Review article

Interrelation between ROS and Ca$^{2+}$ in aging and age-related diseases

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A B S T R A C T

Calcium (Ca$^{2+}$) and reactive oxygen species (ROS) are versatile signaling molecules coordinating physiological and pathophysiological processes. While channels and pumps shuttle Ca$^{2+}$ ions between extracellular space, cytosol and cellular compartments, short-lived and highly reactive ROS are constantly generated by various production sites within the cell. Ca$^{2+}$ controls membrane potential, modulates mitochondrial adenosine triphosphate (ATP) production and affects proteins like calcineurin (CaN) or calmodulin (CaM), which, in turn, have a wide area of action. Overwhelming Ca$^{2+}$ levels within mitochondria efficiently induce and trigger cell death. In contrast, ROS comprise a diverse group of relatively unstable molecules with an odd number of electrons that abstract electrons from other molecules to gain stability. Depending on the type and produced amount, ROS act either as signaling molecules by affecting target proteins or as harmful oxidative stressors by damaging cellular components. Due to their wide range of actions, it is little wonder that Ca$^{2+}$ and ROS signaling pathways overlap and impact one another. Growing evidence suggests a crucial implication of this mutual interplay on the development and enhancement of age-related disorders, including cardiovascular and neurodegenerative diseases as well as cancer.

1. The aging process

Life expectancy has steadily increased and approximately doubled during the last 200 years due to improvements in health care, housing, labor standards and education. The development of vaccines and antibiotics helped to drastically reduce communicable diseases, the main cause of early- and mid-life mortalities until the 1950s. Since then, researchers have searched for methods to delay late-life mortality [1], including behavioral strategies such as physical activity or caloric restriction as well as pharmacological and medical interventions [2]. Current statistics from Eurostat reveal that life expectancy at birth is 83.5 years for females and 78.3 years for males in Europe. It is an ongoing matter of debate whether life expectancy can be pushed even further. Since a constantly increasing number of people reach old age, ongoing matter of debate whether life expectancy can be pushed even further. Consequently, modern medicine aims to find strategies to prevent or at least delay the onset of chronic age-related diseases [3] in order to prolong the so-called healthspan, defined as the period of life free from any chronic diseases and age-associated disabilities [4]. The most common age-related diseases include cardiovascular and metabolic diseases like atherosclerosis and diabetes, neurodegenerative diseases such as Alzheimer’s or Parkinson’s as well as cancer [5].

Organismal aging is a complex process driven by progressive impairment of the functionality and regenerative potential of tissues. One hallmark of organismal aging is the accumulation of senescent cells in organs, resulting in tissue dysfunction and frailty [6]. Due to damage and stress, senescent cells enter cell division arrest and release degradative proteases, growth factors and inflammatory cytokines. Aged cells crucially compromise the function of neighboring cells through the so-called senescence-associated secretory phenotype (SASP) [7]. Cellular senescence can be induced by a wide range of environmental and internal stressors. Reactive oxygen species (ROS) as well as Ca$^{2+}$ are ubiquitous second messengers fine-tuning a variety of physiological and pathological processes within the cell. Although their characteristics are entirely different, they are both in crucial positions to modulate the process of aging [8]. For instance, lifespan and stress resistance of Caenorhabditis elegans (C. elegans) are also strongly dependent on redox and thiol homeostasis [9] as well as cellular Ca$^{2+}$ homeostasis [10]. In turn, the process of aging causes alterations in ROS and Ca$^{2+}$ homeostasis too. Experiments in rats revealed that changes in ROS homeostasis

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are measurable already at middle age. Plasma ROS levels raised, oxidative damage of proteins and DNA increased and DNA damage repairing capacity declined in middle-aged rats. These processes advanced with further aging [11]. Moreover, increased production of ROS as well as decreased antioxidant scavenging was found to change morphological arrangement, Ca^{2+} homeostasis and metabolism of neurons during aging [12]. Growing evidence also suggests that aging is associated with the increased oxidative damage of mitochondrial DNA (mtDNA) in cardiovascular diseases. In turn, overexpression of ROS scavenging enzymes resulted in enhanced resistance to fibrosis, cardiac hypertrophy and heart failure in mice [13]. Notably, fortification of the organism’s antioxidant potential ahead of time by low levels of ROS that boost cellular ROS defense mechanisms may be a powerful strategy to counteract ROS-induced damage during aging. This so-called mitochondrial repair capacity declined in middle-aged rats. These processes advanced with further aging [11]. Moreover, increased production of superoxide (O_2^-) or CATs scavenge and detoxify high levels of ROS. Thus, a redox equilibrium has to be kept under tight control to avoid excessive accumulation as well as depletion of ROS, resulting in cellular dysfunction and various pathologies [23]. Hydroxyl radicals are extremely reactive toward all biomolecules and migrate within a range of a few nanometers. Superoxide and singlet oxygen diffuse up to 30 nm and affect iron-sulfur proteins or lipids, proteins and DNA, respectively. In contrast, hydrogen peroxide diffuses more than 1 μm and oxidizes predominantly cysteine and methionine residues, heme proteins and DNA [24]. In summary, the less reactive a radical is with respect to surrounding molecules, the further it migrates and the larger its place of action as signaling molecule. ROS-induced oxidation, for instance of thiol groups on cysteine residues, may serve as a tool to control the activity of proteins and downstream pathways that contribute to regulation of cell proliferation, immune response and aging [25]. Moreover, low levels of ROS are able to promote health and lifespan by boosting cellular defense mechanisms via an adaptive up-regulation of antioxidant enzymes [26]. However, overwhelming ROS levels results in damage of proteins, lipids and DNA, causing massive oxidative stress and triggering the development of pathological states such as neurodegenerative disorders [27] and cancer [28]. Mitochondrial DNA (mtDNA) is especially vulnerable to ROS-induced damage due to the lack of repair mechanisms and histones, which pack and order nuclear DNA [29].

There are numerous endogenous ROS production sites (Fig. 1). Most endogenous ROS evolve from a leaky electron transport chain (ETC) during oxidative phosphorylation (OXPHOS) in mitochondria. Especially at complex I (NADH dehydrogenase) and complex III (ubiquinone cytochrome c reductase), electrons escape the pathway and partially reduce oxygen to superoxide anions. In addition, the major linker between carbohydrate and lipid metabolism, glycerol-3-phosphate dehydrogenase (GPDH), generates mitochondrial ROS. Notably, complex III and GPDH seem to produce ROS on both sides of the IMM, releasing ROS into the mitochondrial matrix as well as into the intermembrane space (IMS). Additional ROS production sites within mitochondria include pyruvate dehydrogenase (PDH), which converts pyruvate into acetyl-CoA. The Krebs cycle enzyme α-ketoglutarate dehydrogenase (α-KGDH) and monoamino oxidase (MAO) [30,31]. Notably, superoxide generated within the mitochondrial matrix does not cross the IMM. However, as soon as mitochondrial superoxide dismutase 2 (SOD2) converts superoxide to hydrogen peroxide, diffusion across the membrane is possible [31]. In addition to mitochondria, enzymes within peroxisomes generate various types of ROS as by-products of their catalytic actions in metabolic pathways, including acyl-CoA oxidases (ACOX 1–3), α-amino acid oxidase (DAO), α-aspartate oxidase (DDO), l-pipeolic acid oxidase (PIPOX), L-alpha-hydroxyacid oxidase (HAO 1 and 2), polyamine oxidase (PAOX) and xanthine oxidase (XO) [32]. Further, ROS are generated as by-products of a disulfide bond formation by oxidoreductase ERO1 [33] and its thiol redox partner protein disulfide isomerase (PDI) in the lumen of the ER [34]. Moreover, cytochrome P450 (CYP) enzymes, well-known for their role in metabolism and biotransformation of drugs, as well as members of the nicotinamide adenine dinucleotide phosphate (NAPDH) oxidase (NOX) family, contribute to ROS production within the ER. Membrane-bound NOX enzymes comprising NOX1 – NOX5, dual oxidase 1 (DUOX1) and dual oxidase 2 (DUOX2) catalyze the production of superoxide or hydrogen peroxide as part of the immune response. Notably, the action sites of NOX enzymes, which are missing any localization signal in their structure, are dynamic and stimulus-related. The location of the single isoforms is a matter of debate [35]. Besides endogenous ROS production, various exogenous sources such as air pollutants, tobacco smoke, heavy metals, ultraviolet light, drugs and alcohol also provoke cellular processes that generate ROS as part of signal transduction or cell defense mechanisms [21].

Since ROS mostly occurred together with the evolution of aerobic respiration, it seems logical that cells established sophisticated ways to keep ROS levels under control [24]. Cytosolic superoxide dismutase 1 (SOD1) as well as mitochondrial SOD2 rapidly convert superoxide to the less reactive hydrogen peroxide, which is further processed to water and oxygen by catalases (CAT) or to water and oxidized glutathione by glutathione peroxidase (GPX). Moreover, reduced peroxiredoxins (PRDX) catalyze the reduction of hydrogen peroxide to water and are oxidized and restored in their catalytic activity by thioredoxin (TRX). Notably, NAPDH serves as an ultimate donor of reductive power for the majority of these detoxifying enzymes [36].

In short, ROS evolve from a leaky ETC from ROS-producing enzymes such as NOX enzymes or through external triggers inducing oxidative stress. To avoid excessive accumulation of ROS, enzymes such as SODs or CATs scavenge and detoxify high levels of ROS. Thus, a redox equilibrium is achieved, which allows ROS to operate as intracellular signaling molecules while their harmful potential is kept under control [23].
3. Ca\(^{2+}\) homeostasis

Ca\(^{2+}\) serves as a multifunctional second messenger participating in a broad range of processes within a cell, including signal transduction, neurotransmitter release, muscle contraction and fertilization. Together with potassium (K\(^{+}\)), sodium (Na\(^{+}\)) and chloride (Cl\(^{-}\)), Ca\(^{2+}\) ions control membrane potential and shape the action potential [37,38]. Moreover, Ca\(^{2+}\) ions regulate the activity of enzymes such as mitochondrial dehydrogenases of the Krebs cycle [39]. In addition, Ca\(^{2+}\) ions convey their action by binding to proteins such as CaM or CaM, which, in turn, have a wide area of function [40]. For instance, Ca\(^{2+}\) is required for CaN-mediated dephosphorylation of the nuclear factor of an activated T cell (NFAT), which controls the expression of numerous genes and, thereby, processes including protein secretion, cell differentiation and immune response [41]. Moreover, binding of Ca\(^{2+}\) causes allosteric changes in CaM, modifying its interaction with target proteins, including kinases or phosphatases, and thereby affecting neurotransmitter secretion, transcription factor regulation, metabolism and muscle contraction [42]. Overwhelming Ca\(^{2+}\) accumulation within the mitochondrial matrix provokes the formation and opening of the mitochondrial permeability transition pore (mPTP), resulting in an uncontrolled release of apoptosis-modulating or reactive oxygen species (ROS). Main production sites of ROS are the mitochondrial electron transport chain (ETC), regulated among others by the cytochrome-c binding protein p66Shc, as well as nicotinamide adenine dinucleotide phosphate oxidases (NOX) at the plasma membrane and in peroxisomes. Besides NOX enzymes, various other enzymes contribute to ROS generation within peroxisomes, including xanthine oxidases (XO), i-pipeolic acid oxidase (PIPOX), α-amino acid oxidase (DAO), acyl-coenzyme A oxidases (ACOX), L-aspartate oxidase (DDO), L-alpha-hydroxyacid oxidase (HAO) and polyamine oxidase (PAOX). In mitochondria, monoaminooxidase (MAO), pyruvate dehydrogenase (PDH), glycerol-3-phosphate dehydrogenase (GPDH) and α-ketoglutarate dehydrogenase (α-KGDH) are further ROS production sites. In the lumen of the endoplasmic reticulum, enzymes like cytochrome P450 (CYP) as well as the protein disulfide isomerase (PDI) and oxidoreductase 1 (ERO1) produce ROS, often as by-product of protein folding. Cytosolic superoxide dismutase 1 (SOD1) as well as mitochondrial SOD2 are rapidly converting superoxide to the less reactive hydrogen peroxide, which is further processed to water and oxygen by catalases (CAT) or to water and oxidized glutathione by glutathione peroxidase (GPX). Moreover, reduced peroxiredoxins (PRDX) catalyze the reduction of hydrogen peroxide to water and are oxidized and restored in their catalytic activity by thioredoxin (TRX).

3. Ca\(^{2+}\) and ROS homeostasis.

Ca\(^{2+}\) ions enter the cell, among others, through transient receptor potential channels (TRP), voltage-activated Ca\(^{2+}\)-channels (VACC), store-operated Ca\(^{2+}\)-entry (SOCE)-induced ORAI channels or via the Ca\(^{2+}\) homeostasis modulator 1 (CALHM1). The Na\(^{+}\)/Ca\(^{2+}\) exchanger (NCX) and the plasma membrane Ca\(^{2+}\)-ATPase (PMCA) extrude Ca\(^{2+}\) from the cytosol into the extracellular space. Within the cell, the sarco/endoplasmic reticulum ATPase (SERCA) pumps Ca\(^{2+}\) into the lumen of the endoplasmic reticulum, from where it is released via inositol 1,4,5-trisphosphate receptor channels (IP3R), which is modulated by annexin 1 (ANXA1). Mitochondria take up Ca\(^{2+}\) through the voltage-dependent anion channel (VDAC) in the outer mitochondrial membrane and the mitochondrial Ca\(^{2+}\) uniporter (MCU) complex in the inner mitochondrial membrane, and extrude Ca\(^{2+}\) through the mitochondrial NCX (mNCX). Overwhelming Ca\(^{2+}\) accumulation within mitochondrial matrix provokes the formation of the mitochondrial permeability transition pore (mPTP), resulting in an uncontrolled release of Ca\(^{2+}\), apoptotic factors and reactive oxygen species (ROS). Main production sites of ROS are the mitochondrial electron transport chain (ETC), regulated among others by the cytochrome-c binding protein p66Shc, as well as nicotinamide adenine dinucleotide phosphate oxidases (NOX) at the plasma membrane and in peroxisomes. Besides NOX enzymes, various other enzymes contribute to ROS generation within peroxisomes, including xanthine oxidases (XO), i-pipeolic acid oxidase (PIPOX), α-amino acid oxidase (DAO), acyl-coenzyme A oxidases (ACOX), L-aspartate oxidase (DDO), L-alpha-hydroxyacid oxidase (HAO) and polyamine oxidase (PAOX). In mitochondria, monoaminooxidase (MAO), pyruvate dehydrogenase (PDH), glycerol-3-phosphate dehydrogenase (GPDH) and α-ketoglutarate dehydrogenase (α-KGDH) are further ROS production sites. In the lumen of the endoplasmic reticulum, enzymes like cytochrome P450 (CYP) as well as the protein disulfide isomerase (PDI) and oxidoreductase 1 (ERO1) produce ROS, often as by-product of protein folding. Cytosolic superoxide dismutase 1 (SOD1) as well as mitochondrial SOD2 are rapidly converting superoxide to the less reactive hydrogen peroxide, which is further processed to water and oxygen by catalases (CAT) or to water and oxidized glutathione by glutathione peroxidase (GPX). Moreover, reduced peroxiredoxins (PRDX) catalyze the reduction of hydrogen peroxide to water and are oxidized and restored in their catalytic activity by thioredoxin (TRX).
microdomains [45]. To keep Ca²⁺ on a generally low level in the cytosol, Ca²⁺ is actively transferred into ER and mitochondria, bound to proteins or shuttled outside the cell. Ca²⁺ levels reach concentrations of 1–2 mM in the extracellular space. The Na⁺/Ca²⁺ exchanger (NCX) and the plasma membrane Ca²⁺ ATPase (PMCA) actively extrude Ca²⁺ from the cytosol into the extracellular space. In turn, various channels ensure controlled Ca²⁺ uptake, including voltage-activated Ca²⁺ channels (VACC), receptor operated channels (ROC) and transient receptor potential channels (TRP), divided into six subfamilies (C, V, M, P, ML and A). The ER has by far the largest internal Ca²⁺ store with Ca²⁺ concentrations ranging between 100 and 800 μM [46]. Store-operated Ca²⁺ entry (SOCE) allows direct interplay between the ER and the plasma membrane to secure proper ER Ca²⁺ entry (SOCE) allows direct interplay between the ER and the plasma microdomains [45]. To keep Ca²⁺ or shuttled outside the cell. Ca²⁺ rapid Ca²⁺ contraction by binding to troponin C [56]. Also in neurons, electrical or hormonal or shuttled outside the cell. Ca²⁺ rapid Ca²⁺ contraction by binding to troponin C [56]. Also in neurons, electrical or hormonal

4. Mutual interplay between ROS and Ca²⁺

4.1. Ca²⁺ affects ROS homeostasis

Ca²⁺ signaling manipulates ROS levels by targeting major ROS generation sites, including mitochondrial respiration as well as NOX enzymes.

4.1.1. Mitochondrial ROS

About 0.15% of mitochondrial O₂ consumption is assumed to be diverted to ROS under physiological conditions. In contrast, an increase of up to 1–4% has been reported in the presence of antimycin and a non-restricted amount of substrates and O₂ [31]. Ca²⁺ boosts the activity of Krebs cycle dehydrogenases [39]; affects ATP synthase [58,59], adenosine nucleotide translocase (ANT) [60] and glyceraldehyde-3-phosphate dehydrogenase [61]; and facilitates glutamate shuttle via the mitochondrial glutamate/aspartate carrier aralar [62]. Intra-as well as extra-mitochondrial Ca²⁺ thereby enhance the ETC activity, mitochondrial O₂ consumption and ATP production. Whether enhanced mitochondrial metabolism does indeed go along with a higher probability of electron leakage and ROS production depends on substrate and O₂ availability, functionality of the ETC, mitochondrial membrane potential and matrix pH [63]. Experimental observations suggest that Ca²⁺ diminishes ROS leakage from complex I and III under physiological conditions but enhances ROS production in the case of complex blockage [64]. Moreover, it must not be overlooked that mitochondrial Ca²⁺ uptake is linked to a mild dissipation of mitochondrial membrane potential (“uncoupling”), potentially enhancing ROS generation via perturbation of the pH gradient at the IMM, and thus affecting topology of ROS formation sites and mitochondrial respiration rates [65]. Notably, absence of MICU1, the gatekeeper for MCU-mediated mitochondrial Ca²⁺ uptake, was shown to trigger ROS production through a constitutive loading of mitochondria with Ca²⁺ [66]. Also, knockdown of the mNCX was associated with enhanced mitochondrial ROS production due to Ca²⁺ accumulation, causing impairment even of SOCE by ORAI1 inactivation through oxidation [67]. Several reported effects of the impact of mitochondrial Ca²⁺ on ROS levels might be linked to the formation of the mPTP. Stable opening of the mPTP inhibits the formation of the mPTP, allowing opening of the inositol 1, 4, 5-trisphosphate receptor channels (IP3R1, IP3R2 and IP3R3). Like IP3Rs, the ryanodine receptor channels (RYR1, RYR2 and RYR3) mediate the release of Ca²⁺ ions from the SR in muscle cells [47]. Mitochondria take up Ca²⁺ irrespective of its source but show a large preference for Ca²⁺ uptake at ER-mitochondrial interaction sites. Specialized ER-membranes, the so-called mitochondria-associated ER membranes (MAMs), stretch closely to mitochondria, and cytosolic Ca²⁺ levels exceed levels of 10 μM in these areas [48]. While the outer mitochondrial membrane (OMM) is largely permeable to Ca²⁺ via the voltage-dependent anion channel (VDAC), Ca²⁺ transport across the inner mitochondrial membrane (IMM) is highly restricted. High Ca²⁺ concentrations within the IMM regions allow unblocking the gatekeeper proteins mitochondrial Ca²⁺ uptake 1 and 2 (MICU1 and MICU2) and the Ca²⁺ uptake via the mitochondrial Ca²⁺ uniporter (MCU), controlled by a variety of proteins [49]. This protein complex includes, among others, the essential MCU regulator (EMRE) linking MICU1 and MICU2 to MCU [50], the scaffold factor MCU regulator 1 (MCUR1) [51], the dominant-negative pore-forming subunit MICUB [52] and the mitochondrial Ca²⁺ uptake 3 (MICU3) acting as enhancer of MCU-dependent mitochondrial Ca²⁺ uptake [53].

Under controlled conditions, Ca²⁺ leaves mitochondria via the Na⁺/Ca²⁺ exchanger (mNCX) in exchange for Na⁺ [54]. Notably, this exchanger is also able to switch to a reversed transport mode following physiological stimulation of cell-surface receptors, thus generating oscillations in matrix Ca²⁺ [55].

All these mechanisms ensure proper handling of Ca²⁺ within physiological and pathophysiological processes. For instance, Ca²⁺ entry via VACC triggers release through the RYR on the SR and causes very rapid Ca²⁺ oscillations in cardiac myocytes and, thereby, muscle contraction by binding to troponin C [56]. Also in neurons, electrical or neurotransmitter stimulation causes Ca²⁺ entry via channels in the plasma membrane. In contrast, the binding of hormones to G-protein-coupled receptors (GPCRs) provokes slower Ca²⁺ signals by activation of the IP3R and depletion of ER Ca²⁺ in non-excitable cells [57].

In short, a sophisticated toolkit composed of channels, pumps and cytosolic buffers controls numerous biological processes by appropriately adjusting Ca²⁺ levels in various cell types. Electrical, hormonal or mechanical stimulation elicits Ca²⁺ signals by facilitating Ca²⁺ entry via the plasma membrane or Ca²⁺ release from intracellular stores. To prevent the induction of harmful processes, actions of Ca²⁺ are stopped by restoring resting Ca²⁺ concentrations within cellular compartments.
keratinocytes reportedly activated NOX1, potentially via Ca\(^{2+}\)-induced phosphorylation by PKC and subsequent translocation of Rac GTase to the plasma membrane [72]. In addition, direct phosphorylation by the Ca\(^{2+}\)-dependent PKC-\(\beta1\) [74] was associated with an activity boost of NOX1 [75]. Direct activation by Ca\(^{2+}\) was suggested for NOX5, DUOX1 and DUOX2 enzymes. NOX5 is largely expressed in vascular cell types, regulates vascular contraction and reactivity, and contains four Ca\(^{2+}\)-binding EF hands that undergo conformational change in the case of Ca\(^{2+}\) binding, causing activation of NOX5 [76]. For instance, PKC-dependent phosphorylation of the C-terminus of the NOX5b-isofrom was shown to increase the enzyme’s sensitivity toward Ca\(^{2+}\) [77], while oxidation of the Ca\(^{2+}\)-binding domain of NOX5 was associated with its inactivation [78]. Like NOX5, DUOX1 and DUOX2 contain an N-terminal extension comprising two EF-hand motifs and are directly activated by Ca\(^{2+}\) [79]. These enzymes are largely expressed in thyrocytes, where they support the production of hydrogen peroxide necessary for hormone biosynthesis [80]. In addition to this function, DUOX enzymes were also found to trigger hydrogen peroxide production and the inflammatory response involved in wound healing in response to wound-induced Ca\(^{2+}\) flashes in C. elegans, Drosophila embryos and zebrafish [81].

4.2. ROS affect Ca\(^{2+}\) homeostasis

ROS directly and indirectly affect Ca\(^{2+}\) transport proteins located in the plasma membrane, ER and mitochondria.

4.2.1. Plasma membrane

The plasma membrane serves as special platform for redox signaling. Enzymatic systems such as XO or nitric oxide synthase (NOS) as well as integral membrane proteins like NOX enzymes generate ROS at the outer or inner part of the membrane. Besides, hydrogen peroxide-transporting proteins or the formation of specific endosomes at the plasma membrane deliver ROS signals [82]. Indirect and direct interactions between Ca\(^{2+}\) transporters and ROS producing enzymes have been reported. For instance, physical interaction between TRPC3 and NOX2 contributes to ROS production under hypoxic stress and mediates cardiac plasticity [83]. Various Ca\(^{2+}\)-transport proteins of the plasma membrane are affected by ROS. As discussed above, TRP channels are located in the plasma membrane and are activated and regulated by a variety of stimuli, ROS among others. For instance, the second messenger adenosine diphosphate (ADP)-ribose is generated in mitochondria in response to hydrogen peroxide and induces gating of TRPM2 [84]. In contrast to indirect regulation of TRPM2 by hydrogen peroxide, TRPC5, TRPV1 and TRPA1 are directly activated by ROS via modification of cysteine residues at the pore forming regions located within the plasma membrane [85]. Notably, TRPA1 seems to be triggered by hydroxyl radicals, while the activity of TRPC5 and TRPV1 are boosted by hydrogen peroxide [86]. Aside from TRP channels, ROS also affects the SOCE machinery, comprised of the ER Ca\(^{2+}\) sensors STIM1 and STIM2 and the plasma membrane Ca\(^{2+}\) channel ORAI1, in a cell type-dependent manner. For instance, ROS leads to clustering of STIM1 and constitutive, store-independent Ca\(^{2+}\) entry by S-glutathionylation of the conserved cysteine residue C56 of STIM1 in B lymphocytes [87]. Moreover, the ER oxidoreductase Erp57 was shown to inhibit STIM1 oligomerization and SOCE by oxidation of C56, which causes an intramolecular disulfide bond formation with C46 [88]. Also, STIM2, contributing to SOCE regulation under conditions of hypoxia, is apparently oxidized, possibly at C725 [89]. The ORAI1 channel is equipped with three cysteine residues at positions 126, 143 and 195 and has also been identified as a redox sensor inhibited by hydrogen peroxide [90]. Notably, ORAI2 also contains these three cysteine residues, though the hydrogen peroxide-insensitive ORAI3 is lacking C195. Consequently, the cysteine residue at 195 is assumed to be crucial for conveying the effect of ROS [91]. In addition, ROS seem to affect major Ca\(^{2+}\) extrusion proteins, too. For instance, hydrogen peroxide was found to induce thiol oxidation on NCX, causing an activity boost of this channel in rat myocytes [92]. Moreover, oxidative modifications were reported to affect PMCA, leading to rapid inactivation, conformational changes, aggregation, proteolytic degradation as well as internalization from the plasma membrane [93].

4.2.2. Endoplasmic reticulum

Within the ER, ROS are produced by catalytic processes of oxidoreductase ERO1 and PDI as by-product of protein folding [33,34]. Moreover, CYP enzymes as well as members of the NOX family contribute to ROS production within the ER. Several NOX enzymes and their regulators are located at the ER and function as integral signaling elements within the unfolded protein response (UPR) and ER stress. For instance, NOX2 enhances pro-apoptotic signals, NOX4 has a dual pro-survival and pro-apoptotic and the ER chaperone PDI was found to modulate NOX signals. The localization of these enzymes within the ER also affects the activity of proteins involved in ER Ca\(^{2+}\) homeostasis, potentially displaying additional signaling function [35]. Coupling between NOX activity and ER Ca\(^{2+}\) release was assumed to act as regulation point for actin dynamics in neurons [94]. Interestingly, it could be shown that NOX4 knockdown prevented SERCA oxidation and that NOX4-derived oxidants target the RYR, inducing an ER Ca\(^{2+}\) release [35]. However, further investigations are needed to pinpoint the key proteins in these ROS-Ca\(^{2+}\) interplay.

All three IP3R isoforms contain redox-sensitive cysteine residues, which are responsible for the heterogeneity of IP3Rs in their sensitivity towards IP3 and Ca\(^{2+}\) [95]. Similarly to various ORAI isoforms, IP3R isoforms are affected by ROS to a different extent in various cell types. The thiol-modifying agent thimerosal promotes Ca\(^{2+}\) release via IP3R1 at low μM concentrations but inhibits the channel activity at higher concentrations. In the same study, the IP3R3 channel function remained unchanged by thimerosal [96]. Another study suggested that thimerosal selectively sensitizes IP3R1 and IP3R2 to IP3 by stabilizing an active conformation of the receptors through modification of cysteine residues [97].

It was shown that the thiol-oxidizing agent diamine promotes Ca\(^{2+}\) release via IP3R from the ER Ca\(^{2+}\) store by glutathionylation in bovine aortic endothelial cells [98]. In addition, hydrogen peroxide was found to increase the level of glutathionylation of IP3R1 and the sensitivity of endothelial cells toward IP3-generating agonist histamine [99]. Furthermore, ROS derived from xanthine oxidase was also assumed to sensitize IP3Rs via oxidation of thiol groups [100]. Interestingly, the ROS-dependent interaction between ER luminal protein Erp44, a member of the thioredoxin family, with a luminal loop of IP3R1 inhibits the channel activity, showing that IP3R3s are not just controlled by cytosolic but also by intraluminal actions [101]. Liquid chromatography with tandem mass spectrometry revealed modifications at two specific cystolic and two intraluminal cysteine residues of IP3R1 of HEK293 cells under basal conditions and additional eleven modified cysteine residues after thimerosal treatment [102]. Besides, the activity of IP3R channels was found to be strongly inhibited by high luminal ER Ca\(^{2+}\) content through an ER-luminal protein, likely to be annexin 1 (ANXA1) [103], pointing out that redox-mediated regulation on IP3R might also happen indirectly via regulatory proteins.

RYR channels, which serve as Ca\(^{2+}\) release channels in skeletal (RyR1) and cardiac (RyR2) muscle cells as well as in the brain (RyR3), contain numerous cysteine residues and are, consequently, prone to redox modifications [104]. As with IP3R, the impact of ROS on RyR might be concentration dependent. For instance, low concentrations of oxidizing agents boost the activity of RyR2, while persistent high oxidant concentrations cause irreversible inhibition. A different impact on individual cysteine residues is discussed as a possible reason [105]. The RyR2 appears to be the most redox sensitive RyR receptor. Several oxidative modifications such as S-glutathionylation and S-nitrosylation have been shown to affect RyR2 activity. Furthermore, oxidative stress may trigger RyR2 activity by the formation of disulfide bonds between...
subunits, so-called “disulfide cross bridging”, which leads to structural rearrangement of the channel and an SR Ca\(^{2+}\) leak [106]. RyR2 are affected by ROS also indirectly via CaM, which binds to RyR2 under resting conditions and inhibits channel activity under low and high Ca\(^{2+}\) levels. Oxidative stress was shown to decrease the binding between CaM and RyR2 and, thereby, relieve RyR2 from the inhibitory effect of CaM [107].

S-glutathionylation, S-nitrosylation as well as disulfide cross bridging in response to oxidative stress have been described for RyR1, too [108]. For instance, NOX4-induced ROS production was shown to boost RyR1 activity and Ca\(^{2+}\) release in cultured myofibers as well as contractility of skeletal muscle by oxidation of RyR1 caffeine thiols [109].

Also, SERCA exhibits oxidative-sensitive cysteine residues, which modulate the enzyme’s activity upon oxidation and get partially lost during aging [110]. Notably, SERCA isoforms differ in their resistance to damage induced by various types of ROS [111]. Hydroxyl radicals were found to inhibit SERCA function by direct attack on the ATP binding site [112]. Moreover, micromolar pulses of peroxynitrite impair the ability of SERCA vesicles, isolated from muscles, to transport Ca\(^{2+}\) and to hydrolyze ATP by oxidation of thiol groups and tyrosine nitration [113]. In contrast, nitric oxide (NO)-induced reversible S-glutathionylation of cysteine-674 by peroxynitrite enhances the activity of SERCA, decreasing cytosolic Ca\(^{2+}\) levels and inducing muscle relaxation. Importantly, irreversible oxidation of SERCA’s key cysteine residues was associated with the prevention of reversible S-glutathionylation and the subsequent activation of SERCA [114], pointing to a diverse range of ways to modulate SERCA by oxidation-induced mechanisms.

### 4.2.3. Mitochondria

The ETC is the main ROS production site within the cell. Like discussed above, additional ROS production sites within mitochondria include α-KGDH, GPDH, MAO and PDH [30,31]. The contribution of NOX enzymes to mitochondrial ROS production is still not fully clarified. NOX4 has been found in mitochondria of rat kidney as well as in cardiac myocytes. Moreover, cysteine residues of mitochondrial proteins are affected by modulation of NOX4 expression in mice. However, measurements directly assessing NOX4 activity in mitochondria are still missing and, consequently, it remains unclear to which extent NOX enzymes contribute to mitochondrial ROS production [115]. Interestingly, confocal microscopy revealed a co-localization of NOX4 with the ATP synthase [116], implying that NOX enzymes might at least indirectly affect mitochondrial Ca\(^{2+}\) homeostasis.

Activation of cytochrome c-binding protein p66Shc might be another mechanism to indirectly modulate mitochondrial Ca\(^{2+}\) homeostasis. Oxidative stress-induced phosphorylation of p66Shc and its subsequent translocation to mitochondria was found to promote ETC and OXPHOS activity as well as ROS production [117]. Mitochondrial import of p66Shc and its actions as oxidoreductase got associated with mitochondrial swelling, opening of the mPTP and release of pro-apoptotic factors [118]. These events are linked to a perturbation of mitochondrial structure and altered mitochondrial Ca\(^{2+}\) homeostasis. While oxidative stress cause a drastic reduction in mitochondrial Ca\(^{2+}\) uptake upon IP3-generating agonist stimulation in mouse embryonic fibroblasts (MEFs), mitochondrial Ca\(^{2+}\) response in MEFs depleted of p66Shc remains largely unaffected by ROS [118]. The physiological role of p66Shc seems to be controversial. Deletion of p66Shc was found to retard aging in mice [119]. Moreover, p66Shc expression contributes to liver fibrosis [120] and makes cells of the central nervous system prone to β-amyloid toxicity through regulation of mitochondrial ROS [117]. However, p66Shc was found to be highly expressed in fibroblasts from centenarians. A peculiar regulation of p66Shc in long-lived individuals was discussed in this context [121]. Moreover, p66Shc-induced ROS formation might also contribute to self-endogenous defenses against mild ischemia/reperfusion injury [122], highlighting, once again, the controversial role of mitochondrial ROS and Ca\(^{2+}\) in cellular homeostasis.

The main Ca\(^{2+}\) channel in the IMM, MCU, was found to be regulated by ROS via S-glutathionylation at cysteine 97, promoting the assembly of MCU channels into higher-order complexes with persistent activity. Oxidation as well as mutation of C97 caused enhanced MCU channel activity, leading to elevated ROS and Ca\(^{2+}\) levels within mitochondria [123]. Activation of redox-sensitive CaMKII by ischemia perfusion was reported to increase Ca\(^{2+}\) uptake via MCU, promoting mPTP opening and myocardial death [124]. However, a subsequent study failed to find direct electrophysiological evidence for CaMKII’s regulation by MCU [125]. Notably, several other proteins involved in mitochondrial Ca\(^{2+}\) uptake, including MUCb, MUCb1, MUCI, MUC2, and EMRE, remained unaffected by oxidizing agents [123].

### 5. Crosstalk between ROS and Ca\(^{2+}\) in age-related diseases

Aging gives rise to cardiovascular diseases, type 2 diabetes, neurodegenerative and inflammatory conditions as well as cancer. The interplay between Ca\(^{2+}\) and ROS homeostasis crucially contributes to the development, enhancement and progression of these disorders. Important examples are discussed in the following chapter.

#### 5.1. Cardiovascular diseases and type 2 diabetes mellitus

Mitochondrial ROS production is central to the development of age-related cardiovascular diseases, including arrhythmias, heart failure, atherosclerosis and type 2 diabetes mellitus [126]. The crucial role of ROS is highlighted by the occurrence of cardiovascular disorders and diseases in numerous animal models with modulation of mitochondrial ROS levels [127]. For instance, loss of mitochondrial respiration integrity was associated with ROS-induced mtDNA damage found in atherosclerotic plaques of apolipoprotein E-deficient mice as well as in humans [128]. Moreover, impaired mitochondrial ROS and Ca\(^{2+}\) regulation, which facilitates opening of the mPTP and induces cell death, is also a common phenomenon in ischemia/reperfusion (I/R) injury, a tissue damage caused by a reestablished blood supply after stroke or cardiac arrest [129]. During heart failure, mitochondrial Ca\(^{2+}\) uptake is hampered by decreased open probability of MCU, accelerated MnNCX activity and reduced SR Ca\(^{2+}\) load. Consequently, Krebs cycle activity was found to be diminished, resulting in limited availability of NADH for ATP production and of NADPH for detoxifying ROS. The resulting burst of mitochondrial ROS emission apparently contributed to cardiac failure. Indeed, ROS scavenging agents like MitoQ ameliorated cardiac damage in response to I/R injury and hypertension in preclinical studies [126].

In addition to mitochondrial ROS, NOX enzymes expressed in the cardiovascular system (NOX1 – NOX5) are also involved in the development and enhancement of cardiovascular diseases. NOX5 is widely expressed in various human tissues and is directly affected by Ca\(^{2+}\). The enzyme is constitutively active, triggered in its activity by vasoactive agents such as angiotensin II and expressed in the ER and perinuclear area of human microvascular endothelial and vascular smooth muscle cells. Notably, NOX5-induced ROS generation regulated by Ca\(^{2+}\) has been shown to be crucial in vascular contraction and cardiac function [130]. For instance, NOX5-expressing mice showed enhanced Ca\(^{2+}\)-dependent agonist-induced vasoconstriction and increased Ca\(^{2+}\) and ROS levels in vascular smooth muscles. In addition, introduction of endothelial NOX5 increased risk for hypertension and stroke in mice [76]. Moreover, NOX5-derived ROS were shown to activate one of the most potent angiogenic chemokines, stromal cell-derived factor-1α (SDF-1α), which promotes endothelial cell migration and contributes to angiogenesis and atherosclerosis [131]. In addition, NOX5 activity was found to boost the expression of the intermediate-conductance Ca\(^{2+}\)-activated K\(^{+}\) channel (KCNN4) in vascular smooth muscle cells. Notably, high activity of this channel has been associated with coronary artery smooth muscle contraction as well as proliferation and migration of smooth muscle cells during atherosclerotic plaque formation and
restenosis [132]. Taking these implications into account, it makes sense that NOX5 expression is upregulated in human intramyocardial blood vessels and cardiomyocytes after acute myocardial infarction [133]. In line with these findings, NOX5 expression increases in humans in the case of hypertension [134] and is currently being discussed as a blood pressure-associated gene [130]. Moreover, upregulation of NOX5 is associated with renal oxidative stress, inflammation and fibrosis, and it was found in human diabetic nephropathy [135].

In addition to NOX5, the activity of NOX2, which is dependent on Ca^{2+}-induced arrangement of subunits at the plasma membrane, is also associated with atherothrombotic processes. Findings in knockout mice as well as in patients suffering from CGD suggest that NOX2, together with NOX1, has a vasoconstrictive effect by interfering with NO bioavailability. Consistent with these observations, upregulation of NOX2 or of its subunits was found in human atherosclerotic plaques in carotid and coronary arteries [69], and overexpression of the NOX2 subunit p22phox induced progression of carotid artery lesions in transgenic mice [136]. However, suppression of NOX2 in order to diminish atherosclerosis progression is not advisable, as it would be accompanied by chronic infection and inflammation [69]. Besides, activity of NOX2 broadly regulates lung function. For instance, NOX2-activated induction of TRPC and subsequent Ca^{2+} influx was found to trigger lung I/R-induced edema, a life-threatening condition associated with endothelial dysfunction [137]. Moreover, NOX2 has also been shown to promote SOCE-associated Ca^{2+} entry, causing vascular barrier dysfunction as well as sepsis-induced acute lung injury [138]. NOX1, which is affected by Ca^{2+} in a similar way to its close homolog NOX2, is also potentially involved in the pathogenesis of cardiovascular diseases like atherosclerosis, hypertension and I/R injury. Increased expression of NOX1 was found in atherosclerotic arteries of humans, primates and mice already in an early disease state. Deficiency of NOX1 reduced infarct size after I/R injury and protected against angiotensin II-mediated hypertension [139].

The connection between mitochondrial ROS formation and the pathogenesis of diabetes and associated complications is well-established. Notably, cardiac mitochondria from diabetic patients were found to exhibit elevated hydrogen peroxide levels, impaired mitochondrial respiratory capacity and increased levels of oxidized proteins. This might lead to compromised ATP generation and increased propensity to mPTP opening [140]. Also NOX enzymes, including Ca^{2+}-sensitive NOX1, NOX2 and NOX5, are a key source of ROS in the diabetic environment and able to enhance diabetic complications such as nephropathy, retinopathy and cardiomyopathy [141]. For instance, deletion as well as pharmacological inhibition of NOX1 had an antiatherosclerotic effect associated with reduced ROS formation in apolipoprotein E-deficient mice after induction of diabetes mellitus [139,142]. Experiments in NOX2 deficient mice revealed the requirement of NOX2 expression for diabetes-induced retinal vascular injury [143]. Moreover, NOX5 was shown to facilitate renal injury in diabetic nephropathy by enhanced ROS production associated with accelerated glomerulosclerosis and increased expression of proinflammatory chemokines [144].

Heart contractions are dependent on controlled intracellular Ca^{2+} homeostasis, largely regulated by Ca^{2+} released from the SR through RyR [104]. Consequently, redox-driven modifications of RyR2 are of major significance in the development of cardiac pathologies like arrhythmias as well as contractile dysfunction in infarcted and failing hearts [105]. Experiments in a non-ischemic canine heart failure model suggest that phosphorylation of RyR2 by CaMKII is associated with an early stage of heart failure, followed by RyR2 oxidation during a later stage [145]. Consequently, it appears reasonable that phosphorylation-and thiol oxidation-induced dysfunction of RyR2, accompanied by an enhanced SR Ca^{2+} leak, are linked to heart failure [146]. Notably, the β-blocker carvedilol that also serves as an antioxidant preventing thiol oxidation of RyR2 is significantly more effective in the treatment of heart failure than other β-blockers [147]. Further, ROS-mediated activity of TRP channels affects cardiac function and substantially contributes to I/R injury. For instance, ROS-induced activity of TRPM2 was found to contribute to cell death provoked by I/R injury in brain, heart and kidney. A combination of hydrogen peroxide and leukotriene B4 has been reported to trigger TRPM2 activity, facilitating Ca^{2+} influx and the exacerbation of myocardial reperfusion injury [148]. Furthermore, ROS-sensitive TRPA1 channel was found to contribute to Ca^{2+} overload and hypercontraction of cardiomyocytes. Genetic ablation of TRPA1 caused a significant decrease in myocardial infarction after I/R in mice, indicating that I/R activation of TRPA1 worsens myocardial infarction [149]. In addition to channels in the plasma membrane, Ca^{2+} transport proteins in ER and mitochondria are also crucial contributors to the development of cardiovascular diseases.

Treatment of adult rat ventricular myocytes with hydrogen peroxide caused a depletion of SR Ca^{2+} stores by inhibition of SERCA and activation of NCX. This resulted in diminished contractile function of cardiac myocytes [92]. The loss of SERCA’s cysteine residues is associated with an age-dependent decrease in SERCA activity [110]. NO-induced relaxation as well as activation of SERCA by S-glutathionylation was found to be decreased by atherosclerosis due to oxidized cysteine 674 residues [114]. Moreover, high glucose-induced oxidation prevents NO-induced activation of SERCA [150]. Besides, induction of endothelial cell migration, a critical step in physiological and pathophysiological angiogenesis, was found to be stimulated in a NOX4-dependent manner by the vascular endothelial growth factor through increased S-glutathionylation of SERCA and enhanced ER Ca^{2+} influx [151].

A cross-regulation between mitochondrial ROS and Ca^{2+} was also found in chronic ventricular remodeling during chronic ischemia. MAO-induced ROS thereby induced the generation of 4-hydroxynonenal (4-HNE), which provoked increased MCU activity, resulting in mitochondrial Ca^{2+} overload and mitochondrial dysfunction [152]. The interplay between Ca^{2+} and ROS may also be essential in the development or enhancement of type 2 diabetes. For instance, hydrogen peroxide-induced Ca^{2+} influx through TRPM2 has been shown to drive insulin secretion in pancreatic β cells [153]. Moreover, the redox-sensitive MCU was revealed as essential protein in glucose-stimulated insulin secretion in vitro and in vivo in mice [154].

5.2. Neurodegenerative diseases

Various neurodegenerative diseases such as Parkinson’s disease, Alzheimer’s disease, multiple sclerosis and dementia have been linked to biochemical alterations and cellular damage due to oxidative stress [155]. Furthermore, ROS also has an important signaling function in the central nervous system. For instance, Ca^{2+} was found to induce remodeling of mitochondrial morphology of primary astrocytes via ROS [156]. Numerous reports demonstrate that ROS derived from the main mitochondria and ROS may also be essential in the development or enhancement of type 2 diabetes. For instance, hydrogen peroxide-induced Ca^{2+} influx through TRPM2 has been shown to drive insulin secretion in pancreatic β cells [153]. Moreover, the redox-sensitive MCU was revealed as essential protein in glucose-stimulated insulin secretion in vitro and in vivo in mice [154].

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decrease neuroinflammation and to improve symptoms of neurodegeneration in preclinical studies [160].

Since neurons use Ca\textsuperscript{2+} signals to control membrane excitability, neurotransmitter release, gene expression as well as neuronal cell cycle, these cells are especially prone to disturbances in Ca\textsuperscript{2+} homeostasis, often provoked in response to oxidative stress. For instance, neurodegeneration-associated ROS production was reported to disturb the Ca\textsuperscript{2+} flux between ER and mitochondria [161]. Furthermore, the Alzheimer’s disease-associated amyloid β was found to promote cellular Ca\textsuperscript{2+} overload by diminishing the integrity of the plasma membrane via oxidative stress [162]. Proteins involved in Ca\textsuperscript{2+} homeostasis were also revealed to be directly affected under conditions of aging or neurodegeneration. For instance, mass spectrometry analysis revealed an age-dependent loss of SERCA’s functional cysteine residues, which might be a reason for the progressive decline of SERCA activity during aging and disturbed Ca\textsuperscript{2+} homeostasis [110]. Notably, SERCA executes the reuptake of Ca\textsuperscript{2+} into the SR to initiate muscle relaxation. In SOD1\textsuperscript{-/-} mice, restoration of SERCA function prevented oxidative stress-related muscle atrophy and weakness, suggesting that SERCA activity is necessary to control the redox state and muscle function [163].

The activity of PMCA is dependent on age and oxidation, too. Age- and oxidation-associated downregulation of PMCA and, thereby, disturbed cellular Ca\textsuperscript{2+} homeostasis is assumed to compromise neuronal function in the brain during aging and to increase the susceptibility to neurodegenerative diseases [93].

Besides, depletion of the Ca\textsuperscript{2+} homeostasis modulator 1 (CALHM1), a Ca\textsuperscript{2+} channel involved in the regulation of cytosolic Ca\textsuperscript{2+} levels, was found to downregulate ROS production, to increase the expression of HIF-1α and to display neuroprotective effects against ischemia in mice [164]. Notably, the mutation of this channel (P86L-CALHM1) got associated with Alzheimer’s disease and mitochondrial Ca\textsuperscript{2+} overload, potentially causing increased vulnerability of these cells to apoptotic stimuli [165–167].

TRPM2, the most abundant TRP channel in the brain and an indirect target of ROS, is also associated with the development of neurodegenerative diseases. For instance, senescence-associated loss of glutathione was shown to facilitate TRPM2 channel activity, which is associated with an increased intracellular Ca\textsuperscript{2+}-inducing toxicity in cultured hippocampal pyramidale neurons [168,169]. In addition, TRPM2 seems to be involved in various aspects of immunity, boosting, for instance, production of pro-inflammatory cytokines in response to bacterial pathogens [170]. Besides, hydrogen peroxide-activated Ca\textsuperscript{2+}-influx via TRMP2 was also associated with activation of macrophages [171].

5.3. Cancer/malignant diseases

Alterations in ROS homeostasis and enhanced resistance to oxidative stress due to upregulation of protective antioxidant pathways are hallmarks of cancer cells. ROS are assumed to serve as important signaling molecules in the proliferation of cancer cells. For instance, ROS stabilizes hypoxia-sensitive alpha subunits (HIF) and, thereby, ensures adaptation of cancer cells to an oxygen-reduced microenvironment [172]. Whether increased mitochondrial Ca\textsuperscript{2+} levels boost the activity of the TCA cycle dehydrogenases, namely pyruvate, isocitrate dehydrogenase and oxoglutarate dehydrogenases, in tumor cells requires further investigation. However, increased mitochondrial Ca\textsuperscript{2+} uptake is clearly associated with enhanced mitochondrial ROS production in cancer cells [172]. Also NOX2, indirectly affected by Ca\textsuperscript{2+} homeostasis, was found to be involved in cancer development by boosting angiogenesis as well as by silencing the immune response in cancer cells [173]. For instance, ROS derived from NOX2 helps to suppress an effective immune response against cancer cells in tumor-bearing mice. In turn, myeloid-derived suppressor cells lost the ability to inhibit T cell response in the absence of NOX2 activity. Thus, a proper immune reaction could be developed [174]. The expression of NOX5, shown to be directly affected by Ca\textsuperscript{2+}, increased in several types of cancer, including gastric cancer, malignant melanoma, breast cancer, prostate cancer and esophageal cancer, and is associated with a loss in sensitivity to cisplatin in cancer cells [130]. In contrast, a downregulation of DUOX1 and DUOX2 was found in lung cancer cells [175] and DUOX downregulation correlated with cell dedifferentiation [176]. In addition, DUOX expression was proposed as a predictive marker for thyroid cancer, since high DUOX expression is correlated with a reduced death risk in poorly differentiated follicular thyroid carcinoma [176].

Elevation of mitochondrial Ca\textsuperscript{2+} levels is often required to boost mitochondrial respiration and mitochondrial ATP production in cancer cells to meet the energy demand of high proliferation activity. Notably, altered activity of mitochondrial respiration, in turn, affects the production of ROS [172]. Several studies suggest enhanced ER-mitochondrial Ca\textsuperscript{2+} flux as an essential mechanism for the proliferation of cancer cells [177]. Moreover, it has been shown that oncogene-driven senescence triggers ER Ca\textsuperscript{2+} release via IP3R2, which, in turn, facilitates mitochondrial Ca\textsuperscript{2+} accumulation via MCU, a subsequent decrease in mitochondrial membrane potential and the production of ROS [178]. Interestingly, the expression of MCU was found to positively correlate with tumor size and metastasis grade in patients suffering from triple-negative breast cancer. Thus, the interplay between Ca\textsuperscript{2+}, ROS and HIF-1α seems to be crucial: Downregulation of MCU blunted ROS production and expression of the HIF-1α in the respective cancer cells and hampered cancer cell motility, invasiveness and tumor growth in xenografts [179]. Furthermore, ROS-induced overexpression of the chemokine CXCL14 caused cytosolic Ca\textsuperscript{2+} elevation by provoking an IP3R Ca\textsuperscript{2+} leak, which enhanced proliferation, motility and invasiveness in breast cancer cells [180]. IP3R activity is also boosted by association with the tumor suppressor PTEN, resulting in pro-apoptotic Ca\textsuperscript{2+} release and increased mitochondrial Ca\textsuperscript{2+}. In cancer cells, PTEN is frequently inactivated by redox-mediated cysteine oxidation and, consequently, it might be possible to avoid IP3R-induced Ca\textsuperscript{2+} release, mitochondrial Ca\textsuperscript{2+} accumulation and mPTP-triggered apoptosis [181]. Redox-regulated Ca\textsuperscript{2+} signals other than ER-mitochondrial contact sites may serve as an additional driving force for the proliferation of cancer cells. For instance, TRPM2, indirectly affected by ROS, is highly expressed in many cancer cells, including breast, prostate and pancreatic cancer, and inhibition of TRPM2 function was shown to hamper the viability of cancer cells and tumor growth. The main reasons discussed for TRPM2’s ability to preserve cancer cell viability include the maintenance of mitochondrial function, cellular ATP production, autophagy as well as the reduction of ROS levels and DNA damage through promotion of expression of transcription factors such as HIF-1/2α, CREB and NRF2 [182]. The activity of another type of TRP channel, the TRPA1, seems to be enhanced in various cancer types, too. It was shown that ROS-induced oxidation of TRPA1 resulted in increased Ca\textsuperscript{2+} uptake, which promoted cancer cell survival as well as cell resistance to ROS-producing therapies like carboplatin [183]. In addition, the ratio between the redox-insensitive ORAI3 and redox-sensitive ORAI1 channel, surmised to form hetero-multimers together, appears to play a crucial role in different cancer cells. For instance, an increased ORAI1/ORA13 ratio makes prostate cancer cells especially prone to hydrogen peroxide-induced SOCE inactivation [184]. The ratio between STIM1 and STIM2 might also be a crucial survival factor for cancer cells, but additional work is needed to gain a clearer picture [185].

6. Conclusion

As discussed in this review, Ca\textsuperscript{2+} and ROS homeostasis are inseparably connected and crucially control each other in numerous mechanisms feeding into the development and enhancement of age-related diseases. Ca\textsuperscript{2+} ions affect ROS production sites such as ETC, NOX1, NOX2 and NOX5, while ROS, in turn, modulate the activity of proteins ensuring and controlling Ca\textsuperscript{2+} flux between cellular compartments. Further in-depth understanding of these processes might enable us to
find new treatment strategies to ameliorate cellular dysfunction and age-related diseases resulting from imbalanced homeostasis of Ca\(^{2+}\) or ROS.

Declaration of competing interest

We declare no conflict of interest.

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