Mitochondrial Fission via DRP1-Mediated GTPase Activity Versus MFF/FIS1-Dependent Receptor Scaffolding: A Mechanistic and Functional Comparison in Hypoxia-Induced Cardiomyocyte Apoptosis

Skyler Walker
Professor
Ruprecht-Karls-Universität Heidelberg
Im Neuenheimer Feld 205, 69120 Heidelberg, Baden-Württemberg, Germany

Adrian Scott
PhD
Osaka University Graduate School of Medicine
2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

Robin Miller
Associate Professor
University of Toronto, Department of Cell and Systems Biology
25 Harbord Street, Toronto, Ontario M5S 3G5, Canada

Abstract. Mitochondrial dynamics govern cardiomyocyte fate under hypoxic stress, yet the relative contributions of DRP1-driven GTPase hydrolysis and receptor-scaffolded fission through MFF/FIS1 complexes remain incompletely resolved. Using primary neonatal rat ventricular cardiomyocytes subjected to controlled hypoxia–reoxygenation, we systematically compared pharmacological DRP1 inhibition (Mdivi-1), siRNA-mediated MFF/FIS1 knockdown, and combinatorial suppression across indices of mitochondrial morphology, membrane potential (ΔΨm), cytochrome c release, and caspase-3 activation. Super-resolution STED microscopy and flow cytometric analyses revealed that MFF/FIS1 scaffolding predominantly initiates pathological fission events, whereas DRP1 GTPase activity amplifies downstream apoptotic signaling. Combinatorial inhibition achieved 74% reduction in apoptotic index compared to single-pathway targeting. These findings reframe the hierarchical architecture of the mitochondrial fission cascade and suggest dual-pathway therapeutic targeting as a superior cardioprotective strategy.

Keywords: mitochondrial fission, DRP1 GTPase activity, MFF/FIS1 receptor complex, hypoxia-reoxygenation cardiomyocyte apoptosis, mitochondrial membrane potential, STED super-resolution microscopy, cytochrome c release, caspase-3 activation cascade, cardioprotective dual-pathway inhibition

Introduction

Mitochondrial integrity is fundamental to cellular homeostasis, and its dysregulation underlies a spectrum of pathologies ranging from ischemic heart disease to neurodegenerative disorders. Among the most critically studied processes in contemporary cell biology is mitochondrial fission—the programmatic fragmentation of the mitochondrial

network—which has emerged as a nexus between metabolic adaptation and programmed cell death. As cardiovascular disease continues to represent the leading cause of global mortality in the 2024–2026 epidemiological landscape, dissecting the molecular architecture of cardiomyocyte apoptosis at the organellar level has acquired immediate translational urgency. The dynamin-related protein 1 (DRP1), a cytosolic GTPase recruited to the outer mitochondrial membrane (OMM) upon cellular stress, has long been considered the principal effector of mitochondrial fission. Its oligomerization into ring-like structures drives membrane constriction through GTP hydrolysis, a process extensively characterized in yeast and mammalian models alike. However, the mechanistic primacy of DRP1 has increasingly been challenged by evidence implicating OMM-resident receptor proteins—mitochondrial fission factor (MFF) and fission 1 protein (FIS1)—as essential scaffolding platforms that nucleate DRP1 recruitment and modulate the spatiotemporal pattern of fission events. Whether MFF/FIS1-dependent receptor assembly functions upstream, in parallel, or in a context-dependent hierarchy relative to intrinsic DRP1 GTPase catalytic activity remains a subject of active mechanistic debate. Previous studies have predominantly employed single-pathway perturbation designs, leaving the relative weight of each axis under physiologically relevant hypoxic conditions poorly constrained. The use of Mdivi-1, a selective DRP1 inhibitor, has yielded cardioprotective phenotypes in multiple ischemia–reperfusion models; however, its off-target liabilities and the compensatory upregulation of MFF-dependent recruitment have confounded interpretations. Conversely, RNAi-mediated silencing of MFF or FIS1 individually produces intermediate protective effects, suggesting that functional redundancy and pathway crosstalk significantly modulate apoptotic outcomes. The present study addresses this mechanistic gap through a rigorously controlled, multi-arm experimental design that directly compares DRP1-centric pharmacological inhibition, receptor-scaffold-targeted gene silencing, and dual combinatorial suppression within the same hypoxia–reoxygenation cellular model. Morphometric analyses via STED super-resolution microscopy, coupled with flow cytometric quantification of $\Delta\Psi m$ collapse and biochemical assessment of cytochrome c translocation and caspase-3 cleavage, provide an integrative functional portrait of pathway-specific contributions. This article is structured as follows: Section 2 describes the materials, cell culture protocols, and experimental methodologies employed; Section 3 presents quantitative morphological and biochemical results across all experimental arms; Section 4 discusses the mechanistic implications within the broader context of mitochondrial quality control and therapeutic targeting; and Section 5 offers concluding remarks and perspectives for future in vivo validation.

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