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The role of mitochondrial fission in cardiovascular health and disease

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Abstract

Mitochondria are organelles involved in the regulation of various important cellular processes, ranging from ATP generation to immune activation. A healthy mitochondrial network is essential for cardiovascular function and adaptation to pathological stressors. Mitochondria undergo fission or fusion in response to various environmental cues and these dynamic changes are vital for mitochondrial function and health. In particular, mitochondrial fission is closely coordinated with the cell cycle, and is linked to changes in mitochondrial respiration and membrane permeability. Another key function of fission is the segregation of damaged mitochondrial components for degradation by mitochondrial autophagy (mitophagy). Mitochondrial fission is induced by the large GTPase dynamin-1-like protein (DNM1L; also known as dynamin-related protein 1 (DRP1)) and is subject to sophisticated regulation. Activation requires various post-translational modifications of DNML1, actin polymerization and the involvement of other organelles such as the endoplasmic reticulum, Golgi and lysosomes. A decrease in mitochondrial fusion can also shift the balance towards fission. Although mitochondrial fission is necessary for cellular homeostasis, this process is often aberrantly activated in cardiovascular disease. In fact, strong evidence exists that aberrant mitochondrial fission directly contributes to disease development. In this Review, we compare the physiological and pathophysiological roles of mitochondrial fission and discuss the therapeutic potential of preventing excessive mitochondrial fission in the heart and vasculature.

Introduction

Mitochondria are multifaceted organelles that regulate various important cellular processes including metabolism, ATP generation and activation of inflammation. These organelles are also involved in determining cell fate during differentiation and activating cell death[1]. Regulation of these processes is often associated with changes in mitochondrial morphology, where mitochondria undergo fission or fusion in response to changes in the cellular environment. Studies initially indicated a connection between increased mitochondrial fission and activation of apoptosis[2], but it is now clear that mitochondrial

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morphology is also closely linked to bioenergetics and is affected by changes in metabolic demand[3,4]. In addition, mitochondrial fission contributes to quality control by separating damaged organelles from the healthy network for degradation by mitochondrial autophagy (mitophagy)[5]. Mitochondrial fission is also closely coordinated with the cell cycle[6] and facilitates the equal segregation of mitochondrial DNA (mtDNA) in daughter organelles[7]. Considering the vital role of mitochondria in cellular homeostasis, it is not surprising that dysregulation of mitochondrial dynamics is associated with disease development.

Because of its high energy demand, the heart is enriched with mitochondria and well-balanced mitochondrial fission and fusion are essential for cardiac homeostasis. Although fission is important for functional mitophagy in the heart[,8], excessive mitochondrial fission contributes to various cardiovascular pathologies, including ischaemia–reperfusion injury (IRI)[9,10], pathological hypertrophy[11] and atherosclerosis[12]. In this Review, we detail the molecular mechanisms underlying mitochondrial fission mediated by dynamin-1-like protein (DNM1L; also known as dynamin-related protein 1 (DRP1)) and its function in various cellular processes. In particular, we highlight novel mechanisms for DNML1 activation and interorganellar regulation of mitochondrial morphology. We also discuss the increasing evidence for the role of mitochondrial fission in cardiovascular disease progression, and emerging therapeutic strategies to restrict fission-induced cardiovascular pathophysiology.

Mechanisms of mitochondrial fission

Mitochondrial machinery for fission and fusion

DNML1, a member of the dynamin family of GTP-binding proteins, is the primary mediator of mitochondrial fission. DNML1 translocates from the cytosol to mitochondria where it assembles into helical oligomers that wrap around the outer mitochondrial membrane (OMM) to facilitate constriction and scission in a GTP-dependent manner[13,14] (FIG. 1a). Classical dynamins directly bind phospholipids in membranes through their pleckstrin homology domain, which is lacking in DNML1 and so adaptor proteins such as mitochondrial fission 1 (FIS1), mitochondrial fission factor (MFF), mitochondrial dynamics protein 49 (MID49) and MID51 are used to anchor DNML1 to the mitochondrial surface. FIS1 was the first protein reported to function as a mitochondrial adaptor protein for DNML1 in cells[15–17]. However, whether FIS1 is an essential regulator of DNML1-mediated fission is unclear, because FIS1-deficient mammalian cells have mild or no fission defects[16–20]. Other studies have identified that FIS1 specifically functions in mitophagy[21–23]. Subsequent identification of MFF, MID49, and MID51 have led to additional questions about the specific functions of these adaptor proteins in regulating DNML1-mediated fission. For example, knockdown of *MFF*, but not *FIS1*, in HeLa cells leads to mitochondrial elongation, and reduces recruitment of DNML1 to mitochondria[20]. Similarly, MFF-deficient mouse embryonic fibroblasts (MEFs) have more elongated mitochondria compared with *Fis1*$^{-/-}$ MEFs, and simultaneous deletion of *Fis1* and *Mff* is required to recapitulate the mitochondrial phenotype seen in *Dnml1*$^{-/-}$ MEFs[18]. However, in contrast to these findings, Osellame et al. reported that single deletion of *MiD49*, *MiD51*, *Mff* or *Fis1* has no effect on mitochondrial morphology or recruitment of DNML1 in MEFs[24]. Only simultaneous deletion of multiple adaptor proteins disrupts recruitment of

DNML1 and results in a fused mitochondrial network[24], suggesting that redundancies exist among the various adaptor proteins. Thus, the specific roles of individual DNML1 adaptors and their regulation is still not well understood. Other proteins, including the BCL-2 family members BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3) and induced myeloid leukaemia cell differentiation protein Mcl-1 (MCL-1), as well as FUN14 domain-containing protein 1 (FUNDC1), have also been reported to recruit DNML1 to the OMM to induce fission[25–27].

Studies in DNML1-deficient mice have confirmed the importance of functional mitochondrial fission for cardiac health (TABLE 1). Global DNML1-deficiency is embryonically lethal[28,29], whereas myocyte-specific DNML1-deficient mice develop severe cardiac dysfunction after birth and die within 10 days[30,31]. Deletion of *Dnml1* in the hearts of adult mice also results in rapid heart failure and premature death[8,32]. Interestingly, however, doxycycline-inducible expression of the dominant-negative DNML1^{K38A} for up to 6 months in transgenic mice is reported to have no systemic effect[33]. On the basis of phenotypes in mice with genetic deletion of *Dnml1*, fission is not likely to be completely abrogated in DNML1^{K38A} transgenic mice. Little is known about the various adaptor proteins in the heart, but Chen and colleagues found that MFF is important for heart function because *Mff*-deficient mice develop dilated cardiomyopathy and die from heart failure by 13 weeks of age[34]. The milder cardiac phenotype in MFF deficiency compared with the lethality in *Dnml1*-knockout mice suggests that DNML1-mediated mitochondrial fission can still function in the absence of MFF probably due to partial compensation by other adaptor proteins. However, the specific development of heart failure in mice with global MFF deficiency suggests that MFF has a more important role in regulating DNML1-mediated fission in the mature heart relative to other tissues.

DNML1-mediated mitochondrial fission is opposed by fusion between mitochondria, which is driven by GTPases mitofusin-1 (MFN1) and MFN2 in the outer membrane and dynamin-like 120 kDa protein, mitochondrial (also known as optic atrophy protein 1; OPA1) in the inner membrane. The loss of these fusion factors is sufficient to cause a similar fragmented mitochondrial phenotype seen with DNML1 activation[35]. Studies in mice have demonstrated the importance of mitochondrial fusion in cardiac homeostasis (TABLE 1). Despite the overlapping function between MFN1 and MFN2 in promoting mitochondrial fusion, deletion of either *Mfn1* or *Mfn2* causes embryonic lethality[36] suggesting that these proteins have tissue-specific expression or function during development. By contrast, mice with cardiac-specific deletion of either *Mfn1* or *Mfn2* is well tolerated with mice developing normally, whereas simultaneous ablation of *Mfn1* and *Mfn2* in hearts results lethality during development[37] or shortly after birth[38]. Similarly, a truncating nonsense mutation in *Opa1* is embryonically lethal in mice[39] and imbalanced OPA1 processing leads to pronounced mitochondrial fission in the myocardium of mice eventually leading to heart failure[40]. Interestingly, an imbalance in mitochondrial fission or fusion contributes to the development of heart failure. The lethal cardiomyopathy that develops in mice lacking the fission protein MFF is rescued with the concomitant deletion of *Mfn1*[34]. Similarly, *Mfn1*/*Mfn2*/*Dnml1* cardiac triple-knockout mice with disruptions in both fission and fusion are viable with longer survival and delayed development of cardiomyopathy compared with cardiac-specific *Drp1*- or *Mfn1/2*-deficient mice[41]. The fact that cardiomyocyte-specific genetic disruption of

either mitochondrial fission or fusion leads to faster development of heart failure than with deletion of DRP1 or MFN1/MFN2 alone suggest that disproportionate fission or fusion is more deleterious that a simultaneous disruption in both processes.

Post-translational modifications in DNML1

DNML1 is subjected to various post-translational modifications that regulate its activity in response to changes in metabolic or redox status (FIG. 1b). Phosphorylation of DNML1 is well known to stimulate or inhibit fission depending on the specific residue targeted. Phosphorylation of DNML1 at Ser616 and Ser637 — corresponding to mouse Ser579 and Ser600, respectively — are among the most studied sites in fission regulation. Phosphorylation at Ser616 within the GTPase effector domain is associated with translocation of DNML1 to the mitochondria and activation of fission[42–44], whereas phosphorylation at Ser637 remains controversial. Ser637 phosphorylation was initially reported to restrict fission through cytosolic retention of DNML1[45,46]. However, DNML1 has still been observed to translocate to the mitochondria with Ser637 phosphorylation[47], and Ser637 phosphorylation by protein kinase D and Rho-associated protein kinase 1 is associated with increased fission in myocytes and endothelial cells, respectively[48,49]. Interestingly, in a study published in 2021, Ser637 phosphorylation was shown to promote subsequent Ser616 phosphorylation and phosphorylation at both sites was necessary for maximal fission in MEFs[50]. However, blocking Ser616 phosphorylation downstream of Ser637 resulted in mitochondrial elongation rather than fission[50]. Thus, although the precise mechanism for their cross-talk remains elusive, Ser616 phosphorylation status seems to dictate whether phosphorylation at Ser637 will promote fission or fusion. Additional post-translational modifications could potentially coordinate with Ser637 phosphorylation to determine its effect on mitochondrial morphology. A comprehensive understanding of kinases and phosphatases responsible for Ser616 and Ser637 modulation, and the potential coordination with additional post-translational modifications, is needed and might explain the discrepancy in the findings for Ser637.

In addition to phosphorylation, DNML1 activity is also regulated by SUMOylation[51–53], acetylation[54], O-linked-N-acetylglucosaminylation (O-GlcNAcylation)[55], and S-nitrosylation[56–59] (FIG. 1b). Protein SUMOylation involves the covalent attachment of small ubiquitin-related modifier (SUMO) proteins to lysine residues. By contrast to ubiquitination, SUMOylation does not label a protein for degradation but serves to modify function or location. DNML1 can be SUMOylated by both SUMO1 and SUMO2 or SUMO3 at one or more lysine clusters within the variable region[52,53]. Interestingly, SUMO1 and SUMO2/3 have opposite effects on DNML1 activity, where conjugation of SUMO1 promotes DNML1 association with the mitochondrial membrane[60], whereas SUMO2/3 reduce its binding[53]. The SUMOylation of DNML1 prevents it from interacting with MFF at the OMM[51], and a SUMOylation-resistant mutant of *DNML1* exhibits increased interaction with MFF and association with mitochondria[51]. This finding suggests that DNML1 must be deSUMOylated for induction of mitochondrial fission. Moreover, whereas addition of SUMO2/3 prevents mitochondrial fission and cell death during ischaemia[51], SUMO1 conjugation to DNML1 promotes fission and cell death following treatment with

the apoptosis inducer staurosporine[61]. Complex regulation of DNML1 SUMOylation clearly exists depending on the cellular context.

Acetylation is another major post-translational modification in cells, involving the transfer of an acetyl group onto a lysine residue in a target protein. Protein acetylation rates vary according to nutrient status, and hyperacetylation of mitochondrial proteins is well known to accompany metabolic remodelling during heart failure[62]. Indeed, nutrient overload is associated with DNML1 acetylation at Lys632, which promotes mitochondrial fission in mice and primates fed a high-fat diet[54]. The modification of serine and threonine residues by O-GlcNAcylation is also regulated by metabolic status, and O-GlcNAcylation of DNML1 is evident in cultured neonatal cardiomyocytes as well as in the hearts of mice with type 2 diabetes mellitus[55]. Importantly, both DNML1 acetylation and O-GlcNAcylation seem to facilitate increased Ser616 phosphorylation and subsequent translocation of DNML1 to the mitochondria[54,55,]. Cysteine residues are highly sensitive to oxidative modification, such as nitrosylation, which involves the coupling of a nitric oxide moiety to a reactive cysteine thiol[63]. DNML1 activity can be regulated by S-nitrosylation at Cys644, a residue that is conserved from flies to humans[57]. Although researchers agree that S-nitrosylation of DNML1 promotes mitochondrial fission, the mechanism by which this post-translational modification regulates DNML1 activity is not well understood. For example, although S-nitrosylation of DNML1 generally coincides with Ser616 phosphorylation, whether nitrosylation directly stimulates DNML1 GTPase activity remains controversial[56–59]. Considering the pivotal role of nitric oxide in vascular tone and cardiac function[64], investigation into the role of DNML1 nitrosylation in cardiovascular disease is warranted.

Overall, these studies demonstrate clearly that DNML1 is subject to highly sophisticated regulation. Considering the pleiotropic effects of mitochondrial fission, any single post-translational modification is unlikely to underlie the physiological versus pathological effects of this pathway. Rather, multiple post-translational modifications on DNML1 are likely to have a synergistic or additive effect. Therefore, simultaneous assessment of multiple post-translational modifications in a given cellular context could provide insight into the relative tone of fission activation, which is believed to be a major factor determining functional outcomes.

Interorganelle contacts

Mitochondrial fission is not an autonomous process, but involves the actin cytoskeleton and other organelles, including the endoplasmic reticulum (ER), Golgi and lysosomes. Mitochondria form physical contacts with the ER, which are known as mitochondria–ER association membranes (MAMs, or mitochondria–ER contact sites, MERCSs). These contact sites are important for phospholipid synthesis, calcium signalling and autophagosome formation[65]. MAMs are also sites where fission takes place[66]. Using powerful, high-resolution microscopy techniques, Friedman et al. observed that mitochondrial division events predominantly occur where DNML1 and its adaptor MFF associate with MAMs[66]. In a subsequent study, DNML1 oligomers were reported to assemble at the ER prior to transfer to the mitochondria, suggesting that the ER might function as a platform for DNML1 oligomerization[67]. The ER is also important in driving

constriction of the OMM, as ER tubules wrap around the mitochondria during fission (FIG. 2)[66]. In addition, the cytoskeleton positively regulates fission, as actin filaments at MAMs promote DNML1 recruitment and stimulate its GTPase activity[68–70]. Independent of changes in GTPase activity, a study published in 2022 showed that DNML1 undergoes retrograde transport along actin filaments through the PDZ domain-containing protein GIPC1 (also known as GAIP/RGS19-interacting protein), a scaffolding protein involved in trafficking[71]. This active transport allows for effective delivery of peripheral cytosolic DNML1 to distal mitochondria in the perinuclear region.

In addition to the ER and cytoskeleton, phosphatidylinositol 4-phosphate (PI(4)P) has emerged as a critical factor in the regulation of fission. Vesicles from the *trans*-Golgi network enriched in PI(4)P are routed to MAM constriction sites downstream of DNML1 translocation immediately prior to mitochondrial division[72]. Studies have demonstrated that PI(4)P can also be delivered by lysosomes to the sites of fission[73,74]. However, because depletion of PI(4)P on lysosomes still leads to a decline in mitochondrial fission, PI(4)P-enriched vesicles from the Golgi seem to be unable to compensate for the loss of lysosomal PI(4)P[74]. Although lysosomal contacts with the mitochondria might initially be important for PI(4)P transfer, untethering of lysosome-mitochondrial contacts is clearly necessary for the execution of fission. Here, the DNML1 adaptor FIS1 recruits the GTPase activating protein TBC1 domain family member 15 (TBC1D15), which facilitates the inactivation of Ras-related protein Rab-7 and subsequent organelle untethering for the completion of mitochondrial fission[73]. Interestingly, myocardial infarction is associated with prolonged mitochondria-lysosomal contacts, and TBC1D15 overexpression in mice reverses these outcomes to restore cardiac function after injury[75]. Although the enrichment of PI(4)P at the site of fission could be involved in recruiting proteins required for actin polymerization[76], the exact function for this lipid in fission is unclear and requires further investigation.

Mitochondrial calcium levels

A transient increase in mitochondrial calcium uptake is a general feature of fission, where the influx of calcium into the mitochondrial matrix stimulates inner mitochondrial membrane (IMM) constriction prior to fission[77]. In addition, ER contact sites with the mitochondria and polymerization of actin are both needed for fission-induced mitochondrial calcium uptake[78]. Transport of calcium across the IMM into the mitochondrial matrix occurs through the mitochondrial calcium uniporter (MCU). The MCU is essential for calcium-mediated IMM constriction, and MCU depletion reduces both basal and stimulated mitochondrial fission[78]. Interestingly, loss of DNML1 results in upregulation of MCU in mouse heart[79] and skeletal muscle[80], and disrupts calcium homeostasis in myofibers due to abnormally elevated MCU-mediated mitochondrial uptake[80]. Altogether, these data suggest that DNML1 and MCU coordinate to maintain mitochondrial fission and calcium homeostasis in cells.

Physiological roles of mitochondrial fission

Mitochondrial division and mitophagy

Mitochondrial fission has a role in many basic cellular functions, including cell division. Symmetrical mitochondrial fission (also known as replicative fission) generates two functional daughter mitochondria (FIG. 3a) and is closely coordinated with the cell cycle to facilitate the equal segregation of mitochondria during cell division[81,82]. Indeed, loss of DNML1 reduces proliferation of myofibroblasts[83], vascular smooth muscle[84] and pulmonary artery smooth muscle cells[85], confirming the importance of DNML1 in cell division. Mitochondria contain their own genome that encodes respiratory chain proteins, mitochondrial ribosomal and tRNA. The genetic material is packaged into structures known as nucleoids that contain several copies of mitochondrial DNA (mtDNA) and proteins involved in transcription. Mitochondrial dynamics ensure the correct distribution of mtDNA in cells; defects in fission leads to formation of abnormally large mtDNA nucleoids that cluster in fused mitochondria, which correlates with abnormal heart development in mice[30,86]. Importantly, these abnormal mtDNA nucleoids disrupt mitochondrial respiration and sarcomere organization in developing myocytes[30].

Another key function of fission is the segregation of damaged mitochondrial components through asymmetrical division. This form of fission leads to the formation of a healthy mitochondrion and a dysfunctional fragment, which is subsequently labelled for mitophagy[87] (FIG. 3b). The process of mitophagy involves the formation of a double-membraned autophagosome, which engulfs damaged mitochondria and delivers them to the lysosome for degradation by hydrolytic enzymes[5]. Interestingly, in 2021, Kleele et al. reported that FIS1 recruits DNML1 to the periphery of the mitochondrion for asymmetrical fragmentation, whereas MFF seems to be uniquely involved in symmetrical fission at the midpoint of a mitochondrion[22]. Mitophagy of dysfunctional mitochondria is primarily regulated by loss of membrane potential, whereas daughter mitochondria arising from symmetrical fission retain membrane polarization and re-fuse with the existing mitochondrial network[22,87,88]. DNML1 interacts with zinc transporter ZIP1 at the site of mitochondrial fission to regulate membrane potential[89]. Specifically, DNML1–ZIP1 interaction promotes the influx of Zn^{2+} into the mitochondrial matrix leading to a reduction in mitochondrial membrane potential, which enables the clearance of depolarized mitochondrial fragments through mitophagy[89]. Despite normal mitochondria and cardiac function at baseline, hemizygous *Dnml1*-knockout mice exhibit impaired mitophagy during pathological stress[90], and complete loss of DNML1 in cardiac myocytes results in accumulation of dysfunctional mitochondria in cultured cells[91] and mouse hearts[8]. Similarly, mutations in *Dnml1* that prevent its disassembly during execution of fission also result in lethal heart failure due to defects in mitophagy[92]. Therefore, although the myocardium can tolerate reduced rates of mitochondrial fission under baseline conditions, a minimal threshold of DNML1-dependent fission is essential for mitochondrial quality via mitophagy. Although DNML1-mediated fission is clearly sufficient to promote mitophagy, some studies have demonstrated that DNML1 is not necessary for mitophagy[32,93,94]. Moreover, Song and colleagues reported that preventing mitophagy delays cardiomyopathy in cardiac-specific *Dnml1*-knockout mice[95]. Despite the controversy about whether fission is an essential

prerequisite for mitophagy, the studies discussed here confirm the importance of DNML1-mediated asymmetrical fission in segregating damaged mitochondria from the healthy network for mitophagy.

Bioenergetics

Mitochondrial morphology is closely linked to respiration and ATP generation. Studies have shown that inhibition or depletion of DNML1 in cultured cardiac myocytes is associated with reduced mitochondrial respiration[8,91,96]. This finding is consistent with impaired mitochondrial function and respiratory chain complex activities in cardiac-specific *Dnml1*-knockout mice[30,31]. Cardiac respiratory chain deficiencies are also evident with the loss of MFF in vivo, confirming the importance of mitochondrial fission for respiratory function[34]. A portion of the mitochondrial respiratory chain complexes form supercomplex structures to ensure efficient energy production[97]. Interestingly, mice with muscle-specific loss of DNML1 have impaired respiratory chain supercomplex assembly[80], suggesting that functional mitochondrial fission is important for their formation, stability or both. In addition to supporting basal respiration in myocytes, DNML1-dependent fission in the heart is critical for increased mitochondrial respiratory capacity during exercise[3]. DNML1 is rapidly recruited to cardiac mitochondria following intense exercise; however, elevated basal fission prior to exercise reduces endurance, suggesting that excessive fission could exhaust respiratory function[25]. Indeed, sustained fission through DNML1 in various myocyte models of ischaemia–reperfusion[10,98,99], proteotoxicity[100,101], lipotoxicity[102,103] or inflammation[104,105] is consistently associated with mitochondrial bioenergetic defects. These relationships are also conserved in endothelial cells[106,107] and vascular smooth muscle cells (VSMCs)[108] exposed to pathological conditions. Therefore, although physiological fission supports mitochondrial function by facilitating supercomplex assembly and respiration, its hyperactivation during pathological stress often perturbs respiratory capacity.

Cell death

Mitochondrial fragmentation is often present during activation of cell death, and many studies have demonstrated that DNML1-mediated fission is directly involved in apoptosis. BCL2 Associated X, Apoptosis Regulator (BAX) is a pro-apoptotic protein that translocates to the mitochondria upon activation of apoptosis, where it induces mitochondrial outer membrane permeabilization (MOMP) resulting in cytochrome *c* release and downstream caspase activation. Activated BAX oligomers cluster at mitochondrial fission sites[109], and DNML1-dependent mitochondrial membrane remodelling directly promotes BAX oligomerization during apoptosis[110]. As such, genetic and pharmacological inhibition of DNML1 is sufficient to prevent BAX-mediated MOMP[2,111], consistent with the apoptosis resistance observed in *Dnml1*-null embryonic mouse stem cells[28] as well as MEFs lacking DNML1 adaptor proteins[24]. In a study published in 2022, DNML1 was shown to directly interact with BAX, and forced dimerization of these two proteins was sufficient to cause mitochondrial remodelling, membrane permeabilization and cell death even in the absence of apoptotic stimuli[112]. Clearly, DNML1-dependent mitochondrial fission promotes BAX-dependent MOMP, cytochrome *c* release and caspase activation in cells. However, mitochondrial fission can still occur in MEFs lacking DNML1 or BAX, indicating that

multiple pathways exist for mitochondrial fission during apoptosis[110]. Although the specific functions of individual DNML1 adaptor proteins remain unclear, MID49 and MID51 seem to be uniquely essential for DNML1-dependent cristae remodelling during apoptosis in vitro[113]. The mitochondrial permeability transition pore (mPTP) is a non-selective channel that allows the passage of any molecule with a molecular mass <1.5 kDa. Excessive opening of the mPTP leads to the influx of solutes into the matrix, swelling and rupture of the OMM[114]. Studies have shown that DNML1 induces opening of the mPTP in response to various stressors[42,115,116]. Chronic fission is also linked to mPTP opening in cardiovascular pathophysiology[116,117].

Notably, DNML1-mediated fission alone is not sufficient to induce cell death, as overexpression of DNML1 in cells does not cause death[41,118,119]. Furthermore, transgenic mice overexpressing DNML1 in the heart have normal cardiac function despite extensive mitochondrial fission[41]. This finding suggests that additional stimuli are needed for mitochondrial fission to switch into a pro-death pathway via mPTP opening or BAX-mediated MOMP. Additional studies are needed to determine the molecular mechanism underlying DNML1-mediated cell death and the specific conditions that activate this mechanism.

Immune function

Immune cells defend organisms against infections and other foreign material. Research has demonstrated a role for mitochondrial fission in immune cell function. During chemotaxis, mitochondrial network organization via mitochondrial fission facilitates immune cell migration[120]. Simula et al. reported that T cell infiltration into tumours is dependent on DNML1, and ablation of DNML1 in the T-cell lineage disrupts infiltration of these cells in solid tumours[121] (TABLE 2). Thus, immune surveillance against tumours is compromised in mice with a specific DNML1-deficiency in the T-cell lineage. Moreover, when the immune response is activated, immune cells undergo a metabolic transition from a quiescent to an active state. Naïve T cells in the thymus rely on mitochondrial oxidative phosphorylation, whereas activated T cells depend on glycolysis for cytokine production[122]. As such, mitochondrial dynamics regulate T-cell fate through metabolic reprogramming where mitochondrial fission reduces oxidative phosphorylation by promoting cristae remodelling and disassembly of the electron transport chain complexes to shift substrate utilization to glycolysis[121,123]. The importance of mitochondrial fission for glycolysis during immune cell activation is also evident in myeloid dendritic cells[124] and macrophages[125]. Efferocytosis is a process by which macrophages engulf and eliminate apoptotic cells. Studies have shown that phagocytic uptake of apoptotic cells requires DNML1-mediated mitochondrial fission, and silencing *Dnml1* in macrophages leads to impaired efferocytosis[126]. Thus, mitochondrial fission directly contributes to immune cell activation and function.

Dysregulation of mitochondrial fission

Mitochondrial fusion in heart disease

The balance between mitochondrial fission and fusion dictates the outcome on organelle morphology. An overall decrease in fusion can shift the balance towards fission, leading

to excessive mitochondrial fission. Although our knowledge of how mitochondrial fusion machinery is changed in cardiovascular disease remains limited, studies have shown that levels of mitochondrial fusion proteins are decreased during cardiac hypertrophy and in failing hearts. For instance, MFN2 is downregulated in mouse hearts at 1 and 3 weeks after transverse aortic constriction (TAC) and in hypertrophied hearts from 10-month-old spontaneously hypertensive rats[127]. Another study demonstrated that levels of MFN2 and OPA1 are reduced, whereas DNML1 and FIS1 levels are increased in failing hearts from dogs and humans[128]. In 2021, Hsiao et al. reported that levels of MFN1 are decreased in patients with idiopathic dilated cardiomyopathy who did not respond to established heart failure treatments, and that the reduction in MFN1 correlates with increased mitochondrial fragmentation in human hearts[129]. Thus, the excessive mitochondrial fission that contributes to cardiovascular disease is likely to be caused by a combination of increased DNML1 activation and reduced mitochondrial fusion. However, further studies are clearly needed to determine how altered mitochondrial fusion contributes to the development of cardiovascular disease, as the vast majority of existing mechanistic data to date have focused on DNML1 activation. In the remainder of this Review, we discuss the role of DNML1-driven mitochondrial fission in cardiovascular pathogenesis.

Cardiac hypertrophy

Although mitochondrial fission has an important regulatory role in cellular homeostasis, it is becoming increasingly clear that aberrant activation of fission during cardiovascular disease directly contributes to pathophysiology (FIG. 4). Cardiac myocytes undergo hypertrophy as an adaptive response to increased workload (exercise) or haemodynamic overload (hypertension) to maintain contractility and reduce ventricular wall stress. A growing body of evidence suggests that mitochondrial fission is activated during myocyte hypertrophy. For instance, treatment of neonatal rat myocytes with the hypertrophic agonist norepinephrine activates mitochondrial fission, whereas overexpression of a dominant-negative DNML1 inhibits both mitochondrial fission and hypertrophic growth of myocytes[11]. Exercise is associated with physiological hypertrophy, and DNML1-mediated mitochondrial fission is rapidly activated in hearts as an adaptive response[3,25]. Although enhanced fission alters bioenergetics, it is also possible that DNML1 is activated during exercise to facilitate mitochondrial clearance as exercise is linked to mitophagy in trained skeletal muscle[130] and in mouse hearts exposed to irradiation therapy[131]. Physiological hypertrophy through exercise is reversible, however, persistent pathological stress such as chronic hypertension can lead to a transition to pathological hypertrophy with loss of myocytes, cardiac remodelling and development of heart failure[132]. Evidence exists that excessive fission is an underlying factor in this transition. For example, sustained fission contributes to loss of myocytes and cardiac dysfunction in hypertensive rats[133]. Treatment of myocytes with angiotensin II, a major contributor to hypertension, induces pronounced mitochondrial fission and downstream activation of apoptosis[133]. In a TAC mouse model of pressure overload, DNML1 is rapidly phosphorylated and recruited to the mitochondria, and pharmacological inhibition of DNML1 reduces cardiac hypertrophy[134]. Although this finding suggests that fission contributes to maladaptive cardiac remodelling, not all studies have shown a pathological role for fission in cardiac hypertrophy. For instance, rats subjected to ascending aortic constriction develop diastolic dysfunction

concurrent with increased DNML1-mediated fission, activation of mitochondrial biogenesis and enhanced mitochondrial respiration[135]. Interestingly, relief of the pressure overload by aortic de-banding leads to reversal of cardiac hypertrophy and restoration of cardiac function, despite sustained induction of mitochondrial fission[135]. Similarly, Shirakabe et al. found that DNML1 is essential for mitophagy in mouse hearts during TAC[90]. In this study, haploinsufficent *Dnml1* mice had exacerbated development of both mitochondrial dysfunction and heart failure in response to TAC due to impaired mitophagy. Taken together, these studies suggest that mitochondrial fission is an adaptive component of the hypertrophic response, enhancing bioenergetics and facilitating mitophagy of damaged mitochondria, but that persistent fission during pressure overload becomes detrimental to the heart potentially through excessive mitophagy and activation of apoptosis or necrosis.

Myocardial ischaemic injury

Both coronary occlusion and subsequent reperfusion to restore blood and oxygen delivery to the heart can lead to extensive death of myocytes and irreversible tissue damage. A growing body of evidence suggests that excessive mitochondrial fission is an underlying factor of myocyte death in IRI. Mitochondrial fission is initially activated during ischaemia and is sustained during reperfusion, which contributes to increased mitochondrial generation of reactive oxygen species, calcium overload and mPTP opening in myocytes[9,10,136]. In addition, DNML1 is activated in the peri-infarct region of mouse heart tissue, resulting in pronounced mitochondrial fission and cardiac dysfunction[137]. Excessive mitochondrial fission during ischaemia–reperfusion also disrupts endothelial barrier function and integrity in the heart, and restricting fission in cardiac microvascular endothelial cells reverses mitochondrial damage and cell death in studies of simulated ischaemia–reperfusion[138]. Pharmacologic or genetic inhibition of DNML1-mediated fission reduces mPTP opening and cell death in cardiac myocytes subjected to ischaemia–reperfusion[9,136] and inhibiting fission at the onset of reperfusion prevents long-term cardiac dysfunction[9,136]. By contrast, another study indicates that defective mitophagy in cardiac-specific *Dnml1* heterozygous knockout mice exacerbates myocardial IRI[8]. Conflicting results in these studies could be related to pharmacological targeting of fission in non-myocytes compared with cardiac specificity in genetic models.

Fibrosis and calcification

Mitochondrial fission has also been implicated in cardiac fibrosis, an important component of the wound repair process following myocardial injury. However, excessive extracellular matrix deposition induces maladaptive fibrotic remodelling and disrupts electrical signalling, ultimately leading to heart failure[139]. Transforming growth factor β (TGFβ) drives cardiac fibrosis by activating fibroblasts and the extracellular matrix gene programme. Interestingly, stimulation of cardiac fibroblasts with TGFβ also activates mitochondrial fission, and inhibition of DNML1 reduces TGFβ-stimulated fibroblast proliferation, migration and extracellular matrix synthesis[83]. This finding suggests that mitochondrial fission is directly linked to fibroblast activation in vitro, and that fission is likely to be an early catalyst driving fibrotic remodelling in the heart. Sustained mitochondrial fission also drives proliferation of right ventricular fibroblasts isolated from an animal model of pulmonary arterial hypertension[140] as well as human cardiac fibroblasts stimulated

with lysophosphatidylcholine, a lipid known to directly promote collagen production[141]. Moreover, pharmacological inhibition of DNML1 reduces fibrotic remodelling of hearts in hypertensive rats[142]. These studies suggest that the concurrent activation of mitochondrial fission in fibroblasts during cardiac stress also contributes to pathological remodelling and fibrosis in the heart.

Ectopic calcification is an important factor in the progression of atherosclerosis, and evidence exists that DNML1-mediated mitochondrial fission has a direct role in cardiovascular calcification. DNML1 is enriched in human carotid atherosclerotic plaques and calcified aortic valves[143]. VSMCs initiate calcification by differentiating into osteogenic or chondrocytic cells[144] and knockdown or inhibition of *DNML1* attenuates VSMC-mediated calcification in vitro[143]. Similarly, preventing mitochondrial fission reduces aortic calcification in rats fed an adenine-rich diet[145]. Although mitochondrial fission is clearly linked to osteogenic differentiation, the precise mechanism by which mitochondrial fission promotes calcification remains unclear.

Abdominal aortic aneurysm (AAA) is another increasingly common cardiovascular disease that is characterized by dilation of the abdominal aorta, and its rupture can be fatal. Calcification is a risk factor for AAA severity and rupture[146]. In 2021, Cooper et al. reported that expression of DNML1 is increased in AAA patient samples compared to healthy controls, and that inhibition of DNML1 protects against aortic dilation and rupture in a mouse model of AAA[147]. During AAA, adventitial fibroblasts undergo a phenotypic switch towards the myofibroblast phenotype, which is a crucial component of vascular remodelling. DNML1-dependent fission is required for adventitial fibroblast activation in vitro, and strategies to mitigate mitochondrial fission also reduce adventitial thickness and fibrosis in mice[148].

Metabolic cardiomyopathy

Obesity and Type 2 diabetes mellitus are associated with cardiac lipid overload due to increased myocyte fatty acid uptake and oxidation, and accumulation of lipids in diabetic cardiomyopathy is an independent predictor of contractile dysfunction in patients[149]. Excessive lipid uptake is also linked to changes in mitochondrial morphology, where lipids can activate mitochondrial fission. For instance, ceramide is a reactive lipid that triggers rapid mitochondrial fission in cardiac myocytes and in the hearts of rats with diabetes[150,151]. Blocking ceramide biosynthesis in induced pluripotent stem cell-derived myocytes reverses mitochondrial dysfunction and apoptosis by restricting mitochondrial fragmentation[102]. The saturated fatty acid palmitate is also associated with diabetic cardiomyopathy. Increased palmitate levels in the myocardium of mice and primates fed high-fat diets promote mitochondrial fission through Lys624 acetylation and activation of DNML1[54]. Abrogating Lys642 acetylation in adult myocytes restricts fission, thereby preventing apoptosis and contractile deficits imposed by palmitate[54]. In the liver, mitochondrial fission also contributes to systemic metabolism, as hepatocyte-specific *Dnml1*-knockout mice exhibit reduced cholesterol levels and are protected from high-fat diet-induced obesity[152]. Overall, these studies demonstrate that metabolic dysregulation during lipid overload

promotes aberrant fission and mitochondrial dysfunction, which contributes to cardiac pathophysiology.

Vascular inflammation

Mitochondrial fission has also been implicated as an important driver of vascular inflammation. Chronic inflammation of the arterial wall and migration of immune cells and VSCMs within the vasculature contribute to the formation of atherosclerotic lesions. An atherogenic role for mitochondrial fission in activated immune cells has been identified, as macrophage-specific *Dnml1*-knockout mice are protected from intimal thickening and fibrosis following arterial injury[153]. Loss of DNML1 disrupts macrophage activation through reduced pro-inflammatory cytokine production and chemotaxis, which ultimately abolishes VSMC migration in co-culture studies[153]. These findings are consistent with another study in which reduced VSMC migration and intimal hyperplasia was reported in DNML1^{K38A} transgenic mice subjected to arterial injury[154]. In addition, pharmacological inhibition of DNML1 reduces macrophage accumulation and advanced atherosclerotic plaque formation in the aortic roots of *Apoe*$^{-/-}$ mice with diabetes, highlighting the function of mitochondrial fission in vascular inflammation[12].

Mitochondrial fission in cell types other than macrophages also contributes to vascular inflammation. Forrester et al. found that loss of DNML1 in endothelial cells reduces leukocyte adhesion in mouse mesenteric post-capillary venules following immune stimulation with tumour necrosis factor (TNF)[155]. Aberrant mitochondrial fission in endothelial cells also leads to senescence[106], which is a critical component of atherosclerosis[156]. Whereas senescent cells secrete pro-inflammatory factors for immune recruitment, inhibition of DNML1 reverses these outcomes in endothelial cells treated with angiotensin II[157]. This finding suggests that abnormal mitochondrial fission is sufficient to cause endothelial dysfunction, and point to fission as a potent inducer of vascular inflammation during cardiovascular disease.

Therapeutic approaches

Specific DNML1 inhibitors

Collectively, the plethora of studies on mitochondrial dynamics in cells, animal models and human tissue all indicate that dysregulation of mitochondrial fission contributes to the development of several cardiovascular pathologies. Thus, targeting proteins in this pathway has strong therapeutic potential for treating cardiovascular disease.

Mitochondrial division inhibitor 1 (mdivi-1) is a small molecule inhibitor of DNML1 GTPase activity that has been widely used to abrogate mitochondrial fission in vitro and in vivo. Consistent with a role for fission in immune cell activation and migration, mdivi-1 infusion prevents leukocyte–endothelial cell adhesion in mice treated with TNF, providing similar vascular protection to that seen with genetic deletion of DNML1[155]. Similarly, mdivi-1 administration reduces calcification and vascular remodelling in mouse models of atherosclerosis[12] and AAA[147], respectively. Mdivi-1 also attenuates myocardial infarct size and cardiac dysfunction in mouse models of ischaemia–reperfusion[9,10]. These studies

suggest that inhibition of DNML1-mediated fission by mdivi-1 provides protection against cardiovascular disease development. However, a report published in 2017 questioned the specificity of mdivi-1, because this compound also inhibits respiratory complex I (also known as mitochondrial complex I) in a DNML1-independent manner[158]. The study also suggested that mdivi-1 does not inhibit the GTPase activity of recombinant human DNML1 protein[158]. These findings remain controversial, as other investigators have noted the unreliability of assays with recombinant DNML1 and demonstrate that mdivi-1 inhibits GTPase activity in DNML1 immunoprecipitated from human alveolar cells[159]. Considering that DNML1 is necessary for the assembly of respiratory complex I in mouse skeletal muscle[80], and respiratory complex I protein levels are similarly decreased in neonatal rat cardiac myocytes exposed to mdivi-1 or *Dnml1* knockdown[91], additional studies are needed to clarify potential off-target effects of mdivi-1. Application of mdivi-1 in larger mammals is also needed, as a pilot study published in 2019 showed a lack of cardioprotection in a more clinically relevant swine model of myocardial ischaemia–reperfusion[160].

Additional inhibitors of DNML1 have also been developed. The peptide inhibitor P110 specifically disrupts the interaction between DNML1 and FIS1, and performs similarly to mdivi-1 in protecting against myocardial IRI[136]. P110 also alleviates cardiac dysfunction and mitochondrial damage in sepsis, suggesting that the interaction between DRP1 and FIS1 specifically has a critical role in promoting pathological mitochondrial fission. By contrast, another peptide inhibitor P259 abrogates DNML1–MFF binding[161], although animal studies are needed to test the therapeutic potential of P259 in cardiovascular disease. The newly developed DNML1 inhibitor Drpitor1a has greater potency than mdivi-1 and protects rat hearts during ischaemia–reperfusion ex vivo[159]. On the basis of in vitro and in vivo studies in rodents, pharmacological inhibition of mitochondrial fission clearly represents a promising therapeutic strategy to protect against cardiovascular disease. However, this approach needs to be further explored and confirmed in large-animal models.

Indirect regulation of fission

In addition to specific inhibitors of DNML1, many FDA-approved compounds have been reported to repress aberrant mitochondrial fission. For example, the non-steroidal anti-inflammatory drug sodium salicylate limits mitochondrial fission in endothelial cells, thereby reducing immune cell adhesion in mice treated with TNF, and afforded a similar level of protection against endothelial inflammation as seen with mdivi-1[155]. Metformin is used to treat type 2 diabetes and its administration in mice reduces DNML1 protein levels and mitochondrial fragmentation in the aortic endothelium of $ApoE^{-/-}$ mice with diabetes[162]. Importantly, restricting mitochondrial fission through metformin attenuates diabetes-induced endothelial dysfunction and inflammation independent of changes in blood glucose[162]. Empagliflozin, a sodium–glucose co-transporter 2 inhibitor, is also approved for the treatment of type 2 diabetes and its administration in mice limits hyperglycaemia-induced mitochondrial fission, thereby attenuating cardiac microvascular remodelling and dysfunction in vivo[163].

Two different FDA-approved drugs used to treat hypertension have also been shown to inhibit mitochondrial fission. Hydralazine causes vasodilation by interfering with calcium

transport to relax arteriolar smooth muscle cells. Interestingly, acute infusion of hydralazine immediately prior to reperfusion protects against myocardial IRI in mice[164]. Similarly, treatment of cultured adult myocytes with hydralazine significantly reduces mitochondrial fission and cell death in response to simulated ischaemia–reperfusion[164]. Molecular docking analysis indicates that hydralazine directly binds to the Asp218, Asn246 and Ser248 residues of DNML1, which leads to inhibition of GTPase activity. Cilnidipine is a dihydropyridine calcium channel blocker that is used clinically to treat hypertension. Although this drug acts predominately on VSMCs, Nishimura et al. reported that cilnidipine also has off-target effects that are independent of this mechanism[137]. Specifically, cilnidipine prevents hypoxia-induced mitochondrial fission in cardiac myocytes, thereby improving cardiac function in mice subjected to myocardial infarction[137].

In addition to these exogenous compounds, research has identified that some endogenous hormones can regulate mitochondrial fission. For example, melatonin supplementation protects cardiac myocytes[165], endothelial cells[117] and VSMCs[166] from pathological DNML1-mediated fission in multiple disease models. In addition, treatment with the steroid hormone vitamin D_3 prevents mitochondrial fragmentation, oxidative stress and myocyte death induced by ischaemia–reperfusion in vitro and attenuates cardiac injury in mice[167]. Together, these data lend further support for targeting mitochondrial fission in cardiovascular disease and indicate that several FDA-approved drugs, as well as endogenous peptides, could potentially be repositioned for this purpose.

Conclusions

Aberrant mitochondrial fission through DNML1 directly contributes to cardiovascular pathophysiology. Genetic ablation of DNML1 is well-characterized to restrict pathological cardiac and vascular remodelling in mice, and proof of concept in genetic models is validated by pharmacological inhibitors. Despite strong therapeutic promise, the physiological functions of mitochondrial fission warrant careful consideration when developing treatment strategies. Along these lines, fission is needed for mitochondrial division, which supports mtDNA segregation[7] and cellular proliferation[81]. As such, restricting fission might not be advisable during particular developmental stages, or in patients with heritable mtDNA mutations. Mitochondrial fission also has an important role in facilitating mitophagy[8,90]. Therefore, pharmacological strategies to limit fission must consider dose and duration of treatment with respect to mitochondrial quality control through mitophagy. Nevertheless, the fact that pharmacological inhibition of unchecked mitophagy during anthracycline cardiotoxicity also restricts excessive mitochondrial fission[168] indicates that the coupling of these two processes could synergistically promote cardiovascular pathophysiology in particular situations. Cell-type specificity is a major obstacle when evaluating drug efficacy and safety, and could be a potential barrier for targeting mitochondrial fission, as macrophages[153] and endothelial cells[155] are purported to drive vascular inflammation through hyperactive fission. Because DNML1 is essential for immune cell maturation[121] and phagocytosis[126], ensuring that inhibitor regimens do not disrupt physiological immune function will be vital before animal studies can be translated to the clinic. Targeting specific DNML1 adaptor proteins could enable regulation of distinct subtypes of mitochondrial fission contributing to pathogenesis, while leaving physiological

mitochondrial division intact[22]. However, more studies are needed to determine whether this strategy is viable. Currently, there are no ongoing clinical trials evaluating the effects of specific inhibitors for mitochondrial fission[169]. However, a wealth of data indicating a distinct pathological threshold for this process, coupled with the repurposing of several FDA-approved compounds, argues for increased translational application. In summary, mitochondrial fission has emerged as a pivotal hallmark of cardiovascular disease and future research is likely to pave the way for novel therapies in patients.

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Key points

- Mitochondria are involved in regulating many important cellular processes, including metabolism, ATP generation, immune response and activation of cell death pathways.
- Mitochondria are dynamic and undergo changes in morphology in response to various environmental cues, which impacts organelle function.
- Mitochondrial fission is subject to sophisticated regulation, and activation involves various post-translational modifications of dynamin-1-like protein (DNM1L), actin polymerization and the involvement of other cell organelles.
- Although mitochondrial fission is critical for cardiac homeostasis, strong evidence exists that dysregulation of DNM1L-mediated fission contributes to the development of several cardiovascular pathologies.
- Targeting proteins that regulate mitochondrial dynamics has strong therapeutic potential for cardiovascular disease.

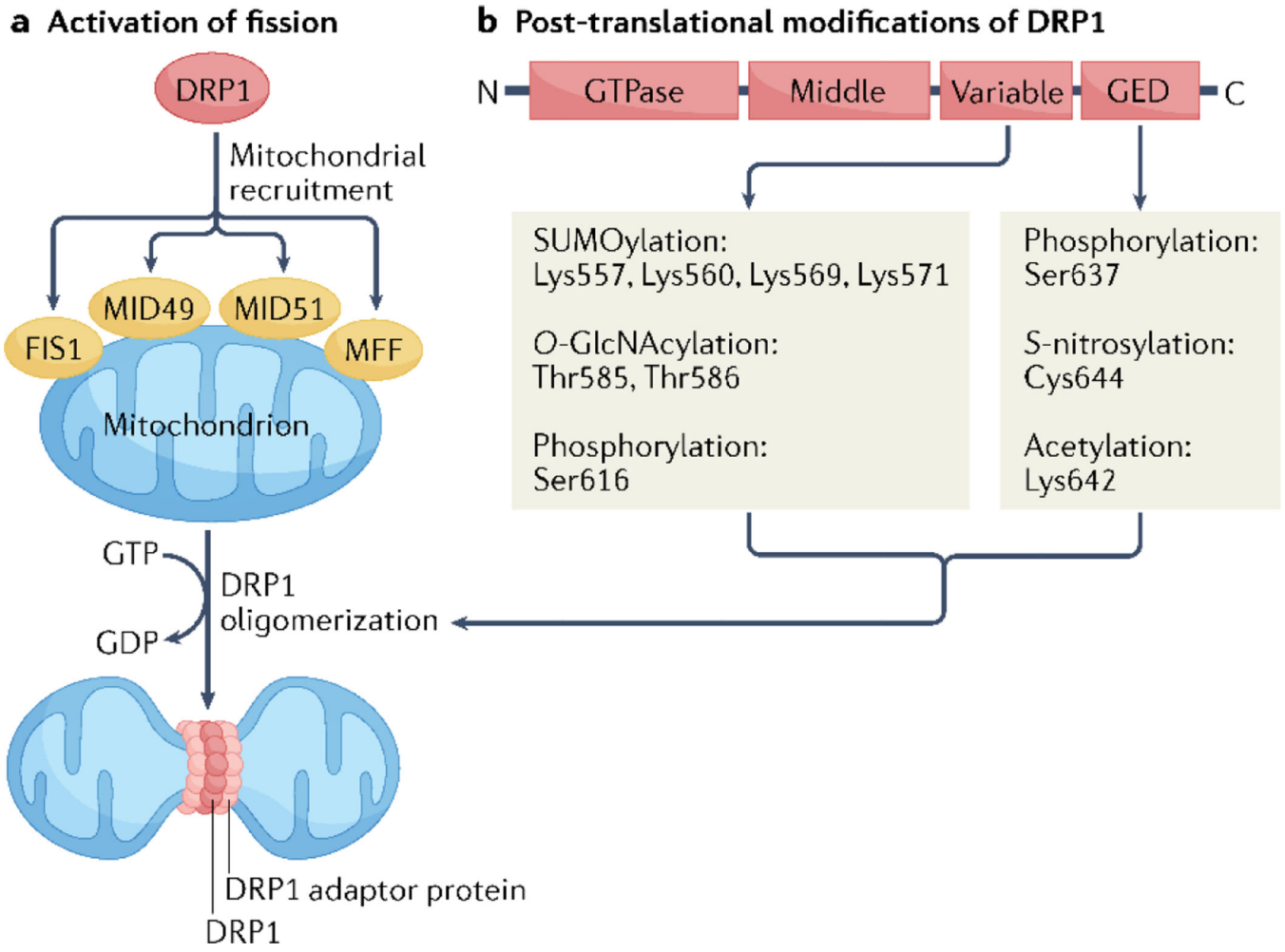

Fig. 1 |. Structure and function of DNM1L in mitochondrial fission.

a | Cytosolic dynamin-1-like protein (DNML1; also known as dynamin-related protein 1 (DRP1) monomers are recruited to the outer mitochondrial membrane by the adaptor proteins mitochondrial fission 1 (FIS1), mitochondrial dynamics protein 49 (MID49) or MID51 and mitochondrial fission factor (MFF), which facilitates the formation of helical ring DNML1 oligomers. GTP hydrolysis by DNML1 stimulates OMM constriction and subsequent scission. **b** | Several post-translational modifications within the variable region and GTPase effector domain (GED) of DNML1 regulate fission. O-GlcNAcylation, O-linked-N-acetylglucosaminylation.

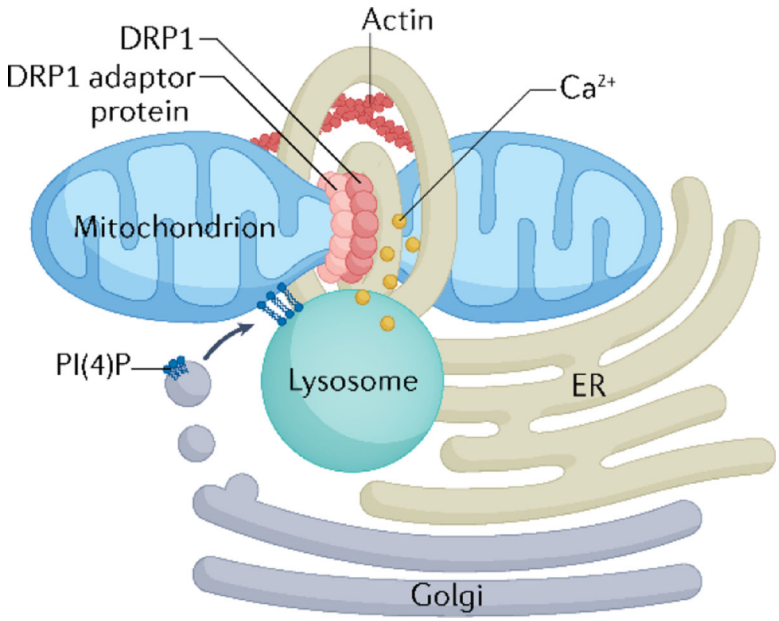

Fig. 2 |. Interorganelle contacts promote mitochondrial fission.

Mitochondria–endoplasmic reticulum (ER) association membranes, in conjunction with polymerized actin, promote DNML1 recruitment and outer mitochondrial membrane OMM constriction. Uptake of calcium (Ca^{2+}) into the mitochondria stimulates inner membrane constriction. phosphatidylinositol 4-phosphate (PI_4P) is also critical for the execution of mitochondrial fission and can be transferred to the OMM through lysosomes or trans-Golgi-derived vesicles.

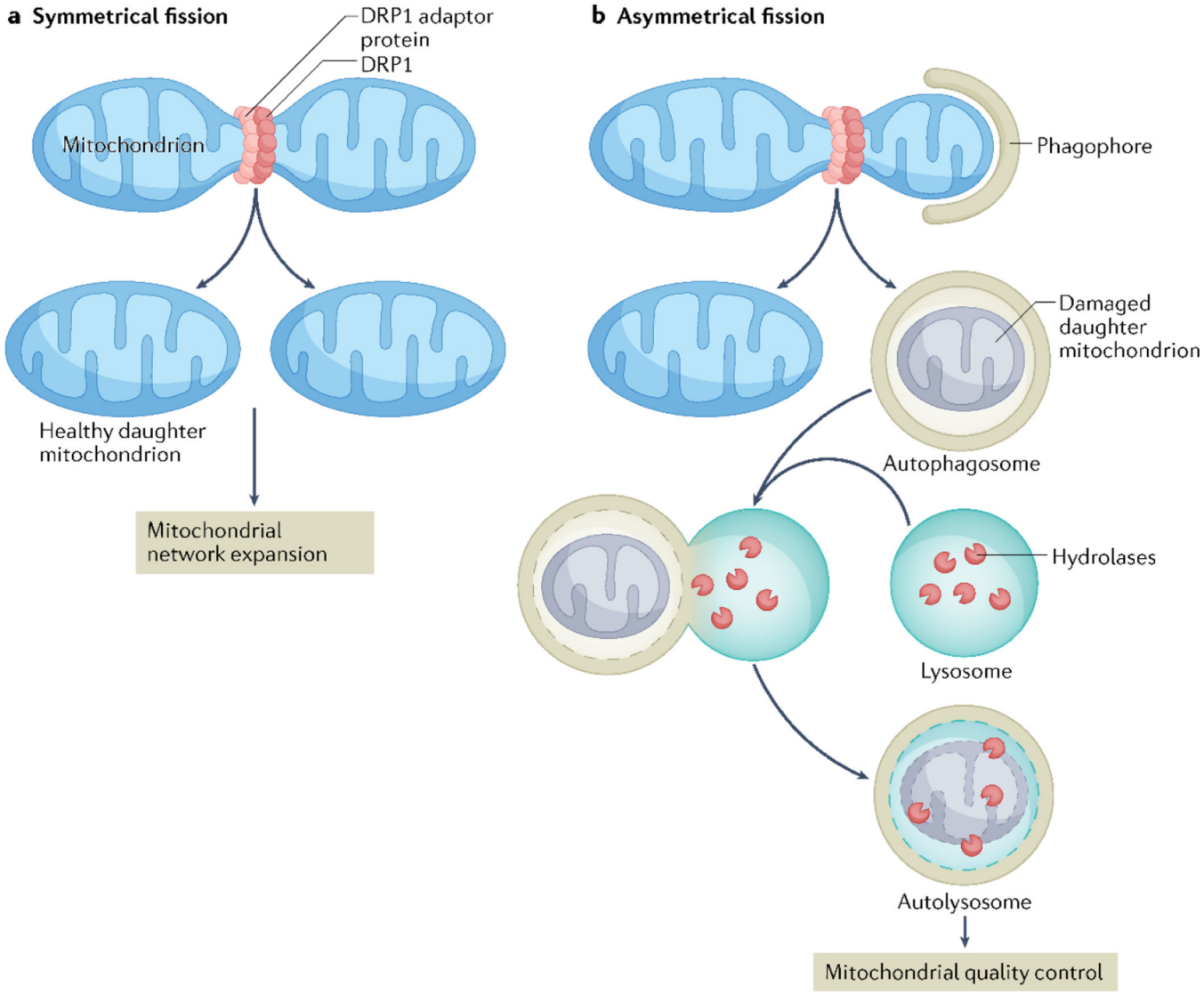

Fig. 3 |. Distinct mitochondrial fission subtypes produce divergent fates.

a | Symmetric fission generates two healthy daughter mitochondria during cellular division.

b | Asymmetric fission facilitates engulfment of damaged mitochondria by autophagosomes, which fuse with lysosomes for organelle degradation via mitophagy.

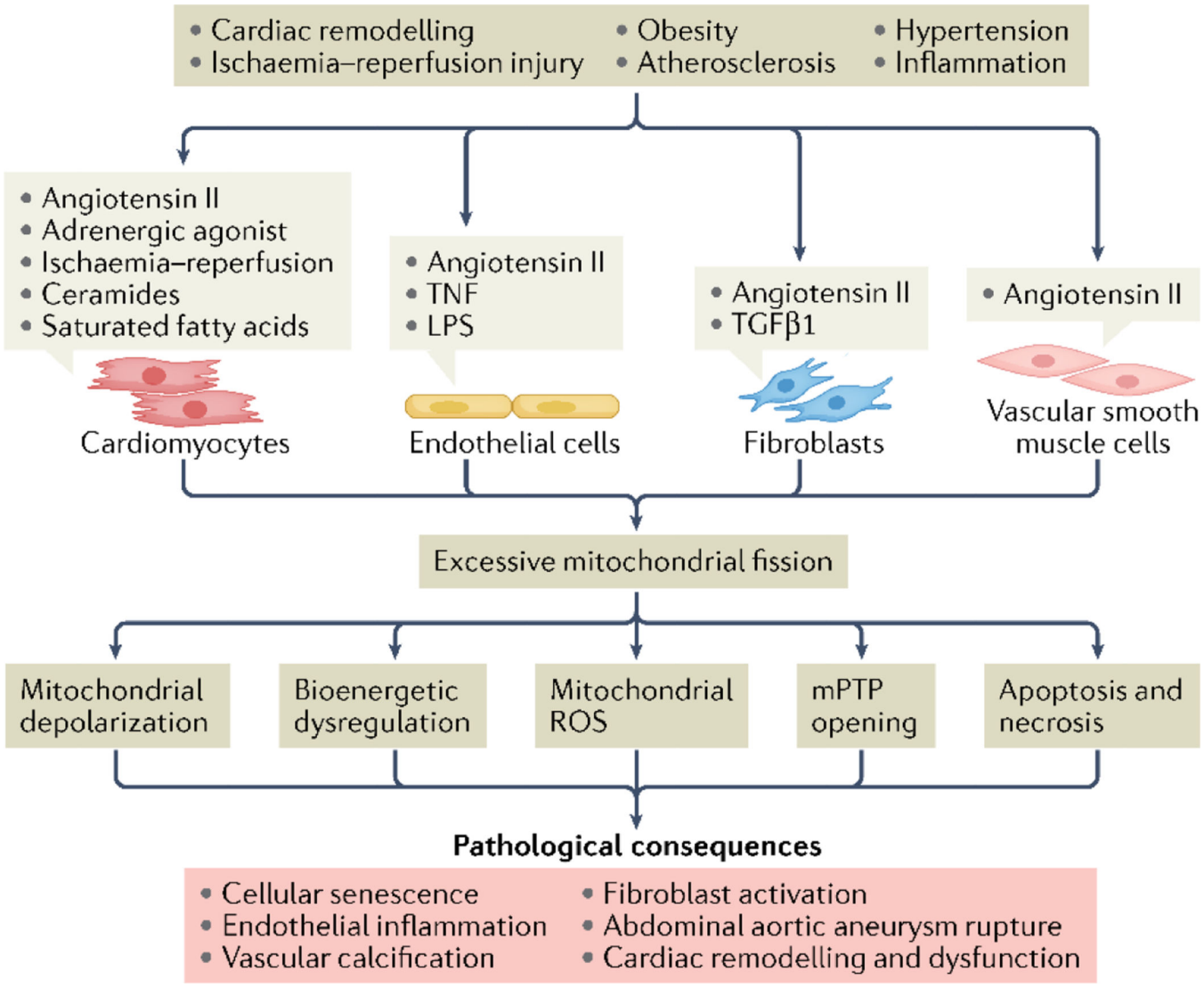

Fig. 4 |. Sustained mitochondrial fission promotes cardiovascular pathophysiology.
Several upstream signals shared and distinct among cardiac myocytes, endothelial cells, fibroblasts and vascular smooth muscle cells (VSMC) cause excessive mitochondrial fission, which culminates in organelle dysfunction and cell death. These effects directly promote cardiac and vascular pathophysiology across multiple disease models. AAA, abdominal aortic aneurysm; FA, fatty acids; LPS, lipopolysaccharide; mPTP, mitochondrial permeability transition pore; ROS, reactive oxygen species; TGF- β1, transforming growth factor β1; TNF, tumour necrosis factor.

Table 1 |

Systemic and cardiac-specific genetic mouse models of mitochondrial dynamics

Target(s)	Genetic model	Outcome		Refs
		Basal characterization	**Effects of pathological stress**	
Mff	Systemic homozygous knockout	Lethal cardiomyopathy at 13 weeks. Neuromuscular and fertility defects. Impaired mitochondrial respiration and ATP production. Loss of pro-fusion MFN1 rescues phenotype.	NR	34
Dnm1l	Systemic homozygous knockout	Lethality by embryonic day 10.5. Developmental defects due to brain hypoplasia and apoptosis.	NR	28,29
	Systemic heterozygous knockout	Viable with no obvious cardiac phenotype.	Reduced immune infiltration and protection against angiotensin II-mediated AAA. No effect in AAV9–PCSK9 atherosclerosis model. Protection against TNF-driven endothelial leukocyte adhesion.	12,29,147,155
	Systemic C452F mutation	Progressive cardiac mitochondrial dysfunction and sterile inflammation resulting in heart failure.	NR	92
	Cardiac-specific homozygous knockout	Lethal dilated cardiomyopathy by postnatal day 7. Cardiac mitochondrial respiratory defects, mitophagy impairment and mtDNA nucleoid aggregation.	NR	30–32
	Cardiac-specific heterozygous knockout	Normal cardiac structure and function despite reduced mitochondrial ATP production.	Increased myocardial IRI. Impaired mitophagy and exacerbated cardiac pathophysiology following pressure overload.	8,31,90
	Tamoxifen-inducible, cardiac-specific knockout	Lethal dilated cardiomyopathy at 4–8 weeks post-tamoxifen. Mitochondrial dysfunction evident prior to cardiac pathophysiology. Dysregulation of mitophagy and activation of apoptosis in the heart. Diminished maximal exercise performance.	NR	3,8,32,79
	Systemic, doxycycline-inducible, K38A dominant negative transgenic	No phenotype evident after modest induction of transgene for 6 months.	Protection against diabetes-induced oxidative stress in the kidney and liver. Protection against vascular hyperplasia following arterial injury.	33,154
	Cardiac-specific inducible bitransgenic (tetracycline-off)	Normal mitochondrial and cardiac function.	NR	41
Mfn1	Systemic homozygous knockout	Lethality by embryonic day 11.5.	NR	36
Mfn2	Systemic homozygous knockout	Lethality by embryonic day 10.5.	NR	35
Mfn1, *Mfn2*	Cardiac-specific double homozygous knockout	Lethality by embryonic day 9.5 when Cre recombinase expression is driven by the NKX-2.5 promoter. Lethal cardiomyopathy when cre expression is driven by the MYH6 promoter. Abnormal mitochondrial structure and reduced mtDNA content.	NR	37,38
Mfn1, *Mfn2*	Tamoxifen-inducible, cardiac-specific double knockout	Eccentric ventricular remodelling and wall thickening at 6 weeks after gene deletion. Reduced mitochondrial size and activation of the mitochondrial unfolded protein response. 50% premature mortality by 9 weeks post-tamoxifen.	Protection against myocardial IRI when induced ~4 weeks after gene deletion (prior to cardiomyopathy) due to impaired mitochondria-sarcoplasmic reticulum tethering.	32,41,170

Target(s)	Genetic model	Outcome		Refs
		Basal characterization	Effects of pathological stress	
Dnm1l, *Mfn1*, *Mfn2*	Tamoxifen-inducible, cardiac-specific triple knockout	Concentric cardiac hypertrophy eventually progressing to heart failure, despite minimal changes in myocyte viability. Extended survival with *mfn1-mfn2-dnml1* triple knockout compared with *mfn1-mfn2* double knockout or *dnm1l* single knockout (7 vs 17 weeks post-tamoxifen).	NR	[41]

AAA, abdominal aortic aneurysm; AAV9, adeno-associated virus serotype 9; DNM1L, dynamin-1-like protein (also known as dynamin-related protein 1 (DRP1)); IRI, ischaemia–reperfusion injury; mtDNA, mitochondrial DNA; MFN, mitofusin; MYH6, myosin-6; NKX-2.5, homeobox protein Nkx-2.5; NR, not reported; PCSK9, proprotein convertase subtilisin/kexin type 9; TNF, tumour necrosis factor.

Table 2 |

Genetic mouse models of *Dnm1l* knockout in non-cardiac tissues

Genetic model	Outcome		Refs
	Basal characterization	**Effects of pathological stress**	
Skeletal muscle-specific knockout	Reduced body growth, muscle fibre number and size. Defects in respiratory complex assembly and function. Lethality by postnatal day 30.	NR	80
Tamoxifen-inducible, skeletal muscle-specific knockout	Reduced body weight at 50 days post-tamoxifen. Reduced muscle mass and fibre size 70–180 days post-tamoxifen. Mitochondrial dysfunction and impaired mitophagy.	NR	80
T cell-specific knockout	Impaired migration and expansion of developing thymocytes.	Accelerated tumour growth.	121
Myeloid/macrophage-specific knockout	Impaired macrophage-mediated uptake of apoptotic cells (i.e. efferocytosis).	Accelerated plaque necrosis in the $Ldlr^{-/-}$ model of atherosclerosis. Protection against vascular remodelling and fibrosis following arterial injury. Reduced macrophage activation and cell proliferation in injured arteries.	126,153
Liver-specific knockout	Reduced liver and white adipose tissue weights. Reduced serum triacylglycerol and total cholesterol levels.	Protection from high-fat diet-induced obesity.	12,152
Endothelial cell-specific knockout	NR	Protection against TNF-driven endothelial leukocyte adhesion.	155

DNM1L, dynamin-1-like protein (also known as dynamin-related protein 1 (DRP1)); LDLR, low-density lipoprotein receptor; NR, not reported; TNF, tumour necrosis factor.