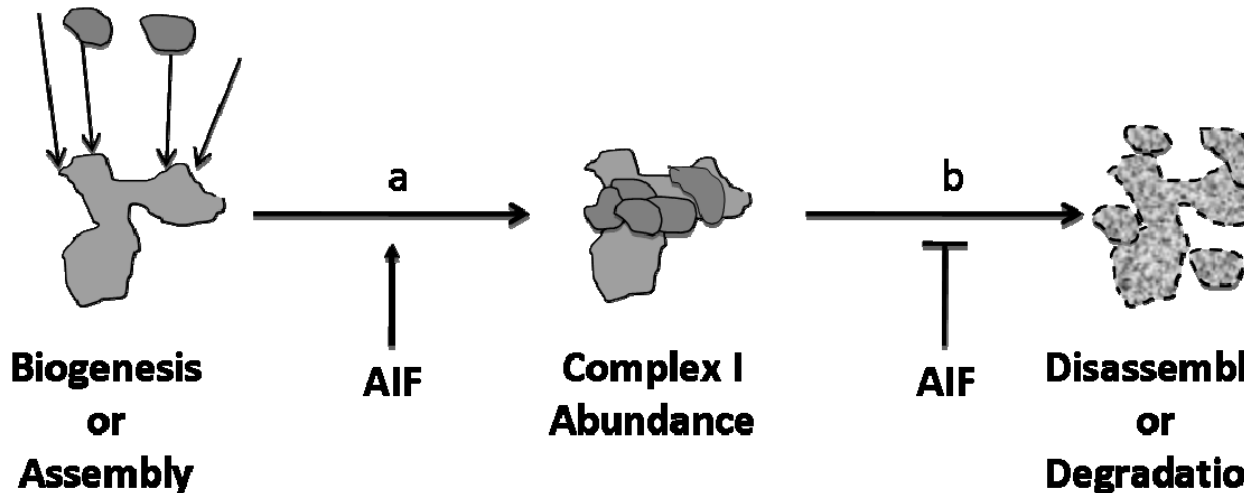


Figure 1: Vital functions of AIF. Two hypothetical models describing AIF's mitochondrial function.

a) AIF regulates the biogenesis or assembly of complex I subunits. b) AIF controls the maintenance or stability of the formed complex.

mice that specifically lost AIF gene in the telencephalon (29). AIF's conditional deletion in muscle and liver had a pronounced impact on the metabolic behavior of the mutant animals. Compared to control littermates, muscle- and liver-specific AIF mutant mice were resistant to diet-induced obesity and diabetes (30). Moreover, the observation of the same phenomenon in young Harlequin (Hq) mutant mice indicates that the global down-modulation of AIF gene expression is enough to confer the resistance against obesity and diabetes (30). With aging, mutant mice with muscle-specific loss of AIF develop severe skeletal muscle atrophy and a dilated cardiomyopathy before becoming lethargic around the age of five months (26).

AIF regulates the respiratory chain complex I

The deletion or depletion of AIF led to the dysfunction of the most important energy-producing machinery of the cell, which is the mitochondrial respiratory chain (16,26,27,30-32). Among the 5 protein complexes, which constitute the respiratory chain, complex I is the one mostly

affected by the loss or down-modulation of AIF. Occasionally, a dysfunction of complexes III or IV could also be detected in specific cells or tissues lacking AIF (26,31). Vahsen *et al.* (31) showed that the decrease in the amount of complex I subunits could be explained by a post-transcriptional regulation, as it was not accompanied by reduced mRNA levels. So far, it is elusive whether AIF is required for the optimal assembly of complex I subunits, or whether AIF maintains its stability (Figure 1) (22,31). Complex I activity was measured in various organs of mutant Hq mice. Importantly, the deficiency provoked by the hypomorphic mutation of AIF was tissue-specific and restricted mainly to degenerating organs like brain and retina (31,32). The molecular basis for the regulation of complex I by AIF or the tissue-specificity of AIF's action on the respiratory chain activity is yet to be determined.

PERSPECTIVES

The phenotype of AIF-defective mice is likewise linked to dysfunctions in respiratory chain, reminiscent of human

“mitochondriopathies”, which also lead to a large spectrum of complex organ-specific manifestations (33). Mice engineered to express reduced AIF levels represent the first reliable murine model of complex I deficiency, which in human accounts for approximately about 30% of all mitochondriopathies (34). This family of disorder is very heterogeneous, insufficiently understood and untreatable. We believe that the pathophysiological characterization of murine AIF deficiency, the comprehension of the tissue-specificity of complex I regulation by AIF, as well as the identification of the molecular pathway linking complex I to AIF will facilitate the therapeutic correction of this type of mitochondriopathy.

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