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INVITED REVIEW

Mitochondrial fission – a drug target for cytoprotection or cytodestruction?

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Keywords

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Introduction

Under both physiological and pathological conditions, mitochondria change their shape through fusion and fission. These processes play central roles in quality control of mitochondria and are important for maintaining various cellular functions and viability, as well as regulating bioenergetic metabolism. Mitochondrial fusion is required for appropriate distribution of mitochondrial DNA, lipids, and proteins across all mitochondria. The main purpose of fusion is to ensure optimal conditions for mitochondria to carry out key cellular processes, such as energy metabolism, cellular differentiation, and calcium

Abstract

Mitochondria are morphologically dynamic organelles constantly undergoing processes of fission and fusion that maintain integrity and bioenergetics of the organelle: these processes are vital for cell survival. Disruption in the balance of mitochondrial fusion and fission is thought to play a role in several pathological conditions including ischemic heart disease. Proteins involved in regulating the processes of mitochondrial fusion and fission are therefore potential targets for pharmacological therapies. Mdivi-1 is a small molecule inhibitor of the mitochondrial fission protein Drp1. Inhibiting mitochondrial fission with Mdivi-1 has proven cytoprotective benefits in several cell types involved in a wide array of cardiovascular injury models. On the other hand, Mdivi-1 can also exert antiproliferative and cytotoxic effects, particularly in hyperproliferative cells. In this review, we discuss these divergent effects of Mdivi-1 on cell survival, as well as the potential and limitations of Mdivi-1 as a therapeutic agent.

Abbreviations

AMPK, 5' adenosine monophosphate-activated protein kinase; CREB, cAMP response element binding; MOMP, mitochondrial outer membrane permeabilization; PLD, phosphatidylcholine hydrolyzing phospholipase D; RISK, reperfusion injury salvation kinase; ROS, reactive oxygen species; SENP3, SUMO1/Sentrin/SMT3-specific peptidase 3; SLP2, stomatin-like-protein 2.

homeostasis (Palmer et al. 2011). A homogeneous mix of mitochondrial matrix proteins, mitochondrial DNA, and maintenance of optimal pH and mitochondrial membrane potential are essential for successful mitochondrial fusion (Kane and Youle 2010). Mitochondrial fusion is thus a complex sequential process which involves integration of the outer mitochondrial membrane, inner mitochondrial membrane, and matrix content. The main regulators of these processes are the GTP-ase dynamin-related proteins: mitofusin 1 (Mfn1), mitofusin 2 (Mfn2), and optical atrophy 1 (Opa1). Other profusion proteins include prohibitin 2, stomatin-like-protein 2 (SLP2), and the phosphatidylcholine hydrolyzing phospholipase D (PLD)

(Fig. 1). The integration of these enzymatic processes has been reviewed elsewhere and will not be detailed here (Palmer *et al.* 2011; Da Silva *et al.* 2014; Kasahara and Scorrano 2014).

The opposite process, mitochondrial fission, plays an important role in mitochondrial proliferation following mitosis and is involved in removing damaged mitochondria from the cells through mitophagy (Otera and Mihara 2012). Mitochondrial fission is regulated by the large GTP-ase dynamin-related protein, Drp1 (the human homolog of the yeast mitochondrial dynamin, Dnm1). Similar to other dynamin-related proteins, Drp1 has a GTP-ase effector domain which is important for its GTP-ase activity. However, it lacks membrane binding domains and thus is heavily dependent on proteins at the outer mitochondrial membrane for anchorage to the mitochondrion (Chan 2012; Dorn 2013). These pro-fission docking proteins include Fis1 (mitochondrial fission 1), Mff (mitochondrial fission factor), MiD49 (mitochondrial dynamic protein of 49 kDa), MiD51 (mitochondrial dynamic protein of 51 kDa, also known as mitochondrial elongation factor 1, MIEF1), miR-30, and miR-499 (Fig. 1). Again these mechanisms have been thoroughly reviewed recently (Chan 2012; Da Silva *et al.* 2014; Lee and Yoon 2014).

Drp1 predominantly localizes in the cytosol as a tetramer and translocates to the outer mitochondrial membrane during mitochondrial fission, where it polymerizes into ring-like structures around the mitochondria to induce fission (Shin *et al.* 1999; Cassidy-Stone *et al.* 2008; Bossy *et al.* 2010). Translocation of Drp1 from the cytosol to the mitochondria is regulated by multiple post-translational modifications including phosphorylation, ubiquitination, SUMOylation, and S-nitrosylation (Karbowski *et al.* 2007; Taguchi *et al.* 2007; Wasiak *et al.* 2007; Cho *et al.* 2009). Phosphorylation is the most well-studied mechanism, with known sites for phosphorylation being the serine residues 616 and 637 (equivalent to serine 585 and 656 in rats) which promote and inhibit translocation of Drp1 into the mitochondria, respectively (Taguchi *et al.* 2007; Qi *et al.* 2011).

Mitochondrial dynamics have been implicated in determining survival of many cell types including cardiomyocytes and neurons. Mitochondria fragmented as a result of fission are associated with apoptosis and autophagy (Ong *et al.* 2010; Chan 2012). Shifting the balance of mitochondrial morphology toward fission enhances susceptibility to death in various cell types. In contrast, fused mitochondria are energetically more active, preserve cell functions, and can better withstand oxidative stress (Ong

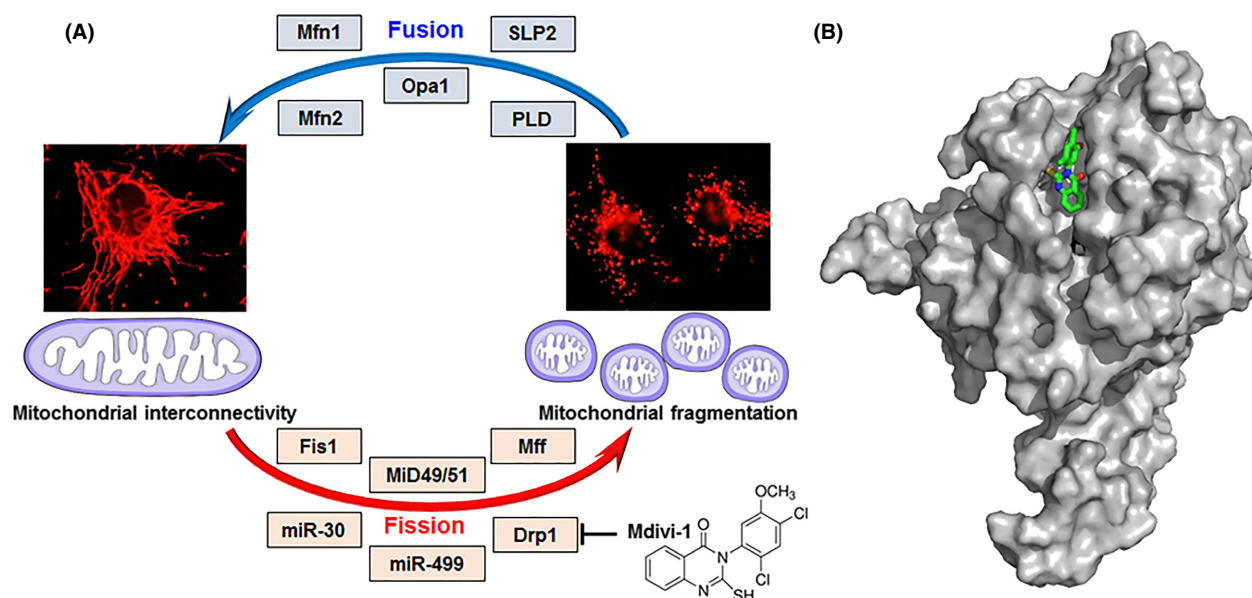


Figure 1. Mitochondrial fusion and fission cycle and its key players. (A) Mitochondrial interconnectivity is maintained by fusion which is regulated by proteins such as Mfn1, Mfn2, Opa1, SLP2, and PLD. Mitochondrial fragmentation follows fission, governed by several factors including Fis1, MiD49/51, Mff, miR-30, miR-499, and Drp1. Mitochondrial fission is suppressed via inhibition of Drp1 by the synthetic small molecule Mdivi-1. (B) Proposed binding orientation of Mdivi-1 (green carbons, sticks) to Drp1 (gray, surface rendered). Mdivi-1 was computationally docked onto the Drp1 crystal structure (Wenger *et al.* 2013) using default conditions for the Geom-dock module in Sybyl-X 2.1.1 (Certara L.P.). Shown is a representative of the highest scoring cluster of solutions. Drp1, dynamin-related protein 1; Fis1, fission 1; Mff, mitochondrial fission factor; Mfn1, mitofusin 1; Mfn2, mitofusin 2; MiD49/51, mitochondrial dynamics protein-49/51; miR30/499, micro-RNA 30/499; Opa1, optic atrophy protein; PLD, phosphatidylcholine hydrolyzing phospholipase D; SLP2, stomatin like protein-2.

et al. 2010). The discovery of Mdivi-1, a small molecule that selectively and reversibly inhibits the mitochondrial fission protein Drp1 (Cassidy-Stone et al. 2008), has led to a better understanding of the role of mitochondrial dynamics in the survival of various cell types under different pathophysiological conditions.

Mdivi-1 as an Inhibitor of Drp1

Mdivi-1 (mitochondrial fission inhibitor-1) is the first selective inhibitor of the mitochondrial fission protein Drp1 (Cassidy-Stone et al. 2008). It contains a quinazolinone core substituted with a thiol moiety and an aryl (2,4-dichloro-5-methoxyphenyl) side chain attached to the N3 position (Figs. 1, 2) (Cassidy-Stone et al. 2008; Qian et al. 2015). Structure–activity relationship analysis has shown that Mdivi-1 is a mixture of two atropisomers which arise due to hindered rotation at its chiral axis around the nitrogen-phenyl bond. The axial chirality at the aryl side chain greatly influences the selectivity of Mdivi-1 for Drp1 (Cassidy-Stone et al. 2008; Qian et al. 2015).

Mdivi-1 has been shown to target Drp1 selectively in mammalian cells by binding at an allosteric site and suppressing Drp1 capacity to catalyze GTP hydrolysis as well as self-assembly into ring-like structures around the mitochondria. Mdivi-1 can induce rapid and reversible formation of interconnected mitochondria without affecting

other cellular structures such as the cytoskeleton and endoplasmic reticulum, suggesting selectivity for mitochondrial fission. The half maximal inhibitory concentration of Mdivi-1 ranges from 1 to 50 $\mu\text{mol/L}$ depending on the cell and assay types (Cassidy-Stone et al. 2008; Qian et al. 2015). Since its discovery in 2008 (Cassidy-Stone et al. 2008), Mdivi-1 has been widely employed as an inhibitor of Drp1 in multiple cell types (Table 1) and organs in different disease settings (Table 2). Interestingly, Mdivi-1 exerts divergent effects on cell survival depending on the cell type and experimental setting.

Divergent Effects of Mdivi-1 on Cell Survival

The cytoprotective effect of Mdivi-1 was first demonstrated by Cassidy-Stone et al. (2008). They showed that treatment with Mdivi-1 significantly reduced mitochondrial fragmentation and apoptosis induced by staurosporine, to an extent similar to that observed in cells expressing the dominant negative Drp1K38A mutant (Cassidy-Stone et al. 2008). Furthermore, Mdivi-1 has been shown to attenuate Bax/Bak-dependent mitochondrial outer membrane permeabilization (MOMP) induced by caspase 8-cleaved recombinant Bid (Cassidy-Stone et al. 2008). These findings suggested that Mdivi-1 inhibits Drp1-mediated mitochondrial fragmentation and the intrinsic apoptotic pathway. Other researchers have since

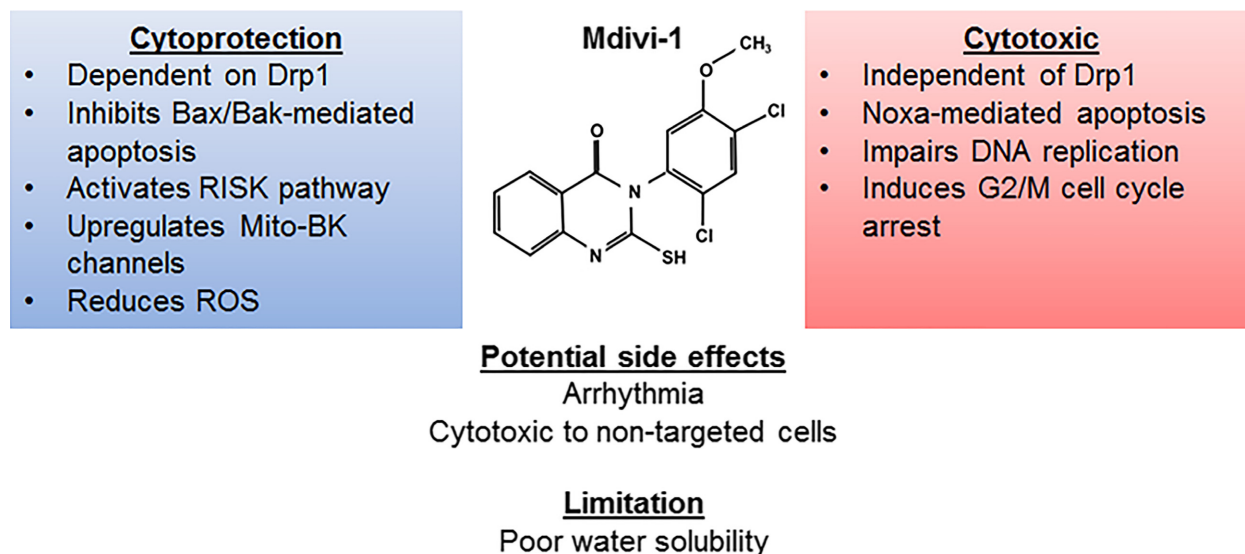


Figure 2. Pharmacodynamic profile of Mdivi-1. Mdivi-1 confers cytoprotection by employing a Drp1-dependent inhibition of Bax/Bak-mediated apoptosis, activating the RISK pathway, upregulating mitochondrial large conductance Ca^{2+} and voltage activated K^+ (Mito-BK) channel as well as reducing ROS. Its cytotoxic effect is exerted independent of Drp1 and through activation of Noxa-mediated apoptosis. Mdivi-1 also exerts an inhibitory effect on hyperproliferative cells by inducing G2/M cell cycle arrest and impairs mitosis. Bak, Bcl2-antagonist/killer 1; Bax, Bcl2-associated X protein; DNA, deoxyribonucleic acid; Drp1, dynamin-related protein 1; G2/M, second gap/mitosis; Mito-BK, mitochondrial big potassium channel; RISK, reperfusion injury salvation kinase; ROS, reactive oxygen species.

Table 1. In vitro studies of Mdivi-1.

Cell types	Models	Treatment regimens		Findings	References
		Doses	Protocols		
Cardiovascular cells Cardiomyocytes (mouse)	Simulated ischemia-reperfusion injury	10 & 50 $\mu\text{mol/L}$	Started 45 min before ischemia	↓Cell death ↑Mitochondrial membrane potential	(Ong et al. 2010)
Cardiomyocytes (mouse)	Simulated ischemia-reperfusion injury	5 $\mu\text{mol/L}$	Started 30 min before ischemia	↓Cell death ↓ROS ↓Cytosolic Ca^{2+} ↑Oxygen consumption rate No significant increase in ATP	(Sharp et al. 2014)
Cardiomyocytes (rat)	Doxorubicin toxicity	1 $\mu\text{mol/L}$	Cotreatment with doxorubicin	Delayed mitochondrial depolarization Delayed hypercontracture	(Gharanei et al. 2013)
HL-1 cells (mouse)	Simulated ischemia-reperfusion injury	10 & 50 $\mu\text{mol/L}$	Started 40 min before ischemia	↓Cell death ↑Mitochondria elongation	(Ong et al. 2010)
HL-1 cells (mouse)	—	1–100 $\mu\text{mol/L}$	—	Delayed mitochondrial depolarization ↑Firing rate & duration of spontaneous action potential ↓amplitude of I_{Kr} ↓Opening probability of K_{Ach}	(So et al. 2012)
Vascular smooth muscle cells (human, pulmonary artery)	Idiopathic pulmonary artery hypertension	5, 10 & 25 $\mu\text{mol/L}$	—	↓Proliferation G2/M cell cycle arrest	(Marsboom et al. 2012)
Vascular smooth muscle cells (human & rabbit, ductus arteriosus)	O_2 challenge on hypoxic cells	20 $\mu\text{mol/L}$	During O_2 challenge	↓Proliferation ↓Oxygen consumption rate ↓Pyruvate dehydrogenase activity ↓Mitochondrial H_2O_2 production ↓ Ca^{2+} release from mitochondria & ER	(Hong et al. 2013)
Vascular smooth muscle cells (rat, aorta)	Angiotensin II or H_2O_2 treatment	1, 10 & 20 $\mu\text{mol/L}$	Cotreatment with Angiotensin or H_2O_2 Pretreatment for 60 min	↓Proliferation G2/M cell cycle arrest ↓Migration ↑Mitochondrial membrane potential ↓ROS	(Lim et al. 2015)
Vascular endothelial cells (human, umbilical cord vein)	—	30 $\mu\text{mol/L}$	24 h	↓Phosphorylated ERK1/2, MEK1/2 ↑Cell senescence ↓Cell migration ↓Angiogenic Tube formation ↑autophagosomes ↓Autolysosomes ↑ROS	(Lin et al. 2015)

Table 1. Continued.

Cell types	Models	Treatment regimens		Findings	References
		Doses	Protocols		
Neurons (rat, hippocampus)	Simulated ischemia-reperfusion injury	50 $\mu\text{mol/L}$	Pretreatment for 40 min	↓Cell death ↓ROS	(Wang et al. 2014)
Neurons (rat, hippocampus)	Simulated epilepsy with magnesium-free culture	10, 25 & 50 $\mu\text{mol/L}$	Pretreatment for 30 min	↓Apoptosis ↓ROS	(Xie et al. 2016)
Neurons (human, embryonic stem cell-derived)	Propofol-induced cell death	25 $\mu\text{mol/L}$	Pretreatment for 60 min	↓Endoplasmic reticulum stress ↓Apoptosis Delayed mitochondrial depolarization	(Twaroski et al. 2015)
Neurons (rat, spinal cord)	Glutamate toxicity	10 $\mu\text{mol/L}$	Cotreatment with glutamate	↓Apoptosis ↓ROS ↑Mitochondrial membrane potential ↑Antioxidant activity ↑Expression of large-conductance Ca^{2+} -activated K^{+} channel	(Liu et al. 2015)
Neurons (rat, cortex)	Glutamate toxicity or simulated ischemia-reperfusion injury	25 $\mu\text{mol/L}$	Cotreatment with glutamate or during simulated ischemia	↓Apoptosis	(Grohm et al. 2012)
Neurons (rat, cortex)	Simulated ischemia-reperfusion injury	25 $\mu\text{mol/L}$	During 4 h of ischemia (cotreatment), without reperfusion During 24 h of reperfusion (posttreatment), after 2 h of ischemia	↓Cell death (cotreatment) ↑Cell death (posttreatment)	(Zhang et al. 2013a)
Astrocytes (mouse)	Hypoxia	5–30 $\mu\text{mol/L}$	1–24 h	↑Exogenous ATP metabolism ↑Extracellular adenosine ↑CD39 ↑cAMP levels & PKA activity ↑CREB expression	(Cui et al. 2016)
HT22 cells (mouse, hippocampal neuronal cells)	Glutamate toxicity	50 & 75 $\mu\text{mol/L}$	Cotreatment with glutamate or 2–12 h after glutamate challenge	↓Apoptosis ↑Mitochondrial Membrane potential ↓ROS & lipid peroxidation Prevent ATP depletion	(Grohm et al. 2012)
N27 cells (rat, mesencephalic dopaminergic cells)	PINK1-induced mitochondrial fragmentation	10 & 30 $\mu\text{mol/L}$	Cotreatment with ponasterone	↑Mitochondrial membrane potential ↑ATP levels	(Cui et al. 2010)

Table 1. Continued.

Cell types	Models	Treatment regimens		Findings	References
		Doses	Protocols		
Skeletal myoblasts	C2C12 (mouse, skeletal muscle)	50 & 150 $\mu\text{mol/L}$	Cotreatment with palmitate for 6 h	$\uparrow$ Glucose uptake $\uparrow$ Mitochondrial membrane potential $\downarrow$ ROS	(Jheng et al. 2012)
	C2C12 (mouse, skeletal muscle)	1, 10 & 20 $\mu\text{mol/L}$	24 h	$\uparrow$ Apoptosis Impaired myotube formation $\downarrow$ Mitochondrial membrane potential $\downarrow$ Mitochondrial mass & DNA $\downarrow$ Expression of myogenic regulatory factors, MHC I & creatinine kinase activity $\downarrow$ H ₂ O ₂ -induced mitochondrial fragmentation	(Kim et al. 2013) (Iqbal and Hood 2014)
C2C12 (mouse, skeletal muscle)	Oxidative stress	25 $\mu\text{mol/L}$	After H2O2 exposure; for 1 h	$\downarrow$ Dexamethasone-induced mitochondrial fission & mitophagy $\downarrow$ oxygen consumption $\downarrow$ autophagic flux	(Troncoso et al. 2014)
L6 (rat, skeletal muscle)	Glucocorticoid-induced muscle atrophy	1 $\mu\text{mol/L}$	Cotreatment with dexamethasone for 6 and 24 h		
Cancer cells	A2780 cells (human, ovarian cancer)	10, 20 & 50 $\mu\text{mol/L}$	Cotreatment with TRAIL for 16 h	$\uparrow$ Apoptosis $\downarrow$ Bid expression	(Wang et al. 2015a)
	A2780cis cells (human, cisplatin-resistant ovarian cancer)	20 & 50 $\mu\text{mol/L}$	Cotreatment with cisplatin for 20–72 h	$\uparrow$ apoptosis $\uparrow$ Bax, Bak & Noxa expression	(Qian et al. 2014)
	A2780cis cells (human, cisplatin-resistant ovarian cancer)	10, 20 & 50 $\mu\text{mol/L}$	Cotreatment with TRAIL for 16 h	$\uparrow$ Apoptosis	(Wang et al. 2015a)
	983A cells (human, melanoma)	10–50 $\mu\text{mol/L}$	Cotreatment with cisplatin for 20 h	$\uparrow$ Apoptosis	(Qian et al. 2014)
	Cal33 cells (human, head, and neck squamous cell carcinoma)	10–50 $\mu\text{mol/L}$	Cotreatment with cisplatin for 20 h	$\uparrow$ Apoptosis	(Qian et al. 2014)
	Epithelial ovarian cancer cells (human)	20 & 50 $\mu\text{mol/L}$	Cotreatment with cisplatin for 72 h	$\uparrow$ Apoptosis	(Qian et al. 2014)
	HeLa cells (human, cervical cancer)	50 $\mu\text{mol/L}$	Cotreatment with staurosporine for 4 h	$\downarrow$ Apoptosis	(Cassidy-Stone et al. 2008)
	LN-428 cells (human, glioblastoma)	10–50 $\mu\text{mol/L}$	Cotreatment with cisplatin for 20 h	$\uparrow$ Apoptosis	(Qian et al. 2014)
	MDA-MB-231 cells (human, breast carcinoma)	20 & 50 $\mu\text{mol/L}$	Cotreatment with cisplatin for 20 h	G2/M cell cycle arrest & aneuploidy	(Qian et al. 2012)
		10–50 $\mu\text{mol/L}$	48 h		(Qian et al. 2014)

Table 1. Continued.

Cell types	Models	Treatment regimens			Findings	References
		Doses	Protocols			
MDA-MB-231 cells (human, breast carcinoma)	Cisplatin or carboplatin-induced apoptosis		Cotreatment with cisplatin or carboplatin for 2–72 h		↑Apoptosis ↓cell proliferation	
MDA-MB-231 cells (human, breast carcinoma)	—	10–50 $\mu\text{mol/L}$	16–48 h		M phase cell cycle arrest Abnormal karyokinesis Impaired cytokinesis Hyperploidy ↑Cell viability ↑Mitochondrial membrane potential ↑Cellular ATP	(Wang et al. 2015b)
SH-SY5Y cells (human, neuroblastoma)	Simulated ischemia-reperfusion injury	5, 10 & 20 $\mu\text{mol/L}$	Started 5 min before ischemia		No effect on 3NP-induced autophagy, ROS production, mitochondrial fragmentation, and Bax translocation to mitochondria	(Zhao et al. 2014)
SH-SY5Y cells (human, neuroblastoma)	3NP-induced autophagy	10 $\mu\text{mol/L}$	Cotreatment with 3NP for 5 h		↑Apoptosis ↑Cleavage of caspase-9 & -3 ↑Cytochrome c release ↑Noxa expression ↑Apoptosis	(Solesio et al. 2013)
MCF7 cells (human, breast carcinoma)	Mitosis	50 $\mu\text{mol/L}$	16 h		M phase cell cycle arrest Impaired assembly of mitotic spindle & cytokinesis	(Wang et al. 2015b)
H1299 cells (human, nonsmall-cell lung carcinoma)	Cisplatin-induced apoptosis	10–50 $\mu\text{mol/L}$	Cotreatment with cisplatin or carboplatin for 2–20 h		↑Apoptosis ↑Cleavage of caspase-9 & -3 ↑Cytochrome c release ↑Noxa expression ↑Apoptosis	(Qian et al. 2014)
H1299 cells (human, nonsmall-cell lung carcinoma)	Mitosis	50 $\mu\text{mol/L}$	8–24 h		M phase cell cycle arrest Impaired assembly of mitotic spindle & cytokinesis G2/M cell cycle arrest	(Wang et al. 2015b)
Malignant mesothelioma cells (human)	PRX3-deficiency model					(Cunniff et al. 2014)
Brain tumor initiating cells of glioblastoma (human)	—	10 & 20 $\mu\text{mol/L}$	2–4 days		↑Apoptosis ↑AMPK activation ↓Amplitude of $I_{K(ERG)}$	(Xie et al. 2015)
GH3 cells (rat, pituitary tumor)	—	30 $\mu\text{mol/L}$	—			(So et al. 2012)
HL-60 cells (human, leukemia)	Doxorubicin toxicity	1 $\mu\text{mol/L}$	Cotreatment with doxorubicin		No effect on doxorubicin-induced toxicity	(Gharane et al. 2013)
U2OS cells (human, osteosarcoma)	Mitosis	50 $\mu\text{mol/L}$	8–24 h		↑Apoptosis M phase arrest Impaired assembly of mitotic spindles & cytokinesis	(Wang et al. 2015b)
A375 & A2058 cells (human, melanoma)	Death receptor-induced apoptosis	50 $\mu\text{mol/L}$	Cotreatment with αDR4		↑Apoptosis	(Suzuki-Karasaki et al. 2015)
	High glucose treatment	10 $\mu\text{mol/L}$	—			(Huang et al. 2015)

Table 1. Continued.

Cell types	Models	Treatment regimens		Findings	References
		Doses	Protocols		
SK-N-SH cells (human, neuroblastoma)				↑Mitochondrial density ↑complex I activity Maintained mitochondrial length ↓Cell death ↓ROS	(Tian et al. 2014)
PC12 cells (rat, pheochromocytoma)	Ischemia-reperfusion injury	25, 50 & 100 $\mu\text{mol/L}$	Pretreatment for 30 min	↑Mitochondrial membrane potential ↓Mitochondrial Ca^{2+} uptake & ER Ca^{2+} release	
Others					
Immortalized fibroblasts (mouse, embryo)	Cisplatin-induced apoptosis	50 $\mu\text{mol/L}$	Cotreatment with cisplatin for 20 h	↑Apoptosis (Drp1 independent)	(Qian et al. 2014)
Immortalized fibroblasts (mouse, embryo)	—	20 $\mu\text{mol/L}$	20 h	↑Apoptosis (Bax/Bak independent)	(Qian et al. 2015)
Immortalized fibroblasts (mouse, embryo)	Fas-induced apoptosis	50 $\mu\text{mol/L}$	16 h	↑Apoptosis (Drp1 & Bax/Bak independent)	(Wang et al. 2015a)
Immortalized fibroblasts (mouse, embryo)	—	50 $\mu\text{mol/L}$	6–48 h	↑Apoptosis (Bax/Bak-dependent)	(Wang et al. 2015b)
Immortalized fibroblasts (mouse, embryo)	—	50 $\mu\text{mol/L}$	6–24 h	M phase cell cycle arrest (Drp1 independent) ↑Apoptosis M phase cell cycle arrest	(Wang et al. 2015b)
COS cells (monkey, kidney cell line)	Staurosporine-induced apoptosis	1–200 $\mu\text{mol/L}$	Cotreatment with staurosporine for 4 h	↓Apoptosis	(Cassidy-Stone et al. 2008)
Immortalized small airway epithelial cells (human)	Irradiation	50 $\mu\text{mol/L}$	Started 2 h before irradiation	No effect on irradiation-induced mitochondrial respiratory dysfunction	(Zhang et al. 2013a)
HEK293 cells (human embryo, kidney cell line)	Simulated ischemia-reperfusion injury	50 $\mu\text{mol/L}$	4 h	↓SENK3-induced cytochrome c release	(Guo et al. 2013)

3NP, 3-nitropropionic acid; AMPK, AMP-activated protein kinase; ATP, adenosine triphosphate; Bak, bcl2-antagonist/killer-1; Bax, bcl2-associated X protein; Bid, BH3-interacting domain death agonist; cAMP, cyclic adenosine monophosphate; Chk1, checkpoint kinase 1; CREB, cAMP response element binding; Drp1, dynamin-related protein 1; ER, endoplasmic reticulum; $I_{\text{K}(\text{erg})}$, erg-mediated K^+ current; I_{Kr} , rapidly activating delayed-rectifier K^+ current; I_{Na} , Na^+ current; I_{tail} , amplitude of tail current; I_{ACH} , muscarinic K^+ channel; MHC I, myosin heavy chain I; MPTP, mitochondrial membrane permeability transition pore; Noxa, latin for damage, alternative name for immediate-early-response protein APR; PKA, protein kinase A; PRX3, Peroxiredoxin 3; ROS, reactive oxygen species; SENK3, SUMO1/Sentrin/SMT3 Specific Peptidase 3; SOD, superoxide dismutase; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.

Table 2. In vivo and ex vivo studies of Mdivi-1.

Organ/Tissue	Model	Regimen		Findings	Reference
		Dose	Mode of treatment		
Heart (mouse)	Ischemia-reperfusion injury	0.24 & 1.2 mg/kg	Intravenous; 15 min before ischemia	↓Infarct size	(Ong et al. 2010)
Heart (mouse)	Pressure overload-induced heart failure	50 mg/kg	Intraperitoneal; daily for 7 days	↓Apoptosis ↓LV Dysfunction ↓Fibrosis ↑Angiogenesis ↑Mitochondrial density ↓Mitophagy ↓Expression of antiangiogenic factors, MMP-9 & TIMP-3	(Givvimani et al. 2012)
Heart (mouse)	Ischemia-reperfusion injury and doxorubicin toxicity (ex vivo)	1 μ mol/L	Perfusion; Cotreatment with doxorubicin during 120 min reperfusion	↓Infarct size ↑Coronary flow ↓Heart rate ↑phosphorylated Akt expression ↓Phosphorylated Erk1/2 & p53 expression ↑Diastolic function	(Gharane et al. 2013)
Heart (rat)	Ischemia-reperfusion injury (ex vivo)	5 & 25 μ mol/L	Perfusion; 10 min before ischemia or during 20 min reperfusion	↑Animal survival	(Sharp et al. 2014)
Heart (mouse)	Potassium-induced cardiac arrest	0.24 mg/kg	Intravenous; cotreatment with epinephrine; after cardiopulmonary resuscitation	↑Heart rate, stroke volume & neurological outcomes ↓Myocardial lactate production ↑Exercise capacity ↑RV function ↓RV hypertrophy ↑Hematocrit	(Sharp et al. 2015)
Pulmonary artery (rat)	Pulmonary artery hypertension (PAH)	50 mg/kg	Intraperitoneal; weekly for 4 weeks (CoCl ₂ study), biweekly for 4 weeks (chronic hypoxia study), or 5 daily injections 3 weeks after monocrotaline-induced PAH	↓Small pulmonary artery thickness Prevents O ₂ -induced constriction Prevent closure of ductus arteriosus ↓Smooth muscle cell proliferation ↓Fibrosis	(Marsboom et al. 2012)
Ductus arteriosus (human & rabbit)	O ₂ -induced constriction (ex vivo)	20 μ mol/L	30 min before exposure O ₂ (PO ₂ 120 mmHg); 6–14 days in normoxic culture (PO ₂ 140 mmHg);		(Hong et al. 2013)
Aortic ring (rat)	Carotid artery balloon injury	50 mg/kg/day	7 days	↓Neointimal formation ↓Smooth muscle cell proliferation ↓Infarct volume	(Lim et al. 2015)
Brain (mouse)	Ischemia-reperfusion injury	3 mg/kg	Intraperitoneal; prior to ischemia		(Grohm et al. 2012)
Brain (mouse)	Ischemia-reperfusion injury	10 & 20 mg/kg	Intraperitoneal	↑Neurological outcome ↓Infarct volume ↓Brain edema ↓apoptosis	(Zhao et al. 2014)

Table 2. Continued.

Organ/Tissue	Model	Regimen		Mode of treatment	Findings	Reference
		Dose				
Brain (mouse)	Ischemia-reperfusion injury	10 & 20 mg/kg		Intraperitoneal; 4 h before ischemia and every 12 h for 10 days after reperfusion	↑Animal survival ↓Infarct volume ↑cerebral blood flow ↓Extracellular ATP ↑Extracellular adenosine ↑CD39 expression ↑Phosphorylation of CREB protein ↑Survival ↑Tumor latency	(Cui et al. 2016)
Brain (mouse)	Implantation of brain tumor initiating cells of glioblastoma	2.5 mg/kg		Intravenous; 3 days after tumor implantation, administered for 5 days	↑Survival ↑Tumor latency	(Xie et al. 2015)
Brain (mouse)	Traumatic brain injury	3 mg/kg		Intraperitoneal; 10 min after injury	↑Motoric and cognitive recovery ↓Infarct volume ↓Brain edema ↓Apoptosis ↓Apoptosis of neuron	(Wu et al. 2016)
Brain (rat)	Ischemia-reperfusion injury	0.24 & 1.2 mg/kg		Intravenous; 15 min prior to ischemia	↓Brain edema ↓Apoptosis ↓Apoptosis of neuron	(Zhang et al. 2013b)
Brain (rat)	Ischemia-reperfusion injury	3 mg/kg		Intraperitoneal; During 24 h of focal ischemia (cotreatment), without reperfusion	No change of infarct volume (cotreatment)	(Zhang et al. 2013c)
Brain (rat)	Pilocarpine-induced seizure	0.25 & 1.25 mg/kg		Intravenous; 15 min prior to pilocarpine injection	↓Apoptosis	(Xie et al. 2013)
Brain (rat)	Pilocarpine-induced seizure	1.2 mg/kg		Intraperitoneal; 30 min prior to pilocarpine injection	↓Apoptosis ↓ROS ↑SOD activity	(Qiu et al. 2013)
Brain (rat)	Cardiac arrest	0.24 & 1.2 mg/kg		Intravenous; after 1 min of restoration of spontaneous circulation	- No effect on latency and intensity of seizure ↑Survival ↑Neurological outcome ↓Apoptosis	(Li et al. 2015b)
Hippocampus (mouse)	Type 2 diabetes	10 & 25 mg/kg		Intravenous; daily for 2 weeks	↑Hippocampal long-term potentiation ↑ATP levels ↑Complex I activity ↑Neurological outcome ↓Spinal cord edema ↑Expression of large-conductance Ca^{2+} & voltage-activated K^{+} channels	(Huang et al. 2015)
Spinal cord (rat)	Ischemia-reperfusion injury	1 mg/kg		Intravenous; at the beginning of ischemia	↑Neurological outcome ↓Spinal cord edema ↑Expression of large-conductance Ca^{2+} & voltage-activated K^{+} channels	(Liu et al. 2015)

Table 2. Continued.

Organ/Tissue	Model	Regimen		Findings	Reference
		Dose	Mode of treatment		
Spinal cord (rat)	Acute spinal cord injury (Modified Allen's method)	0.24 & 1.2 mg/kg	Intravenous; 15 min prior to injury	↑Hind limb motor function ↓Apoptosis ↑Mitochondrial membrane potential ↓ROS ↑Reduced glutathione ↑ATP levels ↑Mechanical allodynia threshold ↓Mitochondrial superoxide	(Li et al. 2015a)
Spinal dorsal horn (rat)	Perineural HIV-1 gp120-induced neuropathic pain	0.3, 1 & 3 µg/10 µL	Intrathecal	↓Apoptosis of retinal ganglion cells ↓Glial fibrillary acidic protein expression ↓Apoptosis of tubular epithelial cells ↓ROS ↑ATP ↑Creatinin kinase ↓Apoptosis of hepatocytes ↑Mitochondrial elongation ↑Respiratory complex activity ↓Insulin resistance index (systemic) ↓Phosphorylated Erk1/2 and p38 MAPK	(Kanda et al. 2016)
Retina (mouse)	Ischemia-reperfusion injury	50 mg/kg	Intraperitoneal; 60 min prior to & 6 h after ischemia	↓Apoptosis of retinal ganglion cells ↓Glial fibrillary acidic protein expression ↓Apoptosis of tubular epithelial cells ↓ROS ↑ATP ↑Creatinin kinase ↓Apoptosis of hepatocytes ↑Mitochondrial elongation ↑Respiratory complex activity ↓Insulin resistance index (systemic) ↓Phosphorylated Erk1/2 and p38 MAPK	(Park et al. 2011)
Kidney (rat)	Rhabdomyolysis-induced acute kidney injury	50 mg/kg	Intraperitoneal; 1 h or 12 h prior to rhabdomyolysis induction	↓Apoptosis of retinal ganglion cells ↓Glial fibrillary acidic protein expression ↓Apoptosis of tubular epithelial cells ↓ROS ↑ATP ↑Creatinin kinase ↓Apoptosis of hepatocytes ↑Mitochondrial elongation ↑Respiratory complex activity ↓Insulin resistance index (systemic) ↓Phosphorylated Erk1/2 and p38 MAPK	(Tang et al. 2013)
Liver (rat)	Sepsis	50 mg/kg	Intraperitoneal; 1 h prior to cecal ligation and puncture	↓Apoptosis of retinal ganglion cells ↓Glial fibrillary acidic protein expression ↓Apoptosis of tubular epithelial cells ↓ROS ↑ATP ↑Creatinin kinase ↓Apoptosis of hepatocytes ↑Mitochondrial elongation ↑Respiratory complex activity ↓Insulin resistance index (systemic) ↓Phosphorylated Erk1/2 and p38 MAPK	(Gonzalez et al. 2014)
Skeletal muscle (mouse)	Leptin deficiency	44 mg/kg	Intraperitoneal; 16 h and 1 h prior to insulin/glucose injection	↓Apoptosis of retinal ganglion cells ↓Glial fibrillary acidic protein expression ↓Apoptosis of tubular epithelial cells ↓ROS ↑ATP ↑Creatinin kinase ↓Apoptosis of hepatocytes ↑Mitochondrial elongation ↑Respiratory complex activity ↓Insulin resistance index (systemic) ↓Phosphorylated Erk1/2 and p38 MAPK	(Jheng et al. 2012)

8-oHdG; 8-Oxo-2-deoxyguanosine; ADP, adenosine diphosphate; AIF, apoptosis-inducing factor; Akt, AKT8 virus oncogene cellular homolog; ATP, adenosine triphosphate; Bax, bcl2-associated X protein; Bcl2, B-cell lymphoma 2; CD39, cluster of differentiation 39; CREB, cAMP response element binding; Drp1, dynamin-related protein 1; Erk 1/2, extracellular signal-regulated kinase-1/2; Fis1, fission 1; gp120, glycoprotein 120; LV, left ventricle; MMP 9, matrix metalloproteinase 9; p53, protein 53; PCNA, proliferating cell nuclear antigen; PO₂, oxygen pressure; ROS, reactive oxygen species; RV, right ventricle; SOD, superoxide dismutase; TIMP 3, tissue inhibitor of metalloproteinases-3.

confirmed the cytoprotective effect of Mdivi-1 in various cell types, particularly in cardiovascular cells and neurons. In contrast, Mdivi-1 has been shown to exert antiproliferative and cytotoxic effects in hyperproliferative cells such as in tumors and immortalized cells (Table 1).

Cardiomyocytes

Studies of Mdivi-1 in cardiomyocytes utilized a wide array of injury models to simulate pathological conditions such as ischemia-reperfusion injury and doxorubicin-induced cardiotoxicity (Ong *et al.* 2010; Gharanei *et al.* 2013; Sharp *et al.* 2014). Death of cardiomyocytes and HL-1 cells (a cardiac cell line derived from a mouse atrial tumor) was moderately repressed when pretreated with Mdivi-1 prior to ischemic insult (Ong *et al.* 2010; Sharp *et al.* 2014). The cytoprotective effect of Mdivi-1 was associated with increased phosphorylation of Drp1 at serine 637, thus preventing translocation of Drp1 into the mitochondria and consequently attenuating mitochondrial fragmentation (Ong *et al.* 2010; Sharp *et al.* 2014). Mdivi-1 has also been shown to confer cytoprotection by reducing production of reactive oxygen species (ROS), attenuating cytosolic calcium overload, restoring mitochondrial membrane potential, and delaying hypercontracture of cardiomyocytes in ischemia-reperfusion injury and doxorubicin-induced cardiotoxicity (Ong *et al.* 2010; Gharanei *et al.* 2013; Sharp *et al.* 2014).

Interestingly, So *et al.* (2012) recently reported that Mdivi-1 can alter the electrical activity of HL-1 cells. Treatment with Mdivi-1 prolonged the duration of the action potential, but increased the firing rate of spontaneous action potentials, inhibited the rapidly activating, delayed-rectifier K^+ current (I_{Kr}) and reduced the open probability of the muscarinic inward rectifier K^+ channels (K_{Ach}) (So *et al.* 2012). The inhibitory effect of Mdivi-1 on I_{Kr} was shown to be concentration-dependent with a half maximal concentration of 11.6 $\mu\text{mol/L}$, similar to that which showed cytoprotection in other studies (Ong *et al.* 2010; So *et al.* 2012; Gharanei *et al.* 2013; Sharp *et al.* 2014). This raises a concern that Mdivi-1 may have arrhythmogenic side effects.

It is important to note that outcomes of studies in HL-1 cells and neonatal cardiomyocytes require cautious interpretation for these cells are not truly representative of primary adult cardiomyocytes. Differences in cell morphology, electrophysiology, and biogenesis can contribute to their individual resistance or susceptibility toward pathological stimuli and pharmacological agents (Bass *et al.* 2001; Milerova *et al.* 2010; Kuznetsov *et al.* 2015). Mitochondria in adult cardiomyocytes have spatio-temporal restraint as well as slower rates of fusion–fission cycle when compared with neonatal cardiomyocytes

and HL-1 cells (Beraud *et al.* 2009; Chen *et al.* 2011; Piquereau *et al.* 2013). The relatively short and discrete mitochondria in adult cardiomyocytes are arranged in a regular pattern between myofibrils alongside the sarcomere. They do not form an interconnected network which might otherwise impose biomechanical restriction during cardiomyocyte contraction (Chen *et al.* 2011; Dorn and Kitsis 2015). In contrast, neonatal cardiomyocytes and HL-1 cells have relatively longer and more dynamic mitochondria which usually form interconnected networks throughout the cell (Amchenkova *et al.* 1988; Anmann *et al.* 2006). Furthermore, cellular metabolism in adult cardiomyocytes is more dependent on oxidative phosphorylation, whereas neonatal rat cardiomyocytes and HL-1 cells rely more on glycolysis (Bass *et al.* 2001; Anmann *et al.* 2006; Monge *et al.* 2009). Taken together, the morphological and bioenergetic differences between these cell types could lead to different outcomes in cell survival within similar experimental settings (Bass *et al.* 2001; Milerova *et al.* 2010; Kuznetsov *et al.* 2015).

Vascular cells

Mitochondrial fission is essential in smooth muscle cells for their proliferation and migration, processes that are relevant to several pathophysiological conditions such as premature closure of ductus arteriosus and pulmonary hypertension (Marsboom *et al.* 2012; Hong *et al.* 2013; Lim *et al.* 2015). Under oxidative stress and angiotensin II stimulation, ROS-induced smooth muscle cell proliferation and migration have been attributed to activation of protein kinase C δ which phosphorylates Drp1, resulting in translocation of Drp1 to the mitochondria and fission (Hong *et al.* 2013; Qi *et al.* 2013; Lim *et al.* 2015). Thus, Mdivi-1 has been shown to suppress smooth muscle cell proliferation and migration through attenuation of ROS production and Drp1 phosphorylation (Hong *et al.* 2013). In arterial smooth muscle cells derived from subjects with pulmonary arterial hypertension, Mdivi-1 was shown to suppress cell proliferation in a dose-dependent manner, an effect attributed to G2/M cell cycle arrest and shown to be independent of cyclin B1/CDK1-mediated phosphorylation of Drp1 at Serine 616 (Marsboom *et al.* 2012).

In addition to regulating proliferation and migration of smooth muscle cells, Drp1-mediated mitochondrial fission plays an important role in metabolism. Oxygen-induced mitochondrial fission in smooth muscle cells derived from ductus arteriosus has been shown to increase oxidative metabolism, oxygen consumption, and cytosolic calcium levels, which were all effectively prevented by Mdivi-1 (Hong *et al.* 2013).

In endothelial cells, inhibition of Drp1 with Mdivi-1 has been reported to induce premature senescence and

impair the angiogenic function of human umbilical cord vein endothelial cells by increasing mitochondrial ROS production and reducing autophagic flux (Lin *et al.* 2015). These studies suggest a key regulatory role of Drp1 in maintaining vascular homeostasis and angiogenesis, and therefore may be a therapeutic target for vascular repair.

Neurons

Similar to cardiomyocytes, neurons contain metabolically active mitochondria and are susceptible to bioenergetic dysfunction and cell death. Therefore, preservation of normal mitochondrial function through manipulation of mitochondrial morphology is a potential therapeutic approach to neuroprotection. The cytoprotective effect of Mdivi-1 in neurons has been well illustrated in several experimental injury models such as simulated ischemia-reperfusion and toxicity of glutamate and propofol (Zhang *et al.* 2013b; Wang *et al.* 2014; Liu *et al.* 2015; Twaroski *et al.* 2015). Mechanistic insights include delayed mitochondrial permeability transition pore opening, preserved mitochondrial membrane potential, increased adenosine levels, attenuated oxidative stress, and reduced endoplasmic reticulum stress (Zhang *et al.* 2013b; Wang *et al.* 2014; Liu *et al.* 2015; Twaroski *et al.* 2015; Xie *et al.* 2016). Cui *et al.* (2016) demonstrated that Mdivi-1 increases release of the neuroprotective agent, adenosine, through the cAMP/PKA/CREB pathway. Under oxidative stress, ROS trigger apoptotic cell death by increasing intracellular calcium levels and promoting outer mitochondrial membrane permeabilization, leading, in turn, to the release of cytochrome c and activation of the caspase cascade (Cardoso *et al.* 2004; Bajić *et al.* 2013). Treatment with Mdivi-1 has been shown to reduce ROS levels partly by augmenting the activity of intracellular antioxidant enzymes such as superoxide dismutase and catalase (Liu *et al.* 2015). The cytoprotective effect of Mdivi-1 in neurons has also been attributed to the opening of the large-conductance calcium- and voltage-activated potassium channels (Liu *et al.* 2015), which have long been implicated in cytoprotection against ischemic injury in the heart and are found in abundance in the central nervous system (Xu *et al.* 2002; Bentzen *et al.* 2014). The influx of potassium through these channels in the inner mitochondrial membrane can cause mild uncoupling of oxidative phosphorylation, ultimately inhibiting ROS production via Complex I (Kulawiak *et al.* 2008).

In a glutamate toxicity model, Mdivi-1 has been reported to protect primary rat cortical neurons and HT-22 cells (immortalized hippocampal neurons), from apoptosis. Moreover, Mdivi-1 remains protective when given

2–8 h after the onset of glutamate challenge (Grohm *et al.* 2012). Using a different injury model, Zhang *et al.* (2013c) has suggested that the therapeutic window of Mdivi-1 in protecting rat cortical neurons against simulated ischemia-reperfusion injury is limited to the ischemic period, for Mdivi-1 fails to confer protection when given during reperfusion. They showed that mitophagy-mediated mitochondrial clearance during reperfusion after ischemia is neuroprotective and inhibition of mitochondrial fission by Mdivi-1 may suppress mitophagy and aggravate ischemia-induced injury (Zhang *et al.* 2013c). These studies have demonstrated the importance of precise temporal regulation of the mitochondrial fission protein Drp1, in neurons under different pathophysiological conditions. Whether a similar therapeutic window for Mdivi-1 is applicable to other types of neurons and other cell types or in other injury models remains unclear and warrants further investigation.

Skeletal myoblasts

Mitochondrial dynamics play an important role in mitochondrial quality control and skeletal muscle homeostasis. Dysregulation of mitochondrial dynamics has been implicated in various pathological conditions of muscular dysfunction (Jheng *et al.* 2015). Inhibiting mitochondrial fission with Mdivi-1 has been shown to attenuate palmitate-induced mitochondrial dysfunction and insulin resistance in C2C12 skeletal myoblasts (Jheng *et al.* 2012). In L6 rat skeletal muscle cells, Mdivi-1 suppressed dexamethasone-induced autophagic flux and enhanced expression of muscle atrophy-related genes. This suggests a regulatory role for mitochondrial fission in mitochondrial quality control in skeletal muscles via activation of autophagy (Troncoso *et al.* 2014). Mitochondrial dynamics also play a significant role in the myogenic differentiation of myoblasts. Inhibition of Drp1-mediated mitochondrial fission with Mdivi-1 impaired myotube formation in both C2C12 myoblasts and primary murine myoblasts, which was accompanied by increased apoptosis and impaired mitochondrial biogenesis (Kim *et al.* 2013).

Cancer cells

In contrast to the cytoprotective effect in cardiovascular cells and neurons, Mdivi-1 exerts a cytodestructive effect in most hyperproliferative cancer and immortalized cell lines (Table 1). A hallmark of cancer cells is their unregulated proliferation and Drp1-mediated mitochondrial fission has been shown to play an important role in cancer cell growth (Rehman *et al.* 2012; Xie *et al.* 2015). Inhibiting mitochondrial fission with Mdivi-1 has been reported to exert a cytotoxic effect on cancer cells by reducing

progression of mitosis and inducing apoptosis (Qian *et al.* 2014, 2015; Suzuki-Karasaki *et al.* 2015; Wang *et al.* 2015a,b). As demonstrated in several cancer cell lines, Mdivi-1 induced G2/M cycle arrest by interfering with DNA replication and synthesis, and activating checkpoint kinase-1 (Qian *et al.* 2014; Wang *et al.* 2015b). The mitotic phase specifically is halted by Mdivi-1 as a result of impaired assembly of mitotic spindles and cytokinesis, consequently disrupting chromosome segregation leading to aneuploidy (Wang *et al.* 2015a). Importantly, the proapoptotic and antiproliferative effects of Mdivi-1 were absent in nontransformed normal human cells such as fibroblasts and epithelial cells, suggesting this effect is selective for tumor cells (Qian *et al.* 2014, 2015; Wang *et al.* 2015a,b; Xie *et al.* 2015).

Mdivi-1 has also been shown to enhance the cytotoxic effect of the anticancer compound cisplatin; it does so by triggering Noxa-dependent mitochondrial outer membrane permeabilization, bypassing the usual Bax/Bak-dependency (Qian *et al.* 2014). However, whether the cytotoxic effect of Mdivi-1 actually involves Drp1 remains controversial. Studies which suggest a Drp1-independent pathway have been conducted in Drp1-deficient, mouse immortalized embryonic fibroblasts without direct evidence in cancer cells (Qian *et al.* 2014, 2015; Wang *et al.* 2015a,b). There is other indirect evidence suggesting the involvement of the Bax/Bak pathway in the cytotoxic effect of Mdivi-1, using mouse embryonic fibroblasts deficient in Bax/Bak (Qian *et al.* 2014, 2015; Wang *et al.* 2015a,b). However, just one study in brain tumor initiating cells showed support for the involvement of Drp1 in proliferation and survival of cancer cells, for Drp1 knock-down or treatment with Mdivi-1 significantly reduced the tumorigenicity of the cells both *in vitro* and *in vivo* (Xie *et al.* 2015). In this study, the anticancer effect of Mdivi-1 was suggested to involve upregulation of AMP-activated protein kinase (AMPK), a downstream enzyme mediator of Drp1 (Xie *et al.* 2015).

Therapeutic Potential of Mdivi-1

Several studies in animal disease models have highlighted the therapeutic potential of Mdivi-1 in settings of ischemia-reperfusion injury (Table 2). In myocardial ischemia-reperfusion injury, treatment with Mdivi-1 increased animal survival rate, reduced myocardial infarct size, and improved heart function (Gharanei *et al.* 2013; Sharp *et al.* 2014, 2015). Consistent with *in vitro* findings, the cardioprotective effect of Mdivi-1 has been associated with activation of Akt signaling, a component of the reperfusion injury salvation kinase (RISK) pathway, and delayed the opening of mitochondrial permeability transition pores (Gharanei *et al.* 2013; Ong *et al.* 2015).

Therapeutic benefits of Mdivi-1 have also been found in other cardiovascular conditions such as pressure overload-induced heart failure (Givvimani *et al.* 2012), cardiac arrest (Sharp *et al.* 2015) and pulmonary artery hypertension (Marsboom *et al.* 2012). Regarding vascular diseases, Mdivi-1 prevented premature ductus arteriosus closure (Hong *et al.* 2013) and reduced neointima formation after carotid artery balloon injury (Lim *et al.* 2015) by suppressing proliferation of smooth muscle cells and fibrosis (Hong *et al.* 2013; Lim *et al.* 2015).

Disruption of mitochondrial dynamics has been associated with impaired mitochondrial biogenesis in the brain, which contributes to several neuropathologies. The lipophilic nature of Mdivi-1 enables the small molecule to penetrate the blood–brain barrier, reaching its peak concentration in brain tissue 4 h after intravenous injection (Cui *et al.* 2016), and treatment has enabled cytoprotection against neuronal loss following ischemia-reperfusion injury, diabetes-induced neuropathy, virus-sensory neuropathy, and seizures (Qiu *et al.* 2013; Xie *et al.* 2013; Zhang *et al.* 2013b; Zhao *et al.* 2014; Huang *et al.* 2015; Cui *et al.* 2016; Kanda *et al.* 2016). The neuroprotective effect of Mdivi-1 is manifest as improved brain hemodynamics and neurological outcome (Zhao *et al.* 2014; Li *et al.* 2015a,b; Liu *et al.* 2015), and the beneficial effects were associated with reduced ROS levels (Qiu *et al.* 2013; Li *et al.* 2015a; Kanda *et al.* 2016), enhanced activity of antioxidant enzymes (Qiu *et al.* 2013; Li *et al.* 2015a), preserved mitochondrial function (Huang *et al.* 2015; Cui *et al.* 2016), and increased expression of large-conductance Ca^{2+} and voltage-activated K^{+} channels (Liu *et al.* 2015).

The therapeutic potential of Mdivi-1 has also been reported in other organs such as retina (Park *et al.* 2011), kidney (Tang *et al.* 2013) and liver (Gonzalez *et al.* 2014), where *in vivo* administration of Mdivi-1 conferred cytoprotection of important cell types in these organs (*i.e.*, retinal ganglion cells, renal tubular epithelial cells, and hepatocytes, respectively). Despite these promising results, many challenges (as discussed in the next section) await before Mdivi-1 might be suitable for patients.

Future Perspectives

In addition to cell survival, mitochondrial fission plays important roles in related cellular functions such as proliferation and differentiation which are particularly important in organ development. Permanent alterations of mitochondrial dynamics are detrimental, often leading to mitochondrial diseases such as autosomal dominant optical atrophy (heterozygous mutation in *Opa1*), Charcot-Marie-Tooth type 2A neuropathy (heterozygous mutations in *Mfn2*) and abnormal brain development

(A395D mutation in Drp1). The physiological importance of mitochondrial dynamics in the heart has also been demonstrated in various mouse models with genetic deletion of mitochondrial fusion (Mfn1, Mfn2, and Opa1) or fission (Drp1, Mff, and Fis1) proteins, exhibiting developmental cardiac defects and increased susceptibility to cardiac injury (Chan 2012; Babbar and Sheikh 2013). Therefore, pharmacological agents such as Mdivi-1, that allow timely and reversible manipulation of mitochondrial morphology in different pathological conditions may have therapeutic potential. However, effective application of Mdivi-1 to manipulate mitochondrial dynamics will require further studies to identify the optimal therapeutic window through a better understanding of the temporal correlation between disease progression and changes in mitochondrial morphology, which can often be disease-specific.

The divergent effects of Mdivi-1 on cell survival is likely to be dependent on cell type. While Mdivi-1 exerts protection of cardiovascular cells and neurons, this small molecule is toxic to hyperproliferative cells such as cancer cells and most immortalized cell lines (Table 1). The differential effects of Mdivi-1 on cell survival could also be attributed to the duration of treatment. Most in vitro studies showing the cytotoxic effect of Mdivi-1 were conducted for longer than 16 h of treatment, whereas studies reporting the cytoprotective effect of Mdivi-1 were performed in much shorter duration (≤ 8 h treatment) (Table 1). This suggests that chronic inhibition of Drp1 with Mdivi-1 might well be detrimental to cell function and survival.

Although the precise mechanisms underlying the differential effects of Mdivi-1 on cell survival remain unclear, Drp1 has been shown to interact with various proteins, such as Cdk1/cyclin B, SUMO1/Sentrin/SMT3 Specific Peptidase 3 (SEN3), Bax/Bak, Noxa, protein kinase A, AMPK, Akt, and Erk2, depending on its posttranslational modification (Chang and Blackstone 2007; Taguchi et al. 2007; Wasiak et al. 2007; Guo et al. 2013; Jheng et al. 2015; Kashatus et al. 2015). In this regard, further investigation of functional outcomes which result from different posttranslational modifications of Drp1 will provide more mechanistic insights on the cytoprotective and cytotoxic effects of Mdivi-1.

The pharmacokinetics and cytotoxic profile of Mdivi-1 remain poorly understood. Cui et al. (2016) is the only in vivo pharmacokinetic profile of Mdivi-1 conducted to date, and they found intraperitoneal administration of Mdivi-1 at 20 mg/kg resulted in peak plasma and brain concentrations 2 and 4 h later, respectively, with a half-life estimated at 12 h (Cui et al. 2016). Future studies should also characterize the pharmacokinetics of Mdivi-1 via intravenous injection, a more common route of drug

administration for patients. Furthermore, the toxicological profile of Mdivi-1 is yet to be fully established. An in vitro study in the HL-1 cardiac cell line has shown that Mdivi-1 can inhibit potassium channels which resulted in longer duration and increased firing rate of action potentials, suggesting a potential arrhythmogenic effect of Mdivi-1 (So et al. 2012). However, the relevance of this observation should be confirmed in primary cardiomyocytes and in vivo by electrophysiological studies of the heart muscle. Future studies should also investigate the physiological effect of the active metabolites of Mdivi-1 to ensure that they are devoid of undesirable biological effects, as prerequisite to advance Mdivi-1 closer to clinical application.

In summary, current preclinical studies have demonstrated therapeutic potential of Mdivi-1 as a cytoprotective, as well as an anticancer agent. However, many challenges and uncertainties remain to be addressed before such drugs might be applied clinically. The mechanism of action by which Mdivi-1 affects cell survival also remains unclear. The pharmacokinetics (absorption, distribution, metabolism, and excretion) and toxicology profiles of Mdivi-1 await further study before clinical translation. Moreover, the lipophilicity (i.e., poor water solubility) of Mdivi-1 may limit its utility, and new Drp1 inhibitors with better specificity, potency, and solubility are highly desirable.

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Disclosures

None declared.

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