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Review article

Mitochondria break free: Mitochondria-derived vesicles in aging and associated conditions

Luigi Ferrucci [a,*], Flora Guerra [b], Cecilia Bucci [c], Emanuele Marzetti [d,e], Anna Picca [e,f,**]

[a] Division of Intramural Research, National Institute on Aging, Baltimore, MD, USA
[b] Department of Biological and Environmental Sciences and Technologies, Università del Salento, Lecce, Italy
[c] Department of Experimental Medicine, Università del Salento, Lecce, Italy
[d] Department of Geriatrics, Orthopedics and Rheumatology, Università Cattolica del Sacro Cuore, Rome, Italy
[e] Fondazione Policlinico Universitario "A. Gemelli" IRCCS, Rome, Italy
[f] Department of Medicine and Surgery, LUM University, Casamassima, Italy

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ABSTRACT

Mitophagy is the intracellular recycling system that disposes damaged/inefficient mitochondria and allows biogenesis of new organelles to ensure mitochondrial quality is optimized. Dysfunctional mitophagy has been implicated in human aging and diseases. Multiple evolutionarily selected, redundant mechanisms of mitophagy have been identified, but their specific roles in human health and their potential exploitation as therapeutic targets are unclear. Recently, the characterization of the endosomal–lysosomal system has revealed additional mechanisms of mitophagy and mitochondrial quality control that operate via the production of mitochondria-derived vesicles (MDVs). Circulating MDVs can be isolated and characterized to provide an unprecedented opportunity to study this type of mitochondrial recycling *in vivo* and to relate it to human physiology and pathology. Defining the role of MDVs in human physiology, pathology, and aging is hampered by the lack of standardized methods to isolate, validate, and characterize these vesicles. Hence, some basic questions about MDVs remain unanswered. While MDVs are generated directly through the extrusion of mitochondrial membranes within the cell, a set of circulating extracellular vesicles leaking from the endosomal–lysosomal system and containing mitochondrial portions have also been identified and warrant investigation. Preliminary research indicates that MDV generation serves multiple biological roles and contributes to restoring cell homeostasis. However, studies have shown that MDVs may also be involved in pathological conditions. Therefore, further research is warranted to establish when/whether MDVs are supporting disease progression and/or are extracting damaged mitochondrial components to alleviate cellular oxidative burden and restore redox homeoastasis. This information will be relevant for exploiting these vesicles for therapeutic purpose. Herein, we provide an overview of preclinical and clinical studies on MDVs in aging and associated conditions and discuss the interplay between MDVs and some of the hallmarks of aging (mitophagy, inflammation, and proteostasis). We also outline open questions on MDV research that should be prioritized by future investigations.

1. Introduction

Mitophagy is an intracellular recycling system that disposes damaged/inefficient mitochondria to allow biogenesis of new organelles and maintenance of mitochondrial quality and functions (Pickles et al., 2018). This constant quality control is pivotal for preserving several mitochondrial activities beyond ATP generation, including the provision of metabolite intermediates for the biosynthetic pathways of biological macromolecules (i.e., carbohydrates, lipids, nucleotides, and proteins), redox signaling, Ca^{2+} homeostasis, biosynthesis of heme and sulfur clusters, and maintenance of mitochondrial DNA (mtDNA), all of which are essential for cellular health (Chakrabarty and Chandel, 2022).

Dysfunctional mitophagy has been implicated in human aging and diseases (Picca et al., 2023a). Because mitochondrial biological functions are essential for the maintenance of organismal health, while persistence of damaged mitochondria is a source of biological stress,

* Correspondence to: Division of Intramural Research, National Institute on Aging, Baltimore, MD 21224, USA.
** Correspondence to: Department of Medicine and Surgery, LUM University, Casamassima 70100, Italy.
E-mail addresses: ferruccilu@grc.nia.nih.gov (L. Ferrucci), picca@lum.it (A. Picca).

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multiple redundant mechanisms of mitophagy have been evolutionarily selected that are typically classified as PTEN-induced kinase 1 (PINK1)/Parkin-dependent and Parkin-independent (Picca et al., 2023a). More recently, the characterization of the endosomal−lysosomal system, the set of organelles and membranous components forming the endocytic complex, has revealed additional mechanisms of mitochondrial disposal and mitochondrial quality control (MQC) that recycle whole damaged mitochondria or mitochondrial fragments through the production of mitochondria-derived vesicles (MDVs) (Hazan Ben-Menachem et al., 2023; Mondal and Towers, 2022; Sugiura et al., 2014). MDVs operate as a mitochondrial disposal system under mild stress conditions and do not necessarily depend on mitochondrial depolarization (like mitophagy), but mostly on mitochondrial reactive oxygen species (ROS) (Cadete et al., 2019; Soubannier et al., 2012a; Vasam et al., 2021; Iorio et al., 2022).

The role of MDVs in health and disease remains undefined, and the isolation of circulating MDVs and their characterization provides an unprecedented opportunity to study mitochondrial disposal *in vivo* and connect it with human physiology and pathology (König and McBride, 2024; Gagliardi et al., 2024; Sansone et al., 2017; Yoshida et al., 2019). However, the characterization of MDVs, their origin, and their role in health and disease are still matter of debate (Picca et al., 2023b). Coalescing the data from different studies into a clear paradigm is difficult because methods to isolate, validate, and characterize MDVs are highly heterogeneous. For instance, while MDVs are vesicles budding from the mitochondrial membranes to extrude damaged components, vesicles containing mitochondrial portions and leaking from the endo−lysosomal system have also been identified and warrant investigation. Preclinical and clinical research indicates that MDV production and signaling in aging and age-related conditions serve as a strategy complementing MQC and used by the cell when the primary mitophagy mechanisms are impaired and the extent of mitochondrial damage is severe (Mondal and Towers, 2022). Both these conditions are typical of aging and age-related diseases. According to this view, high levels of MDVs may reflect conditions of defective primary autophagy mechanisms, which may be targeted for interventions, including the enhancement of MDV production (Picca et al., 2023a).

The study of extracellular vesicles (EVs) and MDVs is an area of active investigation (Théry et al., 2018; Welsh et al., 2024). However, reconciling the data emerging from different studies is difficult because of the heterogeneity of methods used by different investigators to isolate and characterize these special vesicles. Therefore, their role in aging as well as in human physiology and pathology is still unclear.

In the first section of the review, we describe the biological machinery that generates MDVs and downstream signaling pathways. We provide a critical overview of potential criteria that can be used to identify circulating MDVs referring to EVs derived by the fusion of mitochondrial budding vesicles with multivesicular bodies (MVBs) and their release into extracellular space as exosomes and EVs with mitochondrial signature (König and McBride, 2024). Based on the available literature, we draw inferences to support the hypothesis that MDV generation complements mitophagy for recycling damaged mitochondria and becomes particularly important and perhaps a prominent MQC pathway when primary mitophagy mechanisms are impaired by aging or other conditions. We focus on preclinical studies of MDVs and use findings to build on the hypothesis that MDV production in humans plays an important role in opposing loss of mitochondrial health in aging and age-related conditions. We discuss evidence that MDV production interacts with some of the hallmarks of aging, particularly mitophagy, inflammation, and proteostasis. Finally, we outline questions that, in our view, should be prioritized in future MDV research to better understand their relevance for human aging and health.

2. Mitochondria-derived vesicle biology: an overview

2.1. A brief summary on extracellular vesicle biology

EVs are highly heterogenous groups of vesicles characterized by different subtypes, including exosomes (~30–150 nm in diameter), ectosomes [including microvesicles (~200–1000 nm) and large oncosomes (>1000 nm)] (Dixson et al., 2023), and apoptotic vesicles (~50–2000 nm), but also newly discovered exomeres (<50 nm) (Zhang et al., 2019) and migrasomes (Ma et al., 2015) distinguished based on the mechanism of biogenesis, size, and morphology.

Among these, EVs of endosomal origin, called exosomes, are the most studied type of EVs (Meldolesi, 2018). These vesicles are derived by the fusion of MVBs with the plasma membrane and the subsequent release of intraluminal vesicles (ILVs) into the extracellular space, whereby they become “exosomes” (Mashouri et al., 2019; Meldolesi, 2018). Alternatively, MVBs con fuse with lysosomes that degrade their transported cargo through the mediation of the endosomal sorting complexes required for transport (ESCRT) machinery (Van Niel et al., 2018).

In contrast, microvesicles are released into the intercellular compartment by outward budding and fission of the membrane on the cell surface (Tricarico et al., 2017). This mechanism is characterized by alterations in protein and lipid composition of the plasma membrane, ATP-dependent actin and myosin contraction, and regulation by multiple proteins, such as Ras-related GTPase ADP-ribosylation factor 6 (ARF6) (Van Niel et al., 2018).

Considering the wide EV heterogeneity, it is often not easy to distinguish one subtype from another, due to the overlapping sizes and/or shared biogenesis routes. To overcome this issue, Zhou et al. (2023) defined with the term “MitoEVs” all EVs with mitochondrial content, including MDVs and mitovesicles. Albeit further studies to better clarify the mechanisms of biogenesis and release of these vesicles are necessary, it is currently acknowledged that mitovesicles and MDVs have different protein content but may have partially shared mechanism of biogenesis.

As mentioned earlier, in this review, we focus our discussion on the role of circulating MDVs namely EVs derived by the fusion of mitochondrial budding vesicles with MVBs and then released into extracellular space as exosomes and EVs with mitochondrial signature (König and McBride, 2024).

2.2. Mitochondria-derived vesicle biogenesis and delivery

The first identification of MDVs dates back in 2008 when Margaret Neuspiel described a set of intracellular vesicles of mitochondrial origin (~70–150 nm in diameter) shuttling mitochondrial cargoes to peroxisomes (Neuspiel et al., 2008). These were single- or double-membrane vesicles, shedding from the outer mitochondrial membrane (OMM) or inner mitochondrial membrane (IMM), and including portions of mitochondrial matrix (Neuspiel et al., 2008).

Two mechanistic models are currently proposed as potential pathways of MDV biogenesis. The first identifies MDVs as electron-dense structures that bud from mitochondria to be subsequently released via the classical vesicular pathway with a mechanism similar to the clathrin-mediated endocytosis (Neuspiel et al., 2008). The second model poses that these vesicles are born as thin and relatively long membrane protrusions pulled out from mitochondria along microtubules and subsequently portioned at the protrusion tip (König et al., 2021). These two mechanisms of constitutive MDV generation are not mutually exclusive and use overlapping molecular machineries that suggest conserved mechanistic traits.

Regardless of the molecular mechanisms of origin, there is agreement that MDV generation is under the control of cellular metabolic states (i.e., steady states and/or stress conditions) (Peng et al., 2022), although stress-induced MDVs are not part of the constitutive core mechanism of vesicle generation (Gagliardi et al., 2024; Peng et al., 2022). While under stressful conditions MDV biogenesis is regulated by

the Parkinson's disease (PD)-associated protein PINK1/Parkin-(mitophagy)-dependent pathway (McLelland et al., 2014), in the steady state the dynamin-related protein 1 (DRP1)-(mitochondrial dynamics)-dependent pathway is involved (Heyn et al., 2023; Peng et al., 2022; Popov, 2022). Under physiological conditions, PINK1 is continuously imported inside the organelle through the outer membrane mitochondrial import channel, cleaved by the protease of the inner membrane (PARL) (Greene et al., 2012), and retro-translocated to the cytoplasm for rapid proteasomal degradation (Yamano and Youle, 2013). Parkin, instead, resides in the cytosol in the form of an auto-inhibited E3 ubiquitin-ligase (Pickrell and Youle, 2015). Upon mitochondrial depolarization, the activity of mitochondrial import channels is disrupted and PINK1 remains stalled at the import channel or the OMM (Matsuda et al., 2010; Narendra et al., 2010). Such inactivation causes the exposition of the PINK1 kinase domain into the cytosol where it phosphorylates ubiquitin moieties and Parkin, leading to stable Parkin recruitment and activation at the mitochondrial surface (Iguchi et al., 2013; Kane et al., 2014; Kazlauskaite et al., 2014; Kim et al., 2008; Shiba-Fukushima et al., 2012). Thereafter, a series of proteins on the mitochondrial surface are ubiquitinated by Parkin, then recognized by autophagy adaptor proteins and delivered to the autophagosome for mitophagy degradation (Chen and Dorn, 2013; Gegg et al., 2010; Lee et al., 2010; Narendra et al., 2008; Sarraf et al., 2013; Tanaka et al., 2010). Through the same mechanism, MDV formation is triggered by less severe and more localized mitochondrial damage. Indeed, local oxidative damage or complex assembly defects induce protein aggregation at the import site blocking the import process. Conversely, when complete depolarization or organelle dysfunction occurs, the switch of the mechanism from a local removal of a "patch" of mitochondrial content to the global arrest of PINK1 is triggered in all import channels, thereby activating the autophagy machinery and degradation of the whole organelle by mitophagy.

In support to this concept, there is evidence showing that, when cells are exposed to a low dose of the mitochondrial complex III inhibitor antimycin A, which is commonly used at higher concentrations to induce mitophagy acutely, MDV formation is dependent on PINK1/Parkin with a peak after 1−3 h of antimycin A treatment, in contrast to mitophagy that is activated after 12−24 h (McLelland et al., 2014). Similarly, it has been observed that MDV formation represents an early response to stress after the addition of cannabinoids, where MDV formation precedes mitophagy and is dependent on PINK1 and Parkin (Ramirez et al., 2022). MDV generation was also observed in oligodendrocyte precursor cells treated with low dose of carbon monoxide serving as a preconditioning mechanism to protect cells from carbon monoxide toxicity (Guo et al., 2023). These reports suggest that MDVs are a first round of defense against mitochondrial stressors (Cadete et al., 2019; Soubannier et al., 2012a; Vasam et al., 2021) that allow ejection of oxidized/damaged mitochondrial components and avoid whole organelle disposal via mitophagy (Picca et al., 2023b). This early MDV response does not require the activation of the autophagy machinery, as it occurs in the absence of autophagy protein 5 (ATG5), the GTPase Ras-associated binding 9 (RAB9) protein, or Beclin (McLelland et al., 2014; Soubannier et al., 2012a), and sculpts the mitochondrial proteome via removing oxidized/damaged contents (Burman et al., 2017; McLelland et al., 2014; Soubannier et al., 2012b). Indeed, studies have documented the production of vesicles enriched in subunits of the matrix mitochondrial pyruvate dehydrogenase (PDH), translocase of the outer mitochondrial membrane (TOMM)$^{-}$/PDH^{+}, during oxidative stress. These vesicles are different from TOMM^{+}/PDH^{-} MDVs, the most abundant MDVs, generated in the steady state to deliver their contents to MVBs/lysosomes for degradation (König et al., 2021; McLelland et al., 2014).

The constitutive biogenesis of MDVs occurs independent of PINK1/Parkin and involves the microtubule-associated motor, mitochondrial Rho GTPase (MIRO) 1 and MIRO2 and DRP1 proteins (König et al., 2021). MIRO1 and MIRO2 pull thin membrane tubules from mitochondria to separate the selected cargo, while the protein receptors of the mitochondrial dynamics DRP1 (MiD49 and MiD51) or the mitochondrial fission factor (MFF) (König et al., 2021) trigger the recruitment of DRP1 and induce tubule scission from parental mitochondrion leading to MDV generation. Researchers have recently described important roles also for centromere protein F (CENP-F) and several armadillo repeat-containing proteins on the X chromosome (ARMCX) proteins in the generation of TOMM^{+} MDVs (König et al., 2021). Knockdown of CENP-F or double knockdown of ARMCX1 and ARMCX3 by short interfering RNAs has shown to induce a decrease in the amount of TOMM^{+} MDVs. In contrast, single knockdown of either ARMCX1 or ARMCX3 had no significant effects on MDV generation (König et al., 2021). While the exact regulation of these events according to cell cycle is not completely understood, the calcium-regulated GTPases of the OMM, MIROs, can hook mitochondria to the kinesin/dynein family of motor proteins (Eberhardt et al., 2020), while mitochondria are coupled to the growing ends of microtubules during mitosis via interaction between CENP-F and MIRO1 (Kanfer et al., 2017, 2015). Of note, the incorporation of matrix and inner membrane cargoes into MDVs requires the fusion mediator GTPase optic atrophy 1 (OPA1) (Todkar et al., 2021).

Upon generation, MDVs can pursue different fates and be delivered either to MVBs/lysosomes and peroxisomes for degradation or released into the extracellular microenvironment (Gagliardi et al., 2024; Matheoud et al., 2016; Sugiura et al., 2014). The selection of the route is complex, and several mediators are involved. The PINK1, Parkin, Tollip, and syntaxin-17 (STX17) signaling pathway is responsible for MDV delivery to lysosomes, while vacuolar protein sorting ortholog 35 (VPS35) and mitochondrial-anchored protein ligase (MAPL) are involved in the mechanism of peroxisome transport (McLelland et al., 2014; Peng et al., 2022; Ryan et al., 2020).

The preliminary fusion of MDVs with MVBs for subsequent release as EVs is under the control of Parkin inhibition and a set of molecular modulators, including cluster of differentiation 38 (CD38)/cyclic ADP ribose (cADPR) (Suh et al., 2023), sorting nexin 9 (SNX9), and OPA1 (Peng et al., 2022; Todkar et al., 2021). Additionally, the small GTPase RAB7, a key regulator of endocytic trafficking (Guerra and Bucci, 2016), VPS39/41, two homotypic fusion and protein sorting (HOPS) tethering complex subunits, and STX17 are responsible for the delivery of stress-induced PINK1/Parkin-dependent MDVs to the late endosome/lysosomes (McLelland et al., 2016) (Fig. 1).

An additional subpopulation of EVs containing a limited and highly specialized set of mitochondrial components has also been described and named "mitovesicles" (D'Acunzo et al., 2022). These are double-membrane vesicles with electron-dense matrix and peculiar morphology, protein, and lipid composition compared with microvesicles and exosomes (D'Acunzo et al., 2022, 2021). Mitovesicles are enriched of voltage-dependent anion channel (VDAC), cytochrome c oxidase subunit 4 (COX-IV), and PDH E1 component subunit alpha (PDH-E1α) (D'Acunzo et al., 2022, 2021), while they lack mitochondrial structures, such as cristae, ribosomes, and the constitutive proteins TOMM20, mitofusin (MFN) 2, and the polymerase-γ (POL-γ) subunits (D'Acunzo et al., 2022, 2021). The mechanism leading to the generation and secretion of mitovesicles into the extracellular space is unclear. However, some features are shared with MDVs, including budding from the mitochondrial surface and targeting to MVBs. Although the release of mitovesicles into the extracellular space after fusion with MVBs cannot be ruled out (Picca et al., 2019), the tetraspanin CD63, a component of membranes of late endosomes and lysosomes, also indicated as lysosomal associated membrane protein-3 (LAMP-3), is not found in mitovesicles, but only in exosomes and MDVs (Gagliardi et al., 2024; Guerra et al., 2019). Taken as a whole, these findings indicate that distinct mechanisms may be in place to regulate the biogenesis of different subsets of MDVs with specific functions which warrants further investigation.

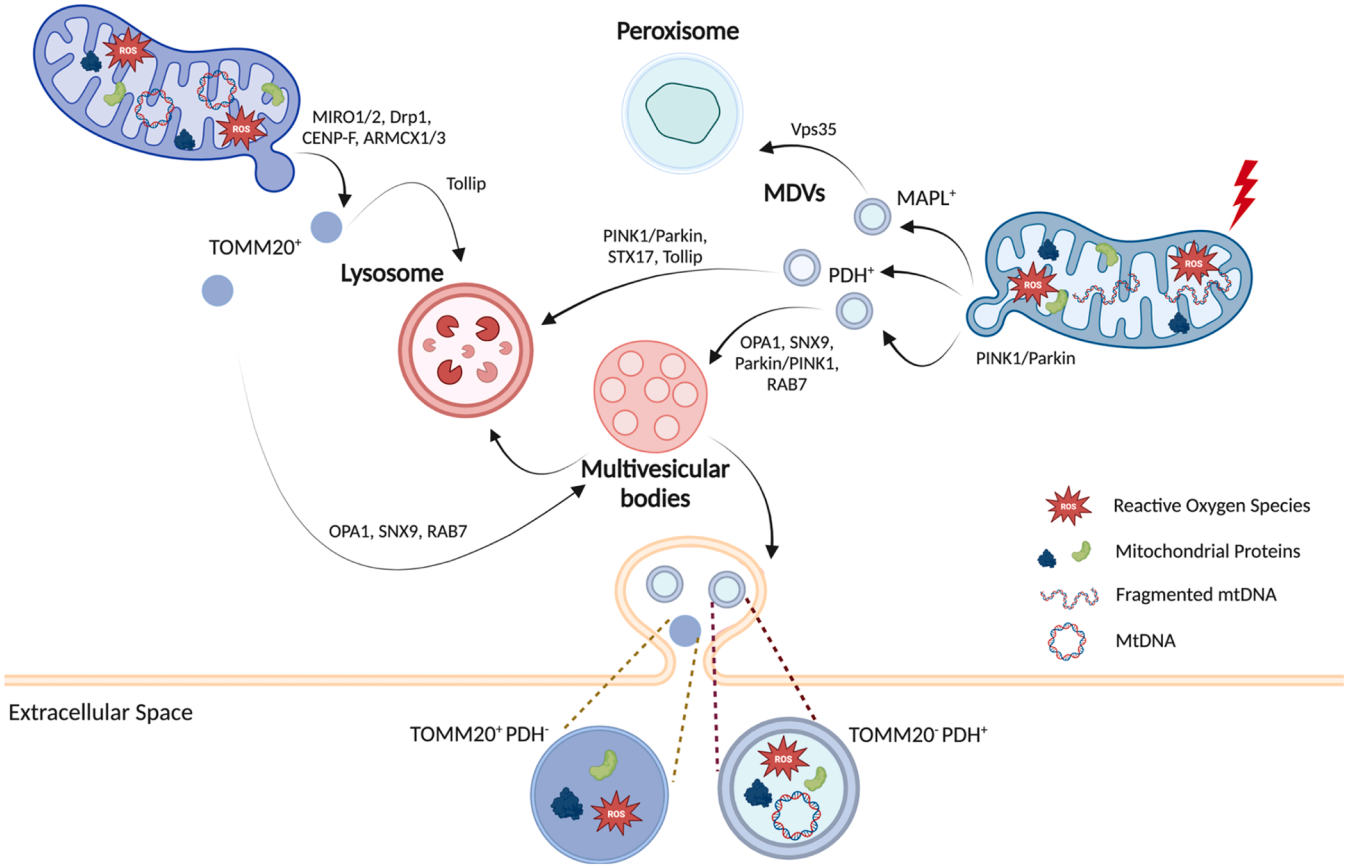

Fig. 1. Schematic representation of mitochondria-derived vesicle generation and classification and overlap with mitophagy pathway. Under basal conditions all types of mitochondria-derived vesicles (MDVs) are constitutively produced. Under stress, the number of all MDV types increases; however, the number of TOMM20^{+} PDH^{-} MDVs roughly doubles, while the number of TOMM20^{-} PDH^{+} MDVs increases by about 4–5 folds. Abbreviations: ARMCX1/3, armadillo repeat-containing proteins on the X chromosome 1/3; CENP-F, centromere protein F; Drp1, dynamin-related protein 1; MAPL, mitochondrial-anchored protein ligase; MIRO, microtubule-associated motor; OPA 1, optic atrophy 1; PDH, pyruvate dehydrogenase; PINK1, PTEN induced kinase 1; RAB7, Ras-related protein in Brain 7; ROS, reactive oxygen species; SNX9, sorting nexin 9; STX17, syntaxin-17; TOMM, translocase of the outer mitochondrial membrane; Vps35, Vacuolar protein sorting ortholog 35. Created with Biorender.com (accessed on 24 September 2024).

2.3. Methods for the isolation and characterization of mitochondria-derived vesicles

Methods for MDV purification are limited mostly due to inconclusive information about size, surface markers, and density. As per today, ultracentrifugation is the elective method to isolate MDVs followed by the analysis and characterization of their content in terms of mitochondrial proteins, DNA, microRNAs, and enzymes (D'Acunzo et al., 2022; Gagliardi et al., 2024; Girolimetti et al., 2024; Sansone et al., 2017). However, the material isolated with this method includes a considerable amount of unwanted particles and other contaminants (Phinney et al., 2015; Todkar et al., 2021). Hence, the task force of the International Society of Extracellular Vesicles (ISEV) provided guidelines on the minimal information for studies of extracellular vesicles 2023 (MISEV2023) (Welsh et al., 2024) and methods for general EV characterization based on morphology, size, and surface markers analyses. Nanotrack analysis (NTA), electron microscopy (EM), flow cytometry (FC) and immunoblotting are used for the detection of morphology, size, and surface markers of circulating MDVs (Welsh et al., 2024). The size and content of MDVs are highly heterogeneous; thus, their specific size distributions and biomarkers remain elusive. After isolation, a series of approaches, such as Western immunoblotting, real-time polymerase chain reaction (RT-PCR), digital droplet PCR (ddPCR), and specific dyes (e.g., MitoTracker, tetramethylrhodamine methyl ester) allow characterization of MDV contents.

Recently, immunoprecipitation and subsequent analysis of EVs with antibodies conjugated with magnetic beads against three tetraspanins (CD63, CD9, and CD81) allowed extracellular MDVs from ovarian cancer cells to be studied (Gagliardi et al., 2024). Although the method did not guarantee selection of pure MDVs but included also non-mitochondrial exosomal fractions, the mitochondrial signature of the immunoprecipitated vesicles was ascertained and their content was quantified (Gagliardi et al., 2024).

The study of extracellular MDVs remains challenging, and optimization of purification methods is needed before MDVs in biofluids can be used as biomarkers of response to chemotherapy (Gagliardi et al., 2024; Sansone et al., 2017), neurodegeneration (Li et al., 2023; Yang et al., 2022), and aging (Yin et al., 2021; Yoshida et al., 2019). Liquid biopsy is the goal of clinical and biological research and MDVs could be accounted among relevant physiological and pathological biomarkers. A method for the detection of circulating cell-free mtDNA has been proposed by Podlesniy and Trullas (2018) by optimizing mtDNA quantification by ddPCR in very small sample volumes. In addi, D'Acunzo et al. (2021), (2022) developed a new approach for isolating mitovesicles from brain tissues by filtration (0.2-μm) and iodixanol-based high--resolution density gradient centrifugation. Although this method allows separation of pure mitovesicles, it has been applied only to brain tissues and it is yet unknown whether mitovesicles exist in tissues other than the brain. Further investigations are needed to optimize MDV purification and to perform targeted MDV analyses.

3. Preclinical studies on mitochondria-derived vesicles: What we know and what we can learn from preclinical models

The characterization of MDVs in preclinical models has shown evolutionary similarities with bacterial vesicles, suggesting that they are likely ancestral remnants of bacterial independent carrier units aimed at inter-organellar and/or cell-to-cell communication that have evolved signaling functions to distant organs and/or even organisms (Deatheragea and Cooksona, 2012). As a conserved trait of microbial life, bacterial EVs may spread virulence factors, antigens, and products of bacterial communication (Deatheragea and Cooksona, 2012), but also antimicrobial and anti-apoptotic signaling as well as other molecules that can protect tissues from hypoxia and stress.

The implication of MDVs in antimicrobial defence is epitomized by their identification within the phagosomes of macrophages infected with methicillin-resistant *Staphylococcus aureus* (Abuaita et al., 2018). The generation of MDVs carrying the mitochondrial antioxidant enzyme superoxide dismutase 2 (SOD2) is promoted upon infection and delivered within bacteria-containing phagosomes. Herein, MDV-carried SOD2 favors the killing of invading bacteria by converting superoxide anions ($O^{-\cdot}$) into hydrogen peroxide (H_2O_2) (Abuaita et al., 2018). A mitochondria-peroxisome shuttling system has recently been identified in cultured cells probably as a remnant of the ancestral MDV-guided antimicrobial role via H_2O_2 generation. The shuttling system requires the contribution of MAPL, a dual ubiquitin and small ubiquitin-like modifier ligase. Through this inter-organellar system, MAPL is shuttled within single-membrane MDVs from mitochondria to peroxisomes to support peroxisome biogenesis from existing organelles and/or for de novo synthesis (Mohanty et al., 2021; Neuspiel et al., 2008). The whole process is assisted by specific peroxisomal proteins, called *peroxines* (Pex), associated with two distinct subpopulations of vesicles that mediate the fusion process. MDVs enriched in Pex3 (from the OMM) and Pex14 (implicated in the import of peroxisomal matrix) fuse with Pex16-rich vesicles produced by the endoplasmic reticulum forming a pre-peroxisomal structure containing proteins of the peroxisomal membrane. Proteins are eventually imported into the lipid bilayer via Pex3 and Pex16, and peroxisomal matrix proteins are recruited from the cytosol (Mohanty et al., 2021; Neuspiel et al., 2008). Once the import is completed, mature peroxisomes grow, elongate, and undergo fission into daughter organelles whose abundance is regulated by the cellular metabolic needs (Mohanty et al., 2021; Neuspiel et al., 2008). Muscle-specific deletion of Pex5 induced disruption of peroxisomes and mitochondria tethering, impairment of lipid metabolism, and physical performance decline in mice (Scalabrin et al., 2024), highlighting the relevance of preserving peroxisomal function and their mitochondrial contact sites to muscle health.

MDV release is observed in mitochondria that are damaged/oxidized but not yet completely depolarized and has been shown to serve multiple roles. For example, MDVs emerging from MVBs as exosomes are packed with proinflammatory, oxidized mitochondrial constituents that, if released directly into the extracellular environment, would act as damage-associated molecular patterns (DAMPs) and trigger innate immunity. Instead, these oxidized mitochondrial contents are shuttled through a Parkin-mediated mechanism to lysosomes that abrogate their activity as DAMPs through acidic clearance (Todkar et al., 2021; Zecchini et al., 2023). Proteomic studies have confirmed that the presence of nucleoid-enriched mitochondrial fragments in the proximity of autophagosomes in brain primary neurons facilitates their autophagosomal capture and mitigates inflammation (Goldsmith et al., 2022). High release of mitochondria in large EVs independent of autophagy and via MVBs has also been described in the setting of lysosomal dysfunction in adult mouse heart (Liang et al., 2023). MDV generation can thus be envisioned as part of the mitochondrial network/quality remodeling process that, via release of MDVs as exosomes from MVBs, contributes to alleviating oxidative cell burden by clearing damaged organelles via the endo−lysosomal system when mitophagy and or lysosomal function is compromised (Poillet-Perez and White, 2021). This housekeeping mechanism may limit the spreading of proinflammatory mitochondrial fragments (Cadete et al., 2016; Liang et al., 2023).

EVs originating from this route have also been found to mediate inter-organ signaling, modulate innate immune responses, and favor cancer progression (Crewe et al., 2021; Guan et al., 2024; Liang et al., 2023; Peng et al., 2022; Suh et al., 2023; Todkar et al., 2021). However, the identity of the molecular switches modulated by these vesicles is unclear.

As mentioned above, the inter-organ signaling role of MDVs is to carry long-distance messages for the purpose of metabolic regulation. MDV secretion has been observed in mice on a high-fat diet, in which the induced expression of mitochondrial ferritin in adipocytes is paralleled by oxidative stress (Crewe et al., 2021). Notably, although stress was specifically induced in adipocytes, ROS increase was observed in the heart. The authors demonstrated that such crosstalk was mediated by the horizontal transfer of EVs bearing oxidized mitochondrial components that retained functional respiratory capacity from adipocytes to cardiomyocytes (Crewe et al., 2021). The receiving cardiomyocytes internalized these EVs, prompting a ROS burst, which later served as a protective preconditioning mechanism (Crewe et al., 2021). Indeed, the injection of EVs isolated from stressed adipocytes of mice prior to coronary artery ligation alleviated cardiac ischemia/reperfusion injury (Crewe et al., 2021). In contrast, adipocytes from Parkin knockout mice produced lower mitochondrial-enriched EVs and lacked a cardioprotective effect (Crewe et al., 2021).

Horizontal transfer of platelet-derived mitochondria via EVs was also implicated in metabolic profiling and cancer progression in human metastatic MDA-MB-231 triple-negative breast cancer cells co-cultured with platelets (Cereceda et al., 2024). The internalization of platelet-derived mitochondria by MDA-MB-231 cells improved mitochondrial bioenergetics (Cereceda et al., 2024). Restoration of cell proliferation and oxygen consumption was also observed in platelets co-cultures with MDA-MB-231 cells depleted of mtDNA, likely indicating mtDNA reconstitution promoted by platelet-derived mitochondria (Cereceda et al., 2024). Beneficial effects of horizontal transfer of mitochondria have also been reported to occur via medium-to-large EVs (Dave et al., 2023). The transfer of these vesicles in a cell line of human brain microvascular endothelium (hCMEC/D3) has been shown to induce an increase in intracellular ATP levels and overall improved bioenergetics in hCMEC/D3 recipient cells (Dave et al., 2023). Uptake and integration of these EVs into the mitochondrial network of human brain microvascular endothelial recipient cells were also observed (Dave et al., 2023). Finally, the transfer of functional mitochondria via EVs from human brain endothelial cells (BECs) to ischemic BECs has been identified and shown to increase ATP levels, mitochondrial activity, and cell survival (D'Souza et al., 2021).

MDV release has also been implicated in antigen presentation by deploying macrophage-guided clearance of mitochondria into phagosomes to limit sterile inflammation and preserve tissue homeostasis. Brown adipocytes stressed by cold exposure undergo mitochondrial uncoupling and ROS bursts, followed by the release of EV-containing oxidized mitochondria (Rosina et al., 2022). These EVs are generated in a PINK1-dependent manner and are double-membrane vesicles with $PDH^{+}/TOMM20^{-}$ phenotype targeting the endo−lysosomal system for subsequent exosome release (Rosina et al., 2022). Treatment with the powerful antioxidant N-acetyl cysteine blunts EV release, whereas oxidized mitochondria-containing EVs are cleared by monocyte-derived macrophages induced by thermic stress. The removal of these EVs by brown adipose tissue-resident macrophages via phagocytosis is pivotal for maintaining metabolic homeostasis (Brestoff et al., 2021; Rosina et al., 2022). Indeed, re-uptake of EV-containing oxidized mitochondrial components by parental brown adipocytes disrupts the thermogenic response by reducing the expression of peroxisome proliferator-activated receptor gamma (PPAR-γ) and uncoupling protein 1 (UCP1), and alters respiration and tricarboxyl acid (TCA) metabolites

in recipient cells, at least partly via activation of the AMP-activated protein kinase (AMPK) signaling pathway (Rosina et al., 2022). A similar mechanism was described in stressed cardiomyocytes of mice subjected to catecholamine infusion or coronary artery ligation (Nicolás-Ávila et al., 2020). In the same study, resident macrophages were shown to execute phagocytosis of EV-containing mitochondria generated via the autophagy machinery (Nicolás-Ávila et al., 2020). Removal of these EVs limits inflammation and is accomplished via recognition of phosphatidylserine residues on the EV surface by the macrophage receptor tyrosine-protein kinase Mer, which mediates phagocytic uptake (Nicolás-Ávila et al., 2020). Stressed cardiomyocytes showed enhanced phagocytosis of EV-containing mitochondria (Nicolás-Ávila et al., 2020).

Horizontal transfer of mtDNA within exosomes has also been described. Seminal work by Guescini et al. (2010a), showed mtDNA transfer via EVs in glioblastoma and astrocyte cell cultures. Later, changes in mtDNA copy number, heteroplasmy, and mutations were verified in plasma-derived exosomes of patients with glioblastoma (Soltész et al., 2022). The shuttling of mtDNA via exosomes has also been confirmed in breast cancer cells resistant to hormonal therapy (Rabas et al., 2021; Sansone et al., 2017). The delivery and integration of mtDNA into recipient cells via EVs, likely released in a PINK1-dependent pathway, induces escape from dormancy of cancer (Rabas et al., 2021; Sansone et al., 2017). mtDNA transfer via EVs has also been observed from colon cancer cells to normal colonic epithelial cells and associated with metabolic reprogramming (Guan et al., 2024). Upon mtDNA transfer, higher mitochondrial respiration and ROS production occurred in recipient colonic epithelial cells triggering upregulation of the transforming growth factor beta 1 (TGFβ1) and activation of TGFβ/Smad pathways driving malignancy in colon cancer cells (Guan et al., 2024).

Endosomal trafficking and exosome release appear also to facilitate cancer cell invasiveness (Rabas et al., 2021). Metabolic rewiring of cancer cells is a necessary step to invasiveness and requires upregulation of a cystine/glutamate antiporter that increases extracellular levels of glutamate to support glutathione synthesis in the tumor microenvironment to cope with oxidative stress (Rabas et al., 2021). This is mediated by the activation of glutamate receptor 3, which supports Rab-dependent endosomal trafficking and exosome release. Chronic glutamate exposure induces mitochondrial stress and triggers the production of EVs containing mitochondrial constituents, including mtDNA (Rabas et al., 2021). Although the mechanism of mitochondrial delivery to the late endosome has not been elucidated, the process is independent of autophagy and requires PINK1 but not its kinase activity for mtDNA inclusion in exosomes. The consequence of EV-mediated mtDNA release is the activation of toll-like receptors, which promote endocytosis, tumor migration, and invasiveness (Rabas et al., 2021). In line with these findings, EM analyses of human renal carcinoma cells revealed a greater release of MDVs in autophagy-deficient clones (Poillet-Perez and White, 2021), supporting the view that MDV generation may be an alternative MQC pathway in the setting of dysfunctional mitophagy.

MDVs have been interpreted as one of the many mitochondrial remodeling forms aimed at preserving optimal mitochondrial function. For instance, under hypoxia changes in mitochondrial morphology ranging from tubular to enlarged, dysmorphic, megamitochondria have been reported (Hao et al., 2023). These megamitochondria make contacts with lysosomes for engulfment in a process termed megamitochondria engulfing lysosome (MMEL) that promotes mitochondrial self-digestion (Hao et al., 2023).

Exospheres, such as extracellular particles containing mitochondria released via membrane blebbing, have also been described in neurons of *C. elegans* to enable mitochondrial horizontal transfer and preserve cell quality under neurotoxic conditions (Melentijevic et al., 2017). The transfer of mitochondria between cells *in vivo* has been shown to occur also via nanotunnels or ectosomes shedding from the plasma membrane (Colombo et al., 2014; Davis et al., 2014; Hekmatshoar et al., 2018; Ortin-Martinez et al., 2021; Yamashita et al., 2018). The mitochondrial changes in morphology under stress are complex and still poorly understood with possible roles in physiology and pathology beyond the model of hypoxia. Similarly, whether this mitochondrial horizontal transfer can be included among the additional noncanonical routes for MQC and whether it is involved in health and disease should be explored in future research.

4. Clinical studies investigating mitochondria-derived vesicles in aging and associated conditions

The discovery of displacement of mitochondrial components via the endo−lysosomal system in human disease can be dated back in 2016 when Caielli et al. (2016) identified portioned oxidized mtDNA within the supernatants of cultured neutrophils from patients with systemic lupus erythematosus. Although the mechanisms were not fully elucidated at that time, the analysis of neutrophils showed that oxidized mtDNA was first accumulated within mitochondria and extruded thereafter in a complex with mitochondrial proteins (Caielli et al., 2016). mtDNA extrusion was promoted under neutrophil stimulation with the oxidative phosphorylation inhibitor carbonyl cyanide m-chlorophenyl hydrazone (CCCP). Of note, CCCP-stimulated neutrophil mitochondria are unable to complete mitophagy triggered by mtDNA nucleoids release and type I interferon response (Caielli et al., 2016). Subsequent studies revealed that mtDNA nucleoids and/or mtDNA fragments can be shuttles within MDVs under specific stimuli (Todkar et al., 2021; Zecchini et al., 2023). The delivery of MDV content to MVBs and their subsequent release as exosomes at the extracellular level increase when lysosomal function is impaired (Liang et al., 2023). This finding led to hypothesize that generation of MDVs may be a compensatory recycling mechanism for damaged mitochondria when primary mitophagy mechanisms are impaired (Poillet-Perez and White, 2021). Hence, it may be possible to study such vesicles to better understand dysfunction and compensation of different forms of mitophagy. Recently, fully respiratory-competent mitochondria have been found in human blood (Al Amir Dache et al., 2020) with potential roles in cell-to-cell communication and/or modulation of immune response and inflammation and implications in homeostasis and disease whose meaning is only started to be understood. Mitophagy is pivotal in maintaining energetic homeostasis that is essential to healthy life and there is strong evidence that mitophagy dysfunction causes a stress condition that contributes to aging (Schmauck-Medina et al., 2022) and age-associated chronic conditions. In this regard, the identification of mtDNA in plasma EVs and the reduced level of EV-derived mtDNA with aging stemmed as a relevant finding (Lazo et al., 2021). Indeed, EV-bound mtDNA holds less proinflammatory signaling and represents a “safe” option for sheltering proinflammatory mitochondrial DAMPs (Trumpff et al., 2021). However, conflicting results have been reported showing high levels of mtDNA and inflammatory mediators in plasma EVs of frail older adults (Byappanahalli et al., 2023). Untangling the mechanisms that selectively modulate MDV formation and the nature of their cargo, with particular reference to mtDNA, is an important step for addressing whether MDVs offset the negative effect of dysfunctional mitophagy with aging. The number of studies on the functional impact of MDVs is steadily growing in several disease paradigms and aging. Indeed, these vesicles have been attributed different roles in skeletal muscle aging, myocardial ischemia, neurodegenerative diseases, and cancer metabolism (Guerra et al., 2019; Li et al., 2020; Picca et al., 2020a, 2020b). In the next sections, we provide an overview of clinical studies investigating MDVs in aging and associated conditions and draw inferences on how the characterization of circulating MDVs may be exploited as mirrors of the mitophagy process in those conditions (Table 1).

Table 1
List of clinical studies on mitochondria-derived vesicles in aging and disease conditions.

Clinical condition	Cell/Tissue/Biofluid	Vesicle subtype	Mechanism/Main finding	Reference
Systemic lupus erythematosus	Neutrophils	EV-containing mitochondrial components	Neutrophil mitochondria unable to complete mitophagy trigger mtDNA nucleoids release and type I interferon response	(Caielli et al., 2016)
Frailty	Plasma	EV-containing mtDNA	High levels of mtDNA and pro-inflammatory mediators released via EVs	(Byappanahalli et al., 2023)
Skeletal muscle disorders				
Sarcopenia	Serum	Total EVs and MDVs	High secretion of EVs and reduced level of MDVs	(Picca et al., 2020a)
	Serum	Total EVs and MDVs	MDV cargo molecules were identified as discriminant mediators for the classification of older adults with physical frailty and sarcopenia and controls	(Marzetti et al., 2020)
Danon disease	Cardiac biopsies	EV-containing mitochondrial components	Inhibition of lysosomal degradation and elimination of mitochondria via EVs	(Liang et al., 2023)
Neurodegeneration				
Parkinson's disease	Serum	EVs and MDVs	High secretion of EVs and reduced level of MDVs	(Picca et al., 2020b)
Huntington's disease	Plasma	Neuronal EVs	High secretion of neuronal EV-containing mtDNA	(Beatriz et al., 2022)
Fragile X-associated tremor/ataxia	Plasma	Neuronal EVs	Dysfunctional mitochondria in neuronal EVs	(Yao et al., 2024)
Down syndrome	Primary fibroblast, brain tissues, and plasma	Mitovesicles	Mitovesicle levels and composition vary according to mitochondrial alterations in the brain	(D'Acunzo et al., 2021)
Alzheimer's disease	Plasma	EV-containing mitochondrial components	High levels of mtRNAs in EVs from individuals with mild cognitive impairment and Alzheimer's disease compared with healthy controls	(Kim et al., 2020)
Cancer				
Glioblastoma	Plasma	EVs	Horizontal transfer of mutated mtDNA via EVs	(Soltész et al., 2022)
Breast cancer	Plasma	EVs	Horizontal mtDNA transfer through EVs and cell's exit from dormancy	(Sansone et al., 2017)
Breast cancer	Triple negative breast cancer cells	EVs	Mitochondrial transfer via EVs induce chemoresistance and promotes tumorigenesis	(Abad and Lyakhovich, 2022)
Malignant glioma cells	Primary glioblastoma cells	EVs	Mitochondrial transfer via EVs and/or nanotunnels induces resistance to radiotherapy and chemotherapy	(Salaud et al., 2020)
Ovarian cancer	Ovarian cancer cells	MDVs	Damaged mitochondrial particles secreted via MDVs are acquired by cisplatin sensitive cells and induce chemoresistance	(Gagliardi et al., 2024)

Abbreviations: EVs, extracellular vesicles; MDVs, mitochondria-derived vesicles; mtDNA, mitochondrial DNA; mtRNA, mitochondrial RNA

4.1. Mitochondria-derived vesicles and sarcopenia

Mitochondrial quality is of utmost importance for skeletal muscle function. Energy demands during periods of intense contraction increase ATP consumption by about 100 folds (Egan and Zierath, 2013). To meet their high energy requirements, cardiac and skeletal muscles rely mostly on oxidative phosphorylation.

Significant reductions in mitochondrial function have been observed in skeletal muscle during aging (Choi et al., 2016) with negative impact on muscle strength and physical performance (Tian et al., 2022; Zane et al., 2017). A reduced mitochondrial function that is not always paralleled by a decrease in mitochondrial mass may also be due to defective mitophagy and inefficient removal of damaged organelles (Balan et al., 2019; Crane et al., 2010; Gouspillou et al., 2014). Indeed, some available data support the hypothesis that a stalling of mitochondrial biogenesis may arise from reduced mitochondrial signaling due to mitophagy impairment (Liu et al., 2021; Ploumi et al., 2017).

There is strong evidence that, in aging and specific muscle diseases, skeletal muscle mitochondria are actively and continuously removed (Balan et al., 2019; Drummond et al., 2014; Gouspillou et al., 2014; Picca et al., 2023c). Muscle proteomic analyses in individuals with peripheral artery disease revealed an unbalanced accumulation of mitochondrial versus nuclear-expressed respiratory complex subunits due to mitophagy impairments, which was associated with a reduction in mitochondrial function (Ferrucci et al., 2023). Recent literature has revealed that EVs enriched in mitochondrial cargo are released by skeletal muscle with various mechanisms and, likely, with different purposes. A pioneer study by Guescini et al. (2010b), and later investigations identified mtDNA among the cargo molecules (histones and nuclear proteins) of EVs generated by human myoblasts and myotubes (Forterre et al., 2014; Le Bihan et al., 2012; Sork et al., 2018). The molecular mechanisms involved in originating these EVs were not completely disentangled. However, subsequent studies revealed a high secretion of small EVs in the serum of older adults with physical frailty and sarcopenia (PF&S) compared with controls (Picca et al., 2020a). In particular, the presence of the three protein tetraspanins CD9, CD63, and CD81 in purified EVs allowed for their classification as a fraction of endosome-derived vesicles originating from the fusion of MVBs with the plasma membrane (Kowal et al., 2017). The different expression of these protein markers in EV populations between older adults with PF&S and controls indicated a heterogenous composition of EVs likely reflecting a different regulation of vesicle trafficking (Andreu and Yáñez-Mó, 2014). In particular, a decreased expression of tetraspanin CD63 in PF&S may be interpreted as resulting from altered late endocytic pathway under the control of RAB7A, a guanosine triphosphatase (GTPase) that modulates exosome secretion (Guerra and Bucci, 2019). The presence of mitochondrial components, such as ATP synthase F1 subunit alpha (ATP5A, complex V), NADH:ubiquinone oxidoreductase core subunit S3 (NDUFS3, complex I), and succinate dehydrogenase complex iron sulfur subunit B (SDH-B, complex II), within the purified EVs suggested their mitochondrial origin. However, lower circulating levels of MDVs were identified in individuals with PF&S (Picca et al., 2020a). A concomitant overall increase in EV secretion and reduced levels of MDVs in older adults with PF&S may reflect a cell's attempt to extrude impaired mitochondria along the secretory endo–lysosomal pathway. Because this becomes difficult in the setting of altered late endocytic pathway and/or impaired MQC, MDV levels may be reduced. This view is in keeping with reports showing MQC derangements in muscle of sarcopenic individuals in whom mitochondrial damage might be too severe to be cleared via MDVs (Marzetti et al., 2016; Picca et al., 2017). Another hypothesis involves a reduced extracellular release of MDVs owing to dysregulated lysosomal signaling and activity, this latter being described in the setting of muscle aging (Picca et al., 2017). Finally, in a subsequent study, MDV cargo molecules were found among the

molecules able to correctly classify older adults with PF&S and non-PF&S controls, suggesting that this pathway may be relevant to PF&S pathophysiology (Marzetti et al., 2020). Similarly, patients with Danon disease bearing a mutation in the gene encoding LAMP-2 and experiencing cardiac hypertrophy and heart failure showed high levels of MDVs (Liang et al., 2023). In this setting, mitochondria may be eliminated in large EVs via the endosomal pathway due to inhibition of lysosomal degradation.

4.2. Mitochondria-derived vesicles and neurodegeneration

Dysregulated mitophagy and MDV signaling have been described in the setting of brain aging, neurodegeneration, and age-related neurodegenerative diseases. The role of *PINK1* and *Parkin* gene mutations in the development of PD has been widely investigated (Cheon et al., 2012). Multiple roles for these mitophagy mediators in preserving mitochondrial quality through different forms of mitophagy and neuronal homeostasis are increasingly demonstrated (Ge et al., 2020). Several neuronal subpopulations, including dopamine neurons of the substantia nigra pars compacta, have large distributed axonal networks, dense synaptic connections, and high basal levels of activity that result in a high bioenergetic cost and high degree of mitochondrial stress during PD-related neurodegeneration (Ge et al., 2020). PINK1 and Parkin are essential for eliminating damaged mitochondria through mitophagy but also for transporting damaged mitochondrial fragments into MDVs for their degradation, thereby playing a dual role in MQC (Sugiura et al., 2014). Although the exact mechanism that leads to MDV generation is not yet clarified, the dual role of PINK1 and Parkin may allow unveiling the fine regulation between mitophagy and MDV generation during PD (Sugiura et al., 2014). MDV formation for the purpose of antigen presentation is repressed by PINK1 and Parkin via inhibition of RAB9 and SNX9 recruitment to mitochondria (Matheoud et al., 2016). Parkin, instead, can regulate the transfer of mitochondrial antigens (i.e., cCOX-I and β-nicotinamide adenine dinucleotide reduced subunit 5) to the nucleus by controlling the adaptive immune modulator and ultimately determining nuclear antigen presentation on major histocompatibility complex (MHC) class I molecules (Roberts and Fon, 2016). High circulating levels of cell-free DNA and overt inflammation have been described in the setting of altered dynamin-like protein 1 (DLP1) and fission protein 1 (FIS1)-guided mitochondrial fragmentation (Chan, 2006). DLP1 is a cytosolic protein that can localize at the sites of fission on the OMM (Smirnova et al., 2001). Turnover of DLP1 is mediated by VPS35, a protein involved in autophagy and a key component of the retromer complex, with a role in PD neurodegeneration (Chan, 2006). MDV production is regulated by VPS35 shuttling mitochondrial cargoes to either peroxisomes or lysosomes (Hanss et al., 2021; Sugiura et al., 2014). In particular, the interaction of the retromer complex with VPS35−DLP1 determines the removal of DLP1 from mitochondria and supports MDV trafficking to lysosomes. This allows efficient mitochondrial fission, which is essential to prevent mitochondrial permanent damage and fragmentation (Wang et al., 2016). Wang et al. (2016) observed that, in sporadic PD, VPS35 mutations increased VPS35−DLP1 interaction that enhanced the retromer-dependent turnover of mitochondrial DLP1 complexes via MDV trafficking, thus leading to excessive fission and mitochondrial dysfunction. While the regulation of mitochondrial dynamics and quality control processes has been shown to be critical in PD pathogenesis and progression, the analysis and role of circulating MDVs in PD are in their infancy and warrant further investigation (Picca et al., 2020b, 2019).

Mitochondrial disorders and MDV release have also been described in late-onset rare neurodegenerative disorders. High circulating levels of neuronal EVs containing mtDNA and mitochondrial proteins have been described in individuals with Huntington's disease with impaired endo−lysosomal pathway (Beatriz et al., 2022). Quantitative and functional abnormalities were also identified in mitochondria of post-mortem brains from people with fragile X-associated tremor/ataxia syndrome (FXTAS) and in plasma neuronal EVs in FXTAS premutations carriers (Yao et al., 2024). A possible application of these vesicles as predictive and disease monitoring markers has been proposed. However, a deeper characterization of vesicles and their originating pathways is warranted.

Mitovesicles have been well characterized in brain tissue of individuals with Down syndrome (DS) and trisomic mouse models (D'Acunzo et al., 2022). DS is the most common aneuploidy characterized by intellectual inability and early onset of Alzheimer's disease (AD) (Capone et al., 2018). Chromosome 21 trisomy is associated with overexpression of several genes, including the amyloid precursor protein implicated in AD-related neuroinflammation, neuronal cell loss, deposition of amyloid plaques, and generation of neurofibrillary tangles (Delabar et al., 2016; Hartley et al., 2015). Mitophagy impairment has been described in DS with consequent buildup of toxic mitochondrial components in brain cells, inflammation, and neurodegeneration (Bordi et al., 2019). Mutations in mtDNA and impaired mtDNA repair systems have also been observed in brain tissues and skin fibroblasts from individuals with DS, respectively (Coskun and Busciglio, 2012; Druzhyna et al., 1998). High levels of brain-derived mitovesicles have been identified in a mouse model of DS compared with controls (D'Acunzo et al., 2024, 2021). Both in the brain of DS models and in an *in vitro* mitochondrial stress model produced by treatment with antimycin A, mitovesicles were enriched in COX-IV and PDH-E1α associated with the mitochondrial matrix (D'Acunzo et al., 2021). These findings point to impaired oxidative phosphorylation as one pathogenic mechanism of DS and provide evidence for mitovesicles as biomarkers of brain mitochondrial dysfunction in neurological disorders. An increase in the levels of mitovesicles was also reported in the brain of aged mice likely reflecting age-dependent alterations of mitochondrial bioenergetics (Kim et al., 2022). Furthermore, sex-specific differences were described in the levels of brain microvesicles and exosomes, with an enhanced turnover in females (Kim et al., 2022). Altogether these findings may indicate a mechanism for successful brain aging advantage of females and sex-dependent susceptibility to age-related neurodegeneration.

The release of mitovesicles may also serve as a mechanism to transfer bioenergetically competent packages from one cell to another. In line with this is the identification of increased transfer of functional mitochondria within EVs from astrocytes to neurons under oxidative stress as a mechanism of neuroprotection (Hayakawa et al., 2016). Furthermore, although mitovesicle levels were found to be increased in murine DS brains compared with diploid controls, they showed reduced levels of proteins of the electron transport chain such as ubiquinol-cytochrome C reductase core protein 2 (UQCRC2) and SDH-B, likely indicating an impaired ATP synthesis (D'Acunzo et al., 2021). Therefore, an increase of ATP demand due to impairment of oxidative phosphorylation may foster mitovesicle production and secretion (D'Acunzo et al., 2021). In support to this view, mitochondrial stress in human skin fibroblasts with trisomic karyotype was associated with a decrease in the expression of proteins involved in ATP production such as ATP synthase and ADP/ATP translocator (Valenti et al., 2011, 2010). Finally, DS is associated with impaired mitophagy and is characterized by accumulation of DAMPs arising from mitochondrial stress (Mills et al., 2017; West and Shadel, 2017). Although the mechanisms of release of DAMPs into the extracellular space are not yet clarified, cellular decision on whether damaged mitochondrial content is encapsulated into EVs or not may depend on the extent of mitochondrial dysfunction and/or damage for selective targeting to the appropriate degradative route (Todkar et al., 2021). Further studies are warranted to clarify whether MDVs support disease progression via inflammatory signaling or extract damaged mitochondrial components to alleviate cellular oxidative burden and restore redox homeostasis in neurodegenerative disease.

Recently, mitochondrial particles and mitochondrial RNA (mtRNA) were identified within EVs isolated from astrocytes, microglia, and neurons exposed to amyloid β (Aβ) aggregates and H_2O_2 to mimic the toxic conditions of AD pathogenesis (Kim et al., 2020). AD is, indeed,

characterized by Aβ plaques, tau-containing neurofibrillary tangles, and neuronal inflammation/loss (Deture and Dickson, 2019; Weller and Budson, 2018). Mitochondrial damage, oxidative injury, and consequent mitochondrial dysfunction were induced by Aβ aggregates (Kim et al., 2020). The secretion of EVs carrying mitochondrial damaged components contributed to the accrual of AD-related cellular features. Moreover, the characterization of circulating EVs from individuals with sporadic and familial AD showed high levels and size of systemic EVs (D'Acunzo et al., 2022). An increase in the levels of endothelial- and leukocyte-derived EVs containing mitochondrial markers were identified in sporadic AD patients compared with those with familial AD in whom an increase in platelet-derived EVs was identified. Finally, increased levels of mtRNAs, such as mt-ND1–6 mRNAs and other protein-coding and non-coding mtRNAs, were found in plasma EVs from individuals with mild cognitive impairment and AD, respectively (Kim et al., 2020). These findings, while highlighting the need of additional research, indicate the MDV trafficking may play a pivotal role in supporting neuronal viability and cognitive function.

4.3. Mitochondria-derived vesicles and cancer

The generation and release of EVs in cancer has been widely investigated and recognized to have a role in mediating intercellular communication, carcinogenesis, chemoresistance, and immunosuppression (Romano et al., 2021). Recently, EV-mediated transfer of mitochondrial components has also been implicated in cancer cell communication and regulation of tumor microenvironment, progression, and metastasis (Takenaga et al., 2021). For instance, the release of mtDNA-rich EVs by breast cancer cells promoted under glutamine starvation increases the expression of matrix metalloproteinase (MMP) and α5β1 integrin in recipient breast cancer cells likely enhancing their invasiveness (Rabas et al., 2021). EV-mediated transfer of mtDNA has also been described in patients with hormonal therapy-resistant (HTR) metastatic breast cancer (Sansone et al., 2017). In preclinical models, the authors demonstrated that, while HT eradicates many cancer cells, some of them can undergo a state of viable dormancy characterized by loss of mtDNA. Restoration of mitochondrial function was induced by mtDNA transfer through EV and accompanied the development of HTR (Sansone et al., 2017). Indeed, cancer-associated fibroblast (CAF)-derived EVs both from patients and xenograft models were associated with increased self-renewal potential of breast cancer cells. Horizontal mtDNA transfer has therefore been envisioned as an oncogenic signal promoting the exit from dormancy of therapy-induced cancer stem-like cells and resistance to endocrine therapy in oxidative phosphorylation-dependent breast cancer (Sansone et al., 2017).

Mitochondrial transfer has also been observed in EVs released by chemoresistant triple-negative breast cancer cells delivering functional mitochondria to sensitive triple-negative cancer cells and inducing chemoresistance phenotype and tumorigenesis (Abad and Lyakhovich, 2022). Delivery of mitochondrial components via EVs from stromal cells to recipient malignant glioma cells was also associated with resistance to radiotherapy and chemotherapy (Salaud et al., 2020). Finally, in the absence of mitophagy, cancer cells can adapt and activate fusion and SNX9- and RAB7-mediated MDV formation to deliver mitochondrial material to lysosomes for maintaining mitochondrial homeostasis and function. Recently, Gagliardi et al. (2024) have demonstrated that synergistic mitochondrial and lysosomal dysfunction induces secretion of MDVs by ovarian cancer cells which may serve as mediators and biomarkers of chemoresistance. Ovarian cancer cells resistant to cisplatin treatment are characterized by a decrease in lysosomal function due to downregulation of the endocytic protein RAB7. These cells also show mitophagy dysfunction and downregulation of mitochondrial biogenesis proteins with a consequent block of mitochondrial turnover and deficit in mitochondrial bioenergetics (Gagliardi et al., 2024). In this setting, MDV secretion may likely serve as an MQC mechanism through which these cells can eliminate damaged mitochondrial particles that are acquired by cisplatin sensitive cell lines to induce a chemoresistant phenotype (Gagliardi et al., 2024). Although additional research is warranted, these findings hold promise toward MDVs being used as diagnostic frontrunners for early cancer detection, disease monitoring, and, possibly, treatment delivery.

5. Interplay of mitochondria-derived vesicles with the hallmarks of aging: the key role of mtDNA

Mitophagy dysfunction impinges on several hallmarks of aging and, as such, may be considered a *trait d'union* between defective mitochondria and the biology of aging. A main feature of inefficient mitophagy is the excessive accumulation of mitochondrial DAMPs in the cytoplasm, including mtDNA, also in an oxidized form, and oxidated cardiolipin. The release of mitochondrial DAMPs increases with aging and contributes to chronic low-grade inflammation. Mitochondrial DAMPs can also be shuttled within MDVs which can act as DAMPs themselves. The signaling role of MDVs cargoes as mitochondrial DAMPs is likely a stress response mechanism that, by unloading unprotected mtDNA, signals the presence of "non-self" elements.

Owing to the lack of histones and the presence of hypomethylated CpG motifs, cell-free mtDNA can bind to a set of receptors of innate immunity and trigger inflammation, probably because of its resemblance to bacterial DNA (Kepp et al., 2011). The inflammatory response requires recruitment of toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-containing protein (NOD)-like receptors (NLRs) (Kepp et al., 2011) and the binding to extracellular pattern recognition receptors (PRRs). Upon these receptor interactions, multiple inflammatory signals are activated such as the NLR family, pyrin domain-containing 3 (NLRP3) inflammasome, the DNA-sensing cyclic GMP–AMP synthase (cGAS)–stimulator of interferon response cGAMP interactor 1 (STING) pathway, and the TLR systems. NLRP3 inflammasome activation also requires nuclear factor kappa B (NF-κB) signaling that triggers the transcription and post-translational stabilization of inactive NLRP3 and the precursors cytokines pro-interleukin (IL)-1β and pro-IL-18. NLRP3 activation culminates into the activation of caspase-1 and subsequent cleavage and release of proinflammatory IL-1 and IL-18 from their precursors (Kepp et al., 2011). Active caspase-1 also cleaves gasdermin D which forms pores in the cell membrane and leads to the extracellular release of proinflammatory IL-1 and IL-18, K^+ efflux, that guide pyroptosis. Activation of TLRs and the cGAS–STING, instead, operates via type I interferon and cellular senescence (Kim et al., 2023) (Fig. 2).

Preclinical studies clearly indicate that enhanced mitophagy can mitigate mtDNA oxidation and release and can attenuate TLR9-triggered inflammation (Bueno et al., 2019). Furthermore, the activation of mitophagy in the microglia enhanced its phagocytic efficiency and mitigated neuroinflammation via inhibition of NLRP3 (Fang et al., 2019). Although in these settings there was no clear evidence of MDV signaling, we speculate that MDVs likely contribute since the release of mtDNA via MDVs and activation of innate immunity have been documented in adult mice with mutations in the mitochondrial enzyme fumarate hydratase (FH) (Zecchini et al., 2023). In these mice, unloading of mtDNA outside the mitochondria has also been associated with altered mitochondrial morphology (Zecchini et al., 2023). Once in the cytosol, mtDNA signals via the cGAS−STING pathway (Zecchini et al., 2023). The inflammatory response elicited is also partly ascribed to the mitochondrial retinoic acid-inducible gene I (RIG-I) signaling pathway (Zecchini et al., 2023).

The release of mtDNA has been reported to amplify tissue and organ injury via inflammation (Simmons et al., 2013; Zhang et al., 2010). Among other signaling roles, the release of mitochondrial DAMPs has been shown to promote neutrophil migration and degranulation at the site of injury and induce cellular damage and local inflammation (Zhang et al., 2010). EV-containing mitochondria produced by M1 pancreatic resident macrophages hold proinflammatory properties and can

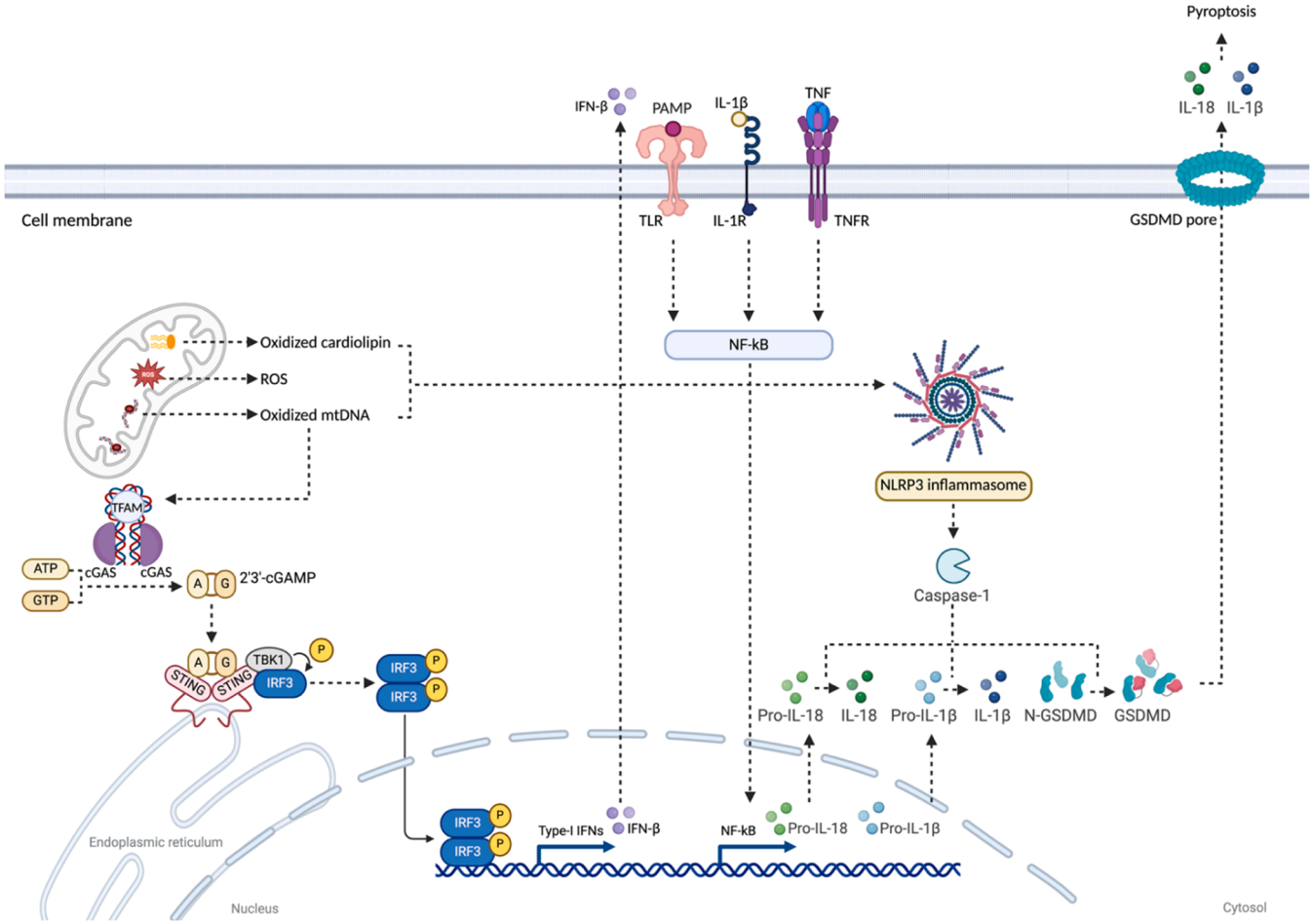

Fig. 2. Schematic representation of the main inflammatory pathways elicited by mitochondrial DNA displacement into the cytosol. Abbreviations: A, adenine; ASC, apoptosis-associated speck-like protein; ATP, adenosine triphosphate; cGAMP, 2′3′-cyclic GMP−AMP; cGAS, GMP−AMP synthase; G, guanine; GSDMD, gasdermin D; GTP, guanine triphosphate; IFN, interferon; IL, interleukin; IL-1R, interleukin-1 receptor; IRF3, interferon regulatory factor 3; mtDNA, mitochondrial DNA; NF-kB, nuclear factor kappa B; NLRP3, NLR family pyrin domain containing 3; P, phosphate; PAMP, pathogen-associated molecular patterns; ROS, reactive oxygen species; STING, stimulator of interferon genes; TBK1, TANK-binding kinase 1; TFAM, mitochondrial transcription factor A; TLR, toll like receptor; TNF, tumor necrosis factor alpha; TNFR, tumor necrosis factor alpha receptor. Created with Biorender.com (accessed on 1 July 2024).

penetrate pancreatic β cells (Gao et al., 2024). Fusion of macrophage-derived inflammatory mitochondria with pancreatic β cells induced lipid peroxidation and mitochondrial derangements (Gao et al., 2024). Furthermore, fragments of mtDNA released into the cytosol, induced ferroptosis via the STING pathway (Gao et al., 2024). Inflammation in the absence of overt infection and triggered by mitochondrial DAMPs has also been documented in individuals transfused with platelets in whom platelet-derived EVs seem to act has a reservoir of proinflammatory mtDNA (Marcoux et al., 2019).

Proinflammatory signals elicited by MDVs other than those mediated by mtDNA unloading have also been described. Mitochondria have been found to be released within microvesicles by lipopolysaccharide-treated monocytes to activate endothelial cells and trigger inflammation (Puhm et al., 2019). However, the immunomodulatory effect of MDV release can also desensitize immune cells and quench inflammation. To manage oxidative stress, mesenchymal stem cells (MSCs) extrude depolarized mitochondria via EVs that are taken up by macrophages for elimination. EV release by MSCs is accompanied by shedding of exosomes carrying microRNAs that blunt macrophage activation by suppressing TLR signaling, thus resulting in a desensitization of macrophages to engulfed mitochondria (Phinney et al., 2015). This coordinated mechanism may be a strategy to outsource mitophagy while circumventing the inhibition of mitochondrial transfer in the setting of sterile inflammation triggered by release of mitochondrial DAMPs.

6. Perspectives on mitochondria-derived vesicle research and impact on human health and aging

We have summarized the scientific evidence that the elimination of damaged mitochondria through MDVs is a physiological mechanism that works in parallel or in conjunction with traditionally described mechanism of Parkin-dependent and Parkin-independent mitophagy. We have also pointed to evidence from preclinical and clinical studies suggesting that the production of MDVs may have compensatory functions in many chronic diseases in humans. Research in this field was prompted by the retrieval of whole mitochondria and/or mitochondrial fragments in EVs (Al Amir Dache et al., 2020; Hazan Ben-Menachem et al., 2023; Mondal and Towers, 2022; Sugiura et al., 2014). The interpretation of these initial findings as methods to ensure MQC comes naturally and has been confirmed by several studies *in vitro* or in animal models (Hazan Ben-Menachem et al., 2023; Mondal and Towers, 2022; Sugiura et al., 2014). More recently, studies demonstrated that circulating cell-free mitochondria and even mitochondrial "fragments" may produce ATP through oxidative phosphorylation in the presence of oxygen and nutrients (Al Amir Dache et al., 2020). These findings raise the question of why cells decide to eliminate through MDVs mitochondria that are still functional (Hazan Ben-Menachem et al., 2023). Indeed, a small number of studies have shown that some cells, especially in the central nervous system, "lend" mitochondria to other cells that are

energetically stressed (Hayakawa et al., 2016; Peruzzotti-Jametti et al., 2021). Whether this mechanism of extending an energetic aid to other damaged or metabolically stressed cells is generalizable across tissues remains unknown. However, therapeutic potential of enhancing them is worth exploration. The availability of energy in the form of ATP is essential to life and becomes even more critical with aging when the accumulation of macromolecular damage through entropy and environmental stress is less prevented and counteracted by resilience mechanisms (Ferrucci et al., 2020). Hence, it should not be surprising that a complex and redundant quality control system has been evolutionary selected to make sure that the energetic machinery remains efficient for as long as possible (Picca et al., 2023a). At least some elements of such redundant machinery have been described and it is likely others will emerge as studies in this field are multiplying. However, the signaling pathways that drive the choice toward one mitophagy mechanism rather than others almost completely escape our understanding. Although there is evidence that inhibiting traditional autophagy may trigger increased production of MDVs, data are conflicting and hampered by methodological heterogeneity. In a young healthy cell, there is plenty of energy to feed all cellular functions that preserve life and allow stress response resilience to ensure maintenance of a healthy allostasis. However, as the macromolecular damage accumulates and the energic needs of resilience strategies expand, cells are forced to make tough choices. For example, impaired oxidative phosphorylation leads to increase production of ROS and decrease of IMM electrochemical gradient essential to produce ATP. The decision to remove a dysfunctional mitochondrion through autophagy and generate a new mitochondrion by biogenesis is a significant investment by a cell that is already challenged by depauperated energy. The biological subroutine used by the cell to make such decision is not known, and similarly very little is known on how decisions are made concerning alternative or parallel forms of mitochondrial disposal. A better understanding of these points is a priority for future research with a strong translational potential.

Ethic approval

Not applicable.

Authors' contributions

All authors contributed to conceptualize the study and writing and editing the manuscript. All authors approved the final version of the manuscript.

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Declaration of Competing Interest

The authors have no conflict of interest to disclose.

Data Availability

No data were used for the research described in the article.

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