Membrane contact sites emerged in the last decade as key players in the integration, regulation and transmission of many signals within cells, with critical impact in multiple pathophysiological contexts. Numerous studies accordingly point to a role for mitochondria-endoplasmic reticulum contacts (MERCs) in modulating aging. Nonetheless, the driving cellular mechanisms behind this role remain unclear. Recent evidence unravelled that MERCs regulate cellular senescence, a state of permanent proliferation arrest associated with a pro-inflammatory secretome, which could mediate MERC impact on aging. Here we discuss this idea in light of recent advances supporting an interplay between MERCs, cellular senescence and aging.

From the second half of twentieth century, the growing use of subcellular imaging through electron microscopy has allowed the identification of local physical contacts between multiple organelles and/or plasma membrane (PM), called membrane contact sites (MCSs), to the point that the field of “contactology” has emerged in the last decade\(^1\)–\(^4\). Indeed, the first structural observations were extended to point out the multiple functional roles of these MCSs in regulating cellular responses in various pathophysiological conditions\(^4\). Firstly, MCSs constitute physical bridges to exchange metabolites (e.g. ions or lipids) between organelles, thus regulating intracellular metabolic fluxes. Secondly and as dynamic molecular microdomains, MSCs create subcellular signalling platforms, allowing local protein modifications and interactions\(^4\).

Endoplasmic reticulum (ER) and mitochondria constitute two key organelles involved in macromolecules synthesis and bioenergetics, but also in stress sensing and integration of cell fate signalling. As they form two independent networks occupying up to 45% of cell volume in eukaryotic cells\(^5,6\). ER and mitochondria are involved in multiple MCSs, including contacts between ER and PM, Golgi or peroxysomes, and between mitochondria and PM, lysosomes or lipid droplets\(^4\). ER and mitochondria are also able to interact with each other through mitochondria–ER contacts (MERCs)\(^7\).

MERCs define tight contacts with a distance shorter than 50 nm specifically between ER and outer mitochondrial membranes\(^7\). MERC structures are dynamic structures known to be present in every eukaryotic cells, along approximately 10–15% of all mitochondrial membranes\(^2\). Highly heterogeneous among tissues and species, MERCs contain up to hundreds of proteins including tethering/structuring proteins, ion channels, transport binding proteins, enzymes and other signalling proteins\(^2\) (Fig. 1). Firstly described in the context of lipid biosynthesis/transfer, MERCs role has been largely extended to other metabolites fluxes, such as calcium\(^8–10\). MERCs are involved in lipid metabolism, calcium and redox signalling but also modulate mitochondrial dynamics\(^11\), autophagy\(^12\), inflammation\(^13\) and apoptosis\(^9\) (Fig. 1).

The use of genetic mouse models targeting MERC components has highlighted MERCs importance in controlling various pathophysiological situations, ranging from vascular remodelling to inflammation and metabolic disorders\(^13–17\). More recently, a role of MERCs in aging was suggested by evidences showing a modulation of their quantity and quality with...
specific age-related diseases or aging\textsuperscript{18–20}. While autophagy and apoptosis are proposed to mediate some of MERCs age-associated effects\textsuperscript{18,19}, whether other critical mechanisms are also involved remains unclear.

Senescent cells accumulate during aging in various animal models\textsuperscript{21,22}. Cellular senescence can be induced by a myriad of stresses and defines a state of permanent cell proliferation arrest and the concomitant acquisition of a senescence-associated secretory phenotype (SASP), including pro-inflammatory factors, pro-fibrotic factors and metalloproteases\textsuperscript{23} (Fig. 2a). Mechanistically, proliferation arrest is mediated by the activation of cyclin-dependent kinase inhibitors, mainly p21 and p16 (Fig. 2a). Besides, the SASP is mostly driven by NF-κB and C/EBPβ, and can be also positively regulated either by Notch, mTOR or NLRP3 pathways\textsuperscript{24–27} (Fig. 2a). Although these factors regulate cell cycle or SASP, the upstream molecular and subcellular mechanisms regulating them are largely unknown. Notably, signalling platforms mediating the link between stresses sensing and molecular activation of senescence effectors are largely unknown (Fig. 2a).

Cellular senescence has been early linked to aging, but the functional demonstration of its involvement in it was recent. The accumulation of senescent cells during aging is the concomitant result of both increased intracellular damage and declined senescence immune surveillance\textsuperscript{28–30} (Fig. 2b). While cell autonomous effects lead to stem cell exhaustion\textsuperscript{31,32} and aberrant cellular function\textsuperscript{33}, non-cell autonomous effects through SASP can mediate paracrine senescence and modify the surrounding
Microenvironment. Subsequently, the chronic and systemic accumulation of senescent cells favours tissue dyshomeostasis, fibrosis, inflammation or immunosenescence, ultimately leading to aging and age-associated pathologies, including chronic metabolic disorders, atherosclerosis, age-related muscular defects and neurodegenerative diseases (Fig. 2b)34–36. Finding strategies to target and specifically eliminate senescent cells or attenuate their pro-inflammatory SASP, namely with senolytics or senomorphics, has thus become a major challenge in aging research field.

In this perspective, we will present and discuss recent advances suggesting a potential interplay of MERCs and cellular senescence in regulating aging. Based on the fact that MERCs and cellular senescence are both involved in aging and age-related pathologies, we compiled evidence that either modulation of MERC components or increased MERCs formation are pro-senescent signals. Lastly, we propose future directions to elucidate molecular mechanisms behind this emerging role of MERCs in regulating cellular senescence.

**MERCs and cellular senescence both control age-associated diseases and aging**

While senescent cells accumulate during aging and participate in multiple age-related pathologies31,37,38, numerous clues indicate that MERCs could also play a role in age-associated diseases.

**Chronic metabolic disorders.** Chronic metabolic disorders such as obesity and type 2 diabetes (T2D) account for two main contemporary diseases linked to overnutrition and aging39. High-fat diet (HFD) and obese pathological contexts promote senescence in several cell types and tissues including adipose tissue, liver, pancreas or brain promoting insulin-resistance and T2D40–43. Of note, a chronic increase of MERCs was observed in HFD-fed and ob/ob mice, two models exhibiting altered glucose homeostasis, insulin resistance and steatosis44. More strikingly, the use of an artificial MERCs linker45 is able to induce this insulin resistance.44 Conversely, reduced MERCs in mice depleted for inositol-triphosphate receptor 2 (ITPR2) are able to improve glucose homeostasis, alleviating age-dependent steatosis and fibrosis46. Accordingly, decreased MERCs through Pdk4 deletion ameliorates glucose homeostasis and insulin response47. Of note, decreased number of hepatic MERCs correlates with reduced mRNA and protein levels of p16 senescence marker46, known to be upregulated during aging37,46. Remarkably and as apparent contradictory result, decreased MERCs using genetic and pharmacological approaches targeting Cyclophilin D (CypD) also perturb insulin response in liver and muscle48,49. Taken together, these data demonstrate that an accurate balanced MERC number is necessary to maintain glucose homeostasis15.

Non-alcoholic fatty liver disease (NAFLD) is characterized by hepatic fat deposits, evolving from steatosis to fibrosis, cirrhosis and/or hepatocellular carcinoma50. As T2D, obesity and aging are three main factors of NAFLD, a detrimental role of cellular senescence has been proposed51. Nonetheless, recent functional studies highlighted the detrimental effects of some senolytics, namely Dasatinib and Quercetin, in the context of fully recapitulated NAFLD52, suggesting a complex role of cellular senescence in NAFLD. Furthermore, the elimination of p16High senescent cells induces liver fibrosis if these senescent cells are not replaced53. In addition, senescence of hepatic stellate cells can inhibit their pro-fibrotic roles28, together showing different roles of senescent cells in liver diseases probably depending on the type of senescent cells and on their dynamic54. The importance of MERCs in NAFLD remains unclear16. For instance, Mfnp2 knockout was suggested to modulate MERCs quantity, though its precise role is still under debate55, and induces non-alcoholic steatohepatitis, one of the advanced stages of NAFLD, in mouse model. The importance of MERCs in the progression of NAFLD still needs to be further tested using specific MERC tools.

Altogether, all these data strongly indicate that MERCs disruption and modulation of cellular senescence are at the crossroads of age and obesity-related alterations of glucose metabolism and chronic liver dysfunction (Table 1).

**Atherosclerosis.** Atherosclerosis is also promoted by overnutrition and aging, constituting the major cause of complications of cardiovascular diseases, including stroke and ischaemic heart failure56. Chronic atheromatous plaques are formed by endothelial dysfunction, the accumulation of oxidized low-density lipoprotein (oxLDL) in blood vessels and the subsequent
aggregation of macrophages foamy cells and vascular smooth muscle cells (VSMCs). Endothelial cells, foamy cells and VSMCs in atheromatous plaques display senescence features. The secretion of these senescent cells inhibits promigratory phenotype switching of medial VSMCs and their lesion entry for fibrous cap assembly. Senolitics treatment rescues this promigratory phenotype-limiting atherosclerosis. Interestingly, recent evidences pointed out that oxLDL treatment of VSMCs or endothelial cells impairs MERCs, notably thanks to PACS-2 and mitochondrial calcium accumulation through increased MERCs is proposed to accentuate apoptosis of endothelial cells. Nonetheless, sub-lethal elevation of mitochondrial calcium can also promote cellular senescence. Taken together, and as correlations, these data suggest that early steps of atheromatous plaques formation involve MERCs modulation and cellular senescence

**Age-related muscular defects.** Muscular aging is characterized by reduced muscle fibre size and number, associated with loss of motoneurons, and terminally results in muscle dystrophy. Muscular aging is driven at least partly by activation of senescence pathways in muscle stem cells through autophagy defect and mitochondrial dysfunction. More importantly, the removal of senescent cells delays this age-associated muscular loss-of-function. Noteworthy, aged muscle was reported to display a reduction of ER–mitochondria calcium fluxes coupling to higher mitochondrial oxidative stress. Functionally, potential MERCs modulation through Mfn2 knockout has detrimental effects in muscle and leads to age-related sarcopenia. Furthermore, CisD2 knockout mice also display premature muscle degeneration. As age-related muscular defects may also come from neuromuscular synaptic loss, it is interesting to note that neuronal Mfn2 is also reduced during aging and its specific deletion is sufficient to trigger skeletal muscle atrophy. Overall, MERCs integrity or MERC components appear to participate in proper neuromuscular function, as MERCs uncoupling drives premature muscular aging (Table 1).

**Neurodegenerative diseases.** Aging is considered as the main risk factor driving neurodegenerative diseases, which include among others Alzheimer disease (AD), Parkinson disease (PD), primary progressive multiple sclerosis (PPMS). While both neuronal and glial senescent cells accumulate during aging and lead to neural stem cells exhaustion, their elimination significantly ameliorates symptoms of AD, PD and PPMS. MERCs were also studied

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### Table 1 MERCs roles in senescence-associated age-related pathologies.

| Roles                          | Cellular senescence | Mitochondria-ER contacts |
|-------------------------------|---------------------|--------------------------|
| Methods                       | Functional studies  | Functional and descriptive studies |
| Age-related diseases          | Beneficial         | Beneficial               |
| Mice                          | → Functional        | → Functional |
| (p16 TG)                      | (SnCs clearance)    | (MERCs linker)           |
| Chronic metabolic disorders   | Detrimental         | Detrimental              |
| Mice                          | → Functional        | → Functional |
| (SnCs clearance)              | (p16 silencing)     | (Pacs-2 silencing)       |
| Atherosclerosis               |                     |                         |
| Mice                          | → Functional        | → Functional |
| (SnCs clearance)              | (CypD → /−)         | (Pacs-2 → /−)           |
| Mice                          | → Functional        | → Functional |
| (SnCs clearance)              | (Mfn2 → /−)         | (Itpr2 → /−)            |
| Neuro-degenerative diseases   |                     |                         |
| AD                            | Mice                | Mice                    |
| → Functional                  | → Functional        | → Functional |
| (SnCs clearance)              | (SnCs clearance)    | (SnCs clearance)        |
| PD                            |                     |                         |
| → Functional                  | → Functional        | → Functional |
| (SnCs clearance)              | (SnCs clearance)    | (SnCs clearance)        |
| FA                            | In vitro → Functional | In vitro → Functional   |
|                               | (Frataxis silencing) | (FxnFrataxis silencing) |
| Lifespan                      |                     |                         |
| Mice                          | → Functional        | → Functional |
| (SnCs clearance)              | (SnCs clearance)    | (SnCs clearance)        |

Summary of the studies reporting a (beneficial or detrimental) role for MERCs and cellular senescence in age-related diseases and lifespan. For each study, the model is indicated in bold and experimental approaches, either descriptive (displaying correlations) or functional (modifying MERCs structure), are indicated underneath. N/D not determined. AD Alzheimer disease, PD Parkinson disease, FA Friedreich's ataxia, SnCs senescent cells, TG transgenic.
in the context of neurodegenerative disorders. In AD patients, MERCs are enhanced and three enzymes involved in amyloid β generation (presenilin-1, presenilin-2 and γ-secretase) colocalize at MERCs. Moreover, increased amyloid β induces expression of multiple MERC components including ITPR3 and voltage-dependent channel 1 (VDAC1), and presenilin-2 mutants display increased MERCs. Importantly, pde4d8 deletion decreases MERCs and rescues AD-associated locomotor decline in fly. In PD, MERCs structure is altered and most of PD-associated proteins, such as a-synuclein, Parkin or PINK1, are found in MERCs fraction. Furthermore, a-synuclein enhances MERCs and mitochondrial calcium uptake. Taken together, these later studies suggest a detrimental role of MERCs in AD and PD. Noteworthy, multiple studies point out also decreased MERCs in PD- and AD-associated conditions, underlying also a beneficial role of these MERCs. For example, recent live FRET imaging of rat neurons displays decreased average length of tight MERCs in an AD model. The use of an artificial linker in vivo also rescues locomotor defects in AD model in fly. In a PD context, a-synuclein displays opposite role in MERCs formation as it can also decrease VAPB-PTPIP51 interaction. Moreover, loss of parkin-induced ubiquitination of MFN2 is responsible for an inefficient coupling of MERCs and restoration of MERCs rescues motricity defects in a PD model in fly. While no consensus, all these results support that MERCs dyshomeostasis could contribute to AD and PD. Finally, reduced MERCs are found in a model of Friedreich’s ataxia (FA), accompanied by decreased mitochondrial calcium and cellular senescence, according to the importance of physiological basal mitochondrial calcium in sustaining mitochondrial bioenergetics. Collectively, these data indicate that MERCs dysregulation and cellular senescence could be linked to neurodegenerative disorders. Nonetheless, while cellular senescence displays detrimental roles, MERCs roles are still under debate because of a low number of functional studies.

Systemic aging and lifespan. Senescent cells accumulate during aging in spite of multifactorial heterogeneity in the speed and level of their accumulation. Removal of these senescent cells or reduction of their SASP result in a decrease of some age-related alterations as described above. Most importantly, this can also result in delayed aging and improve in both lifespan and healthspan (Table 1). Altogether, cellular senescence thus appears to be a key cellular phenotype driving tissue dysfunction and aging (Fig. 2b). Recent reviews have depicted how MERCs dyshomeostasis may be involved in some age-related pathologies and aging. Genetic mouse models studied in the context of MERCs dyshomeostasis, CypD knockout and Mfn2 knockout have been largely studied, while their specific role in MERCs is still under debate. Interestingly, lifespan of CypD haploinsufficient but not CypD knockout mice is increased compared to their control WT littermates. Lifespan of Mfn2 knockout mice, although displaying premature muscular aging, was not monitored. Noteworthy, C. elegans lifespan is extended by knock-in of Hsp9a, encoding the GRP75 (Glucose-Related Protein 75) scaffold protein binding to ER and mitochondria through ITPR and VDAC channel. Though not clearly demonstrated, GRP75 may enhance MERCs in this model, and its muscle constitutive expression could counteract deleterious effects caused by MERCs disruption in muscle, as previously discussed. More recently, the contribution of ER-mitochondrial calcium flux to aging in mice and worms was assessed. On one side, deletion of the murine ER-calcium release channel ITPR2 reduces MERCs and age-related alterations in males and females and it extends lifespan only in females. On the other side, atf6 loss-of-function in worms results in sustained ITPR-mediated ER-mitochondria fluxes enhancing lifespan.

MERCs are heterogeneous in terms of number and activity. MERCs composition is also highly variable according to recent proteomic data in different cell types, challenging its specific study. Nonetheless, MERCs are regulated by abundance of scaffold proteins, defining MERCs quantity, and by other interface proteins, interacting with each other and including calcium channels, receptors and kinases, underlying MERCs activity and function. Noteworthy, quantity and activity/function of MERCs are interrelated as some enzymes and calcium channels, for example Pyruvate Dehydrogenase Kinase 4 (PDK4) and ITPRs, are able to regulate MERCs integrity. Finally, numerous MERC components are regulated by protein interactions such as ITPR3-TOM70, VDAC2-CDKAP4, ITPR2-FUNDC1, but also by post-translational modifications such as DRP1 SUMOylation by MAPL/MUL1, which further adds a layer of complexity in the organisation and function of MERCs.

Role of MERCs scaffold/tethering proteins in cellular senescence. MERCs establishment is regulated by multiple scaffold and tethering factors that include among others Mfn2, GRP75-ITPRs-VDACs, FIS1-BAP31, VAPB-PTPIP51, PDZD8, CYPD

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and SIGMAR112,111 (Fig. 1). MFN1 and MFN2 have been linked functionally to senescence112. MFN1 is a target of ubiquitin ligase MARCH5 and accumulates in MARCH5-deficient cells, which display hyperfused mitochondria and senescence features113. On the contrary, downregulation of Mfn1 extends replicative lifespan114. Concerning Mfn2, its depletion boosts cellular proliferation of both B cell lymphoma cell line and mouse embryonic fibroblasts (MEFs)113; Mfn2 mediates hyperfused mitochondria and promotes senescence of mesenchymal stem cells116. Concerning haematopoietic stem cells, opposite results highlight that Mfn2 deletion induces defect in long-term lymphoid repopulation117, though no senescence markers were investigated. Overall, these data indicate that alterations of Mfn1 and Mfn2, key regulators of MERCs number, are able to regulate senescence-associated phenotypes. However, MFN1 and MFN2 are, independently of MERCs, also involved in mitochondrial fusion, and whether their effects on senescence depend on their function inside the MERCs is still unknown.

Concerning ITPR–GRP75–VDAC complex, GRP75 overexpression leads to increased replicative lifespan through down-regulation of RAS and reduced phosphorylation of ERK118,119 and ITPRs promote cellular senescence46,61,62, as it will be further discussed below.

BAP31 is an ER-resident chaperone transmembrane protein, found in MERCs fraction, forming a bridge with FIS1120 and known mainly to regulate apoptosis121. Although the functional implication of BAP31 has never been investigated in the context of cellular senescence, its deletion reduces cell proliferation of colon cancer cells122 and it is found to be upregulated in both replicative and X-ray induced senescence of fibroblasts123.

Overall, alterations of key MERC tethers are able to drive features of cellular senescence. Aside from these reports, further studies on additional tethers such as VAPB, PTPP51 or PDZD8, or proteins participating in MERCs structure such as SIGMAR1 and CYPD, need to be conducted to determine whether they could also regulate cellular senescence.

Role of MERC components involved in ER–mitochondria exchanges in cellular senescence. MERCs constitute a privileged site for exchanges of metabolites, including calcium and lipids, which have been recently involved in the regulation of cellular senescence.

Calcium exchanges at MERCs interface are regulated by multiple calcium channels, namely ITPRs (ITPR1, 2 and 3) in ER, VDACs (1, 2 and 3) at the OMM and MCU at the IMM for calcium124,125. The hypothesis of a role for MERCs in calcium-mediated activation of mTOR and NLRP3, and subsequent SASP promotion, still needs to be tested. In addition to mTOR and NLRP3, two classical MERC resident proteins, namely PTTPI51 and PACS-2, are involved in the regulation of the NF-κB pathway which drives the pro-inflammatory SASP. While the MERCs tether PTPP51 interacts with RelA and IKBα126, the induction of NF-κB programme upon irradiation in Pacc-2 –/− thymocytes is abolished, through blunted phosphorylation of IκBα, IκBβ and Relα127. Taken together, these data suggest that the mTOR, NLRP3 and NF-κB pathway could regulate SASP through MERC-dependent activation, even if more precise investigations should be conducted in the future to decipher this potential role.

p66Shc was largely studied in the context of senescence and also in relation with aging. Decreased p66Shc delays replicative senescence of human diploid fibroblasts132, and its increase promotes hepatocyte senescence and subsequent senescence-driven steatosis133. In the context of aging, p66Shc is phosphorylated at Serine 36 in an age-dependent manner in old animals, and strongly correlates with enhanced ROS production134. Though p66Shc intracellular localization is still under debate, frequently found in mitochondria, MERCs or PM-associated membranes, it has been suggested that p66Shc accumulates in MERCs with age before re-localizing to the mitochondria134. Exogenous oxidative stress is able to relocate p66Shc to the nucleus, where the oxidative stress response is integrated to establish a senescence response135–137. Collectively, these studies highlight the role of p66Shc as a sensor and regulator of cellular senescence and lifespan135–137.

Promyelocytic Leukemia protein (PML), an essential component of the PML nuclear bodies (PML-NBs), critically regulates cellular senescence138. Not only present in PML-NBs, PML is also located in cytoplasm, in ER and in MERCs139. PML limits phosphorylation of ITPR3, leading to dampened ER mitochondria calcium flux and subsequent failed apoptosis139. A possible role of PML localization at MERCs in its pro-senescence function could be considered as well.
Two other MERCs proteins, CisD2 and PACS-2, were found to also regulate cell proliferation arrest, one of the key features of cellular senescence. CisD2, an ER protein located at the MERCs interface through Bcl2–IP3R interactions, is necessary for proliferation of induced-pluripotent stem cells. The knockdown of PACS-2, mentioned above for its ability to regulate NF-kB activation, also delays cell proliferation arrest induced by DNA damage–p53–p21 axis in thymocytes. Additional cell types and complementary senescence markers should be monitored to better evaluate the contribution of CisD2 and PACS-2 in the modulation of the senescence fate.

Altogether these data indicate that several MERCs components, including tethers, calcium channels and others, regulate key features of cellular senescence (Table 2), although it still has to be proven that this action is MERC-dependent. Of note, it seems particularly interesting that some key SASP regulators are found associated and activated at MERCs interface. Overall, these data strongly suggest that MERCs regulate the senescence fate, which is an idea that has been recently evaluated.

MERCs regulate cellular senescence

A pro-senescent role for MERCs: potential mechanistic. Increased evidence of the role of MERCs proteins during aging and in cellular senescence raised the question of the importance of MERCs in regulating cellular senescence. Expression of an artificial linker tightening ER and mitochondrial membranes led to premature cellular senescence in normal human fibroblasts, with a NF-kB-dependent SASP. This cellular senescence was accompanied by increased mitochondrial calcium and functionally involved ROS production, as antioxidant treatment rescued the onset of this premature senescence. Downstream, p53 was necessary to induce senescence. The importance of MERCs in established senescence models (replicative senescence, Ois, oxidative stress-induced senescence) should be critically tested in further investigations.

Noteworthy, deletion of MERC-associated proteins (such as MFN2, Frataxin or ORP5) and potential MERCs uncoupling were also proposed to mediate a senescence phenotype. Whether these effects are dependent of MERCs is unknown. To properly assess whether MERCs decrease may also impact senescence, it will be necessary to evaluate the impact of a specific MERCs spacer, such as FATE1, in senescence models. While forcing MERCs induces a ROS- and p53-dependent senescence through an increased mitochondrial calcium uptake, an opposite role of MERCs uncoupling is nonetheless still unknown (Fig. 3).

How MERCs dyshomeostasis mechanistically drives cellular senescence remains an open question, but involves necessarily at least one of the three MERCs sub-compartments, i.e., mitochondrion, ER and apposed cytosol.

Mitochondrion side—An important number of studies report that senescent cells display mitochondrial abnormalities and various mitochondrial dysfunctions promote cellular senescence. These mitochondrial dysfunctions are largely driven by non-exclusive mechanisms which include among others defective ETC, excessive ROS production, abnormal dynamics and altered mitophagy. Nonetheless, the upstream mechanisms driving these mitochondrial dysfunctions during cellular senescence remain so far elusive.

In line with increased evidence of the role of calcium fluxes within the cell and specifically between ER and mitochondria, calcium signalling appears to be an interesting candidate to mediate the senescence phenotype. In human endothelial cells and MEFs, replicative senescence is characterized by an increased MERCs number and a subsequent accumulation of mitochondrial calcium. Accordingly, replicative senescent neurons display also an increased transfer of calcium from the ER to mitochondria accompanied by an upregulation of MCU expression. Reduction of this mitochondrial calcium accumulation by knocking down ITPR2 or MCU inhibits the establishment of cellular senescence in several cell models, including hMEC and fibroblasts. When excessively accumulated, mitochondrial calcium leads to mitochondrial membrane depolarization, ROS generation and subsequent cellular senescence or apoptosis. Of note, accumulation of mitochondrial calcium through the suppression of mitochondria calcium efflux by knocking down the mitochondrial Na⁺/Ca²⁺ exchanger triggers superoxide generation and neuronal apoptosis, driving AD-associated pathology.

Mitochondrial calcium regulates mitochondrial bioenergetics mainly through its transfer through MERCs. Indeed, mitochondrial dehydrogenases present EF-hand calcium binding necessary for enzymatic activity. As mitochondrial calcium homeostasis is necessary in order to maintain mitochondrial bioenergetics, massive MERCs uncoupling may also trigger mitochondrial dysfunction. For instance, reduced MERCs through CypD knockout leads to dysregulation of TCA cycle and fatty acid β-oxidation, while no senescence markers were monitored in these studies. Interestingly, this dual role of the importance of a balanced ER-mitochondrial calcium influx has been recapitulated in the context of neural stem cell development in fly. Indeed, deletion of Miro, an OMM GTPase, reduces mitochondrial calcium and leads to mitochondrial metabolic impairment, whereas its constitutive expression triggers mitochondrial calcium overload and apoptosis. Both conditions impaired neural stem cells lineage progression, though no senescence markers were investigated.

To summarize the role of MERC-mediated mitochondrial calcium influx in cellular senescence, it appears that an altered calcium transfer from ER to mitochondria triggers mitochondrial dysfunction, eventually leading to cellular senescence. At this stage, most of the data support the hypothesis of an enhanced calcium influx into the mitochondria during cellular senescence, which could be mediated notably by increased MERCs, without excluding the hypothesis that reduced MERCs could also elicit pro-senescent signals. From calcium, whether lipid fluxes at MERCs interface are involved in mitochondrial alterations promoting cellular senescence remains an open question.

Beyond metabolites transfers, MERCs also promote early steps of mitochondrial fission through ER wrapping of mitochondria at future fission sites. Reducing mitochondrial fission is able to induce senescence in normal cells. Though no evidences were clearly established, MERC uncoupling could lead to accumulation of hyperfused damaged mitochondria by fission and subsequent mitophagy defects, eventually mediating a senescence phenotype. Nonetheless, whether the sole MERC uncoupling induces senescence features should be critically addressed (Fig. 3).

ER side—ER stress is a potent candidate to also mediate senescence phenotype. This role is suggested by the fact that ER stress is observed in several models of senescence in vitro, such as Ois and UV- or X-ray-induced senescence, but also in several senescence-associated pathological contexts, including therapy-induced senescence (TIS) in lymphomas, diabetic nephropathy, age-related sarcopenia and osteoarthritis. ER stress may result from a persistent unfolded protein response (UPR) or from a luminal calcium depletion and includes three main sensors: PERK, ATF6 and IRE1. Interestingly, MFN2 depletion in MEFs heightens the activity of the three ER stress branches. Furthermore, PERK interacts...
Table 2 Regulation of senescence-associated features by MERC-associated proteins.

| Function                  | Name     | Effect of experiment on protein levels/activity (+/−) | Effect on MERCs number | Effects on ER and/or MT | Effects on senescence-associated phenotype |
|---------------------------|----------|------------------------------------------------------|------------------------|-------------------------|-------------------------------------------|
| **ER-MT TETHERING**      | MFN1     | + (Stabilization)                                    | N/D                    | Increased MT mass       | Induction of senescence                   |
|                           | MFN2     | − (KO)                                               | N/D                    | N/D                     | Delayed RS                               |
|                           | GRP75    | + (OE)                                               | N/D                    | N/D                     | Delayed RS                               |
|                           | FIS1     | − (shRNA)                                            | N/D                    | Hyperfused MT           | Induction of senescence                   |
|                           |          | + (OE)                                               | N/D                    | Rescue hyperfused MT    | Rescue of DFO-induced senescence          |
| **ER-MT METABOLITES**     | ITPR1    | − (shRNA)                                            | N/D                    | Limited ER-MT calcium fluxes/ROS/MMD | Limited OiS and delayed RS |
|                           | ITPR2    | − (shRNA)                                            | N/D                    | Limited ER-MT calcium fluxes/MT ROS/MMD | Reduction of senescence |
|                           | − (siRNA)|                                                     | N/D                    | Limited ER-MT calcium fluxes/MT ROS/MMD | Reduction of senescence |
|                           | − (KO)   |                                                     | Decreased              | Limited ER-MT calcium fluxes/MT ROS/MMD | Reduction of senescence |
|                           | ITPR3    | − (shRNA)                                            | N/D                    | N/D                     | Reduction of OiS                          |
|                           | MCU      | − (shRNA)                                            | N/D                    | Limited ER-MT calcium fluxes | Reduction of OiS                          |
|                           | ORP5     | − (siRNA)                                            | N/D                    | Alteration of MT morphology and reduced OXPHOS | Induction of senescence |
| **SIGNALLING PROTEINS**   | PACS-2   | − (KO)                                               | N/D                    | N/D                     | Resistance to p53-dependent CCA and NF-κB programme |
|                           | p66Shc   | − (KO)                                               | N/D                    | N/D                     | Delayed RS                               |
|                           | CISD2    | − (KO)                                               | N/D                    | Enhanced MMD            | Reduction of cell proliferation           |
|                           | RelA     | − (shRNA)                                            | N/D                    | N/D                     | Inhibition of SASP                        |
|                           | NLRP3    | − (KO)                                               | N/D                    | N/D                     | Reduction of age-dependent increase of p53 and p21 |
|                           | mTOR     | − (Rapa)                                             | N/D                    | N/D                     | Inhibition of NF-κB-dependent SASP         |
|                           | PML      | − (KO)                                               | N/D                    | N/D                     | Resistance to OiS                         |
|                           |          |                                                      | N/D                    | Limited ITPR3 phosphorylation | Reduced ER-MT calcium fluxes |

Summary of the studies reporting a potential role for MERC-associated proteins involved in tethering, ER-MT metabolites transfers and signalling in the regulation of features of cellular senescence. For each study, the effect of MERCs protein dysregulation (upregulation + or downregulation −) on MERCs number and also ER and mitochondria are indicated. If investigated, the effects on features of cellular senescence are reported. N/D not determined, KO knockout, OE overexpression, Rapa Rapamycin, ER endoplasmic reticulum, MT mitochondria, OXPHOS oxidative phosphorylation, MMD mitochondrial membrane depolarization, OiS oncogene-induced senescence, RS replicative senescence, CCA cell cycle arrest.
with MFN2 and is found in MERCs fraction to transduce apoptosis triggered by ROS-mediated ER stress\textsuperscript{170}. MFN2 is able to interact with PERK in normal conditions to restrain its activity. Remarkably, another ER-anchored MERCs tether, namely VAPB, interacts with ATF6, repressing transcription of its target genes\textsuperscript{171}. Finally, IRE1 regulates lipid composition at MERCs and thus subsequent calcium fluxes\textsuperscript{151}. Taken together, these data indicate cross-talks between MERC components and ER stress\textsuperscript{169,170}. Concerning calcium fluxes, MERCs may decrease ER calcium content. ER calcium is determinant for chaperones involved in protein folding, such as Calreticulin or BiP/GRP78, and variations of ER calcium content subsequently lead to UPR and ER stress\textsuperscript{172}. How ER stress is regulated upon modulation of MERCs and how it participates in cellular senescence should be addressed in the future.

Altogether, these data strongly suggest the importance of mitochondria in participating in the regulation of senescence phenotype, while ER contribution is still barely understood. Finally, some non-ER and non-mitochondria resident proteins modulating senescence features can be located in MERCs, as for example NLRP3, mTOR or PML. Therefore, MERCs could regulate cellular senescence by impacting signalling pathways in the cytosol through these proteins.

**MERCs: a dynamic platform for integrating pro-senescence signals?** MERCs are constantly modified and some pro-senescence signals were shown to affect MERCs protein levels. For example, persistent DNA damage response at telomeres during replicative senescence and X-ray-induced DNA damage upregulate the MERC tether BAP31 at mRNA levels\textsuperscript{123}. Noteworthy, expression of ITPR2, the most efficient channel for ER-to-mitochondria calcium transfer\textsuperscript{106}, is upregulated by other pro-senescence stresses, including oncogenic stress\textsuperscript{61}, oxidative stress\textsuperscript{173} or high-fat diet\textsuperscript{44}. Aside from transcriptional and translational regulations, some MERCs proteins were found relocated, as previously mentioned for p66Shc, or activated upon stresses inducing cellular senescence. Taken together, these data indicate that pro-senescence signals may modify MERCs composition and function. Proteomic analysis of MERCs during cellular senescence induced by different stresses will be needed to appreciate MERCs rearrangement in composition in response to pro-senescence signals.

MERCs quantity is also regulated upon senescence-inducing stresses. Increased MERCs were found during replicative senescence of endothelial cells and MEFs\textsuperscript{46,146}. MERCs quantity may be regulated by redox stress, as, for example, antioxidant treatment rescues MERCs deficiency in FA model\textsuperscript{85,86}. Importantly, DNA damage promotes the formation of MERCs to promote intrinsic cell death through enhanced mitochondrial calcium uptake\textsuperscript{174}. At sublethal doses, oxidative stress and DNA damage also mediate cellular senescence\textsuperscript{23}.

Altogether, it seems that MERCs are highly modulated in response to pro-senescence signals. Further studies need to be performed in order to evaluate how MERCs evolve during senescence in quantity, composition and function. Upon senescence-inducing stresses, MERCs could behave as platforms...
integrating these signals and modulating signalling to other subcellular compartments such as mitochondria, cytosol and nucleus where senescence features are regulated.

**Perspectives and conclusion**

In conclusion, the observation that MERCs and cellular senescence play a crucial role in aging and age-associated diseases led to the hypothesis that MERCs may regulate aging at least partly through cellular senescence, and indeed, experimental data support that MERCs are key platforms controlling cellular senescence. This idea was first supported by studies showing a role for MERC components in cellular senescence and was strengthened by the observation that forcing MERCs with an artificial linker induces senescence in vitro. MERCs trigger this phenotype through ROS production and p53 activation. However how MERC-mediated mitochondrial calcium accumulation impacts mitochondrial functions and ROS generation and whether other calcium-independent processes are involved remains unknown.

Artificial linkers are powerful tools to investigate MERC functions but face some limitations. Some improvements have been obtained by the generation of inducible linkers when compared to the constitutive artificial linker. However, these linkers do not reflect many aspects of MERC complexity. Indeed, they do not allow to control the distance between ER and mitochondria (<50 nm but heterogeneous) when compared to the constitutive artificial linker. These thickness is defined as the width of the cleft separating OMM from ER, and subdivides MERCs in tight (~10 nm) and loose (~25–40 nm) structures. For instance, while loose MERCs were reported to promote ER–mitochondrial calcium transfers, tight MERCs were shown to limit them. Beyond the distance between ER and mitochondria, the complexity of MERC composition and their plasticity critically orientate the effects of MERCs. There is then an urgent need to develop new tools to better manipulate MERCs to better understand the role of MERCs in controlling cellular senescence.

Whether MERC dysregulation is at the origin of cellular senescence which in turn results in pathological outcomes needs further investigations. For instance, the effects of linkers and spacers allowing to modulate MERCs need to be monitored in vivo, on cellular senescence and aging. Further studies will have to assess in these models and in in vivo models harbouring a knockout of MERC components (Mfn2−/−, GisD2−/−, CypD−/−, Pdk4−/−, Pac2−/−) if the impact of these linkers, spacers and knockout on aging-associated alterations depends on cellular senescence. Cross-study models with models less prone to senescence or treating them with chemical compounds to suppress features of senescent cells (senomorphic) or eliminate them (senolytics) would precise the importance of cellular senescence in the phenotypes induced by MERCs alterations.

We are then proposing that modulating MERCs could be a new avenue to modulate senescence, associated-pathological alterations and healthspan. Either the death of senescent cells could be promoted by increasing ER to mitochondria calcium flux or their accumulation could be reduced by lowering this flux. Chemical compounds targeting MERCs already exist and could be tested for these capacities.

In summary, we presented and discussed new insights into the potential role of MERCs in regulating cellular senescence which could participate in MERCs impact on aging. As it has been proposed for cellular senescence, we propose that the principle of antagonistic pleiotropy could be also applied to MERCs, which have a beneficial role for the cells when tightly regulated and which could be detrimental when dysregulated, leading to cellular senescence and associated consequences such as aging and age-related diseases. More broadly, this new field of research on the role of MERCs in cellular senescence and aging has highlighted the importance of communication between different compartments, sometimes mediated through contacts sites such as MERCs. This will pave the way to investigate the role of other MCs, such as PM-ER, PM-MT or Lyosome-MT, in the context of cellular senescence and aging.

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