

## Lessons Learned from Transgenic Mouse Models for the Therapeutic Use of Drp1 Inhibitors

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### Abstract

Pharmacological inhibition of dynamin-related protein 1 (Drp1) the main mammalian promoter of mitochondrial fission - has emerged as a promising therapeutic target for the treatment of neuronal injuries. Genetic studies, however, have revealed that inhibiting Drp1 during development leads to defects especially in neuronal differentiation. Bypassing this neurodevelopmental impairment, a number of recent studies have genetically ablated Drp1 in different adult neuronal subpopulations. This has led to new insights into the importance of mitochondrial fission in differentiated neurons and has highlighted potentially severe side effects of this new therapeutic strategy.

### Commentary

Neurons have a particularly high energy demand and are heavily dependent on a functional mitochondrial network. Mitochondria constantly engage in membrane fusion and fission cycles, in which single organelles frequently bud off or merge with the mitochondrial syncytium [1]. Single mitochondria sprouting from the network are transported along the cytoskeleton into distant sub cellular compartments such as synapses and neuronal spines where they can respond to local energy demands [2]. Mitochondrial fission is believed to be substantial for the sequestration of defective organelles and their removal from the network through a specialized form of autophagy called mitophagy [3,4]. Fusion, on the other hand, is important for maintaining qualitative homogeneity of the syncytium through complementation [5]. Both mitochondrial fusion and fission are mediated by highly conserved dynamin-like proteins capable of self-assembling, GTP hydrolysis and membrane remodeling [6]. The only known bona fide mammalian pro-fission protein of the dynamin superfamily is the cytosolic dynamin-related protein 1 (Drp1). It is activated by the phosphorylation of one or more residues and translocates to predefined mitochondrial fission sites where it binds to outer mitochondrial membrane-bound adaptor proteins such as mitochondrial fission factor (Mff) or Mid49/51 [7]. The exact molecular configuration of these fission sites is currently not fully understood. According to a currently favored model, Drp1 translocation is preceded by ER tubules wrapping around mitochondria to constrict the organelles. A growing body of evidence suggests that this initial step of mitochondrial fission is driven by the constriction of actin and myosin filaments to create a geometric hotspot for the assembly of multimeric Drp1 complexes which upon GTP hydrolysis constrict further to complete the mitochondrial fission process [8].

The fragmentation of the mitochondrial network is one of the evolutionary conserved hall-mark events of apoptosis. In mammals inhibition of mitochondrial fission by disrupting of Drp1 function leads to a delay of cytochrome c release and consequently protects from apoptosis [9,10]. In cultured neurons Drp1 ablation leads to a super-elongation of the mitochondrial network and has been shown to be neuroprotective [11]. Accordingly, several studies reported neuroprotective effects of Drp1 inhibitors in animal models of brain ischemia [12-15], retinal ganglion cell ischemia [16], spinal cord ischemia and injury [17,18], traumatic brain injury [19], status epilepticus [20-22], as well as Huntington's [23] and Parkinson's disease (PD) [24]. However, seemingly contradictory *in vitro* studies of Drp1 ablation in cultured neurons have also reported the formation of spherically enlarged mitochondria that aggregate in the perikarya.

This phenotype, as opposed to the neuroprotection of the super-elongated phenotype, is linked to neurodegeneration [11]. Further, two Drp1 constitutive knockout mouse models displayed severe neurodevelopmental defects [25,26], and similar observations were made in a human infant born with a *de novo* dominant-negative Drp1 mutation [27] and in patients with mutations in the mitochondrial Drp1 receptor, Mff [28]. Moreover, deleting Drp1 in adult neuronal stem cells leads to defective differentiation [29,30]. The importance of Drp1 function for differentiating and proliferating tissues is also underlined by the decrease of tumor aggressiveness upon treatment with Drp1 inhibitors [31-33]. Mechanistically, disrupting cell proliferation and differentiation by Drp1 inhibition is linked to: (i) cell cycle arrest due to uneven distribution of hyperfused mitochondria to progeny after mitosis, (ii) to favoring in tumor cells a non-glycolytic cellular metabolism counteracting the Warburg effect, and (iii) to defects in cell migration due to the inability of hyperfused mitochondria to concentrate in lamellipodia and growth cones [34].

All of these mechanisms should be less relevant for the survival of postmitotic adult neurons. Neuroprotective effects of Drp1 inhibitors made it likely that the anti-apoptotic effect of Drp1 inhibition would prevail in Drp1-ablated postmitotic neurons *in vivo*. However, genetic Drp1 deletion in adult Purkinje cells in 3-week-old mice led to their complete degeneration within 6 months [35]. Similarly, Drp1 ablation in midbrain dopaminergic neurons of 3-month-old mice caused their degeneration within 2.5 months, sparing only a sub-population apparently resistant to ablation of mitochondrial fission [36]. Greater resilience to genetic ablation of Drp1 was found in hippocampal neurons of 2-month-old mice which did not display signs of neurodegeneration for up to 3 months [37]. In another model, deleting Drp1 in hippocampal neurons of newborn mice rendered neurons viable for up to one year [38]. However, hippocampal Drp1 ablation in these later two studies

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was not without effects: both studies reported that short-term memory and synaptic short-term potentiation was decreased, accompanied by hippocampal atrophy [37,38]. Further, it was shown that the number of presynaptic mitochondria was decreased [37], and similar findings were made in dopaminergic neurons resistant to Drp1 ablation [36]. In all of these models the mitochondrial network was characterized by spherically enlarged mitochondria that aggregated in the perikarya, a mitochondrial phenotype that is associated with neurodegeneration in cultured neurons [11]. In contrast to mitochondria of Drp1-ablated mouse embryonic fibroblasts, mitochondria of Drp1-ablated neurons exhibit rather severe respiratory deficits [25,37].

Collectively, from these studies a picture emerges whereby prolonged neuronal Drp1 ablation leads to spherically enlarged, respiratory-deficient mitochondria whose transport to presynaptic terminals is impaired. The intrinsic resistance to these energetic deficits apparently varies greatly among neuronal subtypes. Purkinje cells and dopaminergic neurons are known to be selectively susceptible to degeneration in other disease-related contexts such as Autism and PD respectively. The super-elongated mitochondrial phenotype reported as anti-apoptotic and neuroprotective *in vitro* has *in vivo* only transiently been observed in Drp1-ablated Purkinje cells [35] while it was not detectable in Drp1-ablated hippocampal neurons [37]. All of these discussed animal models used Cre-driven recombination of a floxed Drp1 locus to genetically ablate Drp1. A recent study infecting adult dopaminergic neurons of 3-month-old mice with a viral construct expressing a dominant negative mutation of Drp1 (Drp1K38A) has reported elongated mitochondria and neuroprotection against a neurotoxin which induces PD-like symptoms 2 months after viral infection [24]. In contrast, Drp1 ablation via recombination of the Drp1 locus in dopaminergic midbrain neurons leads to 90% neurodegeneration within 2.5 months. The dominant-negative mutation Drp1K38A inhibits GTP hydrolysis but not Drp1 recruitment to mitochondria and the most commonly used Drp1 inhibitor (mdivi-1) has a similar mechanism of action [39]. Potentially, the K38A mutation as well as the Drp1 inhibition by mdivi-1 is more tolerable for neurons than the complete genetic deletion of Drp1. It remains to be tested whether low frequency mitochondrial fission is maintained under these conditions. Potentially, new transgenic mice which could be induced to express lowered levels of Drp1 or to express a dominant negative mutation of Drp1 for a limited time would show only the beneficial effects of Drp1 ablation without the neuronal deficit. Taking into account the lessons learned from mouse models using a genetic Drp1 knockout strategy so far, it appears that close monitoring of mitochondrial morphology is mandatory upon treatment with Drp1 inhibitors. This aspect seems particularly important when treatment is targeted to more vulnerable neuronal subpopulations such as dopaminergic midbrain neurons, to prevent neuronal mitochondria from spherical enlargement and aggregation in perikarya.

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