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Review

Calcium homeostasis in cancer: A focus on senescence☆

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ABSTRACT

Senescence is one of the primary responses to the activation of oncoproteins or down-regulation of tumor suppressors in normal cells and is therefore considered as being anti-tumorigenic but the mechanisms controlling this process are still much unknown. Calcium (Ca^{2+}) plays a major role in many cellular processes and calcium channels control many of the "hallmarks of cancer" but their involvement in tumor initiation is poorly understood and remains unclear.

Therefore, in this article we review some striking senescence-associated characteristics and their potential regulation by Ca^{2+}. The main aim is to produce plausible hypothesis on how calcium homeostasis may participate in cancer-related senescence. This article is part of a Special Issue entitled: 13th European Symposium on Calcium.

1. Introduction

Cell division is an essential tool used by multicellular organisms to survive and, where necessary, to renew or repair damaged tissues. However, genomes are continually damaged by environmental and oxidative stresses and, in dividing cells, by errors in DNA replication and mitosis. In fact, dividing cells can sometimes undergo genomic mutations that, if occurring in cell cycle-controlling genes, put the organism at risk to develop cancer [1]. To counteract this phenomenon, complex organisms have developed some mechanisms in the attempt to repair the damage, if possible, or to block proliferation in cells susceptible of oncogenic transformation. The most common way for cells to limit uncontrolled proliferation is to die by apoptosis (or programmed cell death), but cells have developed alternative mechanisms to block proliferation avoiding death.

The concept of cellular senescence was formally postulated five decades ago when Hayflick and colleagues showed that normal cells had a limited ability to proliferate in culture [2]. Further experiments demonstrated that the loss of the proliferative potential was due to the telomere shortening. Indeed, when the length of one or more telomeres falls below a certain threshold, the exposed DNA end is recognized as a DNA doublestrand break by the DNA damage response machinery and triggers the canonical DNA damage checkpoint. As a general mechanism, DNA damage signals contribute to the activation of the p53 and pRB tumor suppressor pathways, key regulators of much of the senescence program. Activation of p53 contributes to senescence-associated proliferation arrest through one of its target genes, CDKN1a, encoding the cyclin/cdk inhibitor $p21^{CIP1}$. By inhibiting cyclin/cdk2 or cyclin/cdk4 complexes, $p21^{CIP1}$ activates the pRB tumor suppressor pathway, which inhibits cell proliferation through numerous downstream targets, including the E2F family of transcription factors, whose response genes are necessary for progression through S phase [3,4]. Senescent cells are characterized by specific features such as irreversible proliferation arrest, resistance to apoptotic cell death and altered differentiated functions, such as morphological modifications, changes in gene expression and production of secreted proteins like metalloproteases, inflammatory cytokines and growth factors. In the context of cancer, senescence would represent an obstacle to overcome for the cells to become fully tumorigenic in that it irreversibly arrests cell growth. As a matter of fact, cells with cellular and molecular characteristics of senescence are found in benign precancerous neoplasms associated with oncogene activation in both humans and mice. For example, human nevi or moles are benign clonal neoplasms containing melanocytes made senescent by a mutated Ras or B-Raf [5]. Evidence for the tumor suppressor role of senescence has been obtained also with mouse models of cancer triggered by ablation of the tumor suppressor *Pten* in the prostate [6] or oncogenic N-Ras expression in the hematopoietic system [7], in which the oncogenic initiating event led to the development of senescent premalignant lesions with little evidence of apoptosis. However, even if cells attempts to block uncontrolled proliferation by triggering senescence, cancer still occurs. Many evidence indicate that at one point, some cells lose the senescent phenotype and re-enter in the cell cycle progression, the so-called senescence escape. It is thus not surprising that the main effectors of senescence, such as p53 and pRB, are the most commonly lost functions in mammalian cancers. Indeed, in experimental models of cancer,

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the progression to cancerous neoplasms instead of benign ones occurs when the oncogenic event is combined with simultaneous deletion of mediators of the senescence response [6,8]. Calcium controls many aspects of the cellular lifecycle and it is conceivable that senescent cells are characterized by a specific calcium signature that could be responsible in part for the senescent "phenotype". Moreover, since the prominent role of Ca^{2+} in cancer progression [9], particular perturbations of Ca^{2+} equilibrium could allow cells to bypass the proliferation arrest and become tumorigenic.

Therefore, the aim of this review is to provide new hypothesis on how Ca^{2+} signaling could participate in senescence during tumor initiation. However, since on the one hand senescence is the tight cellular balance between proliferation, apoptosis and autophagy and on the other hand Ca^{2+} is a key controller of all these processes, we will recapitulate first the current knowledge of how it governs the "cellular life".

2. Ca^{2+} and the regulation of the cellular fates

2.1. Proliferation

Typically quiescent cells exhibit localized short-term Ca^{2+} fluctuations, which represent native Ca^{2+} signaling pathways, required to maintain basic cellular physiological activity. This "versatility" mostly depends on spatial and temporal localization of Ca^{2+} in the so-called nanodomains that activate specific pathways in specific circumstances.

Ca^{2+} transients have been detected in different parts of the cell cycle. For example, anaphase in mammalian fibroblasts and epithelial cells is manifested through sustained Ca^{2+} elevation [10,11]. Ca^{2+} mobilization have also been detected during the cell cycle, in cultured cells and early embryos, taking cells out of G0 into G1, regulating events during G1 and G2, and taking cells out of prophase into mitosis [10]. These mitotic and other cell cycle events, including gene activation, have also been shown to occur after Ca^{2+} injections into eggs or by using Ca^{2+} ionophores, whereas blocking such cytosolic Ca^{2+} increase has led to mitosis prevention [12]. From these considerations, it is suggested that sustained rise in cytosolic Ca^{2+} is important for the activation of quiescent cells and for speeding up one or more phases in cell cycle.

In cancer cells, the enhanced proliferation is usually associated with increased cytosolic Ca^{2+} concentration [13]. Indeed, multiple transcription factors responsible for cell cycle propagation highly depend on Ca^{2+} ions. Among the most common regulators of those are calmodulin, Ca^{2+}/calmodulin-dependent protein kinases, calcineurin and protein kinase C (PKC) [14].

For instance, Nuclear Factor of Activated T-cells (NFAT) signaling pathway has been implemented in multiple features of cellular cycle, including proliferation. As a general mechanism, following phospholipase Cγ (PLCγ) activation the phosphatidyl-inositol-1,4,5-trisphosphate (InsP3) is produced and binds to the InsP3 receptor (InsP3R) on the endoplasmic reticulum (ER) membrane, inducing the efflux of Ca^{2+}. Stromal interaction molecule 1 (STIM1) and STIM2 subsequently detect the decrease of ER Ca^{2+} stores, form small clusters and communicate with the Orai channel protein at the plasma membrane to trigger Store-Operated Calcium Entry (SOCE). Ca^{2+} binds to the Ca^{2+} sensor protein calmodulin, resulting in activation of calcineurin, which assists in dephosphorylation of NFAT, its nuclear localization and subsequent target genes expression. Interestingly, it has been recently shown that Orai1 detects local Ca^{2+} by N-terminal domain in specific nanodomains thus effectively coupling Ca^{2+} entry to NFAT activation explaining such selective activation of a fundamental downstream response by Orai1 [15]. Nevertheless, there is evidence that the same proliferation-promoting Ca^{2+}/calmodulin/calcineurin/NFAT pathway can be also activated by the store-independent Ca^{2+} entry (e.g. through transient receptor potential (TRP) channels, Orai heteromers) [16–18].

Another ubiquitous regulator of genes that control cell proliferation and survival is Nuclear Factor Kappa-light-chain-enhancer of activated B cells (NF-κB). It has been shown to be constitutively activated in some types of cancer cell and can control the expression of genes that are involved in the stimulation of cell proliferation, inhibition of apoptosis and increase of metastatic and angiogenic potential [19]. Ca^{2+} seems to play a crucial role in the activity of NF-kB: using primary cultures of neonatal cerebellar granule neurons, Lilienbaum and Israël found that the opening of Ca^{2+} channels at the plasma membrane and at intracellular stores is indispensable for the basal NF-κB activity [20]. They demonstrated that calmodulin, protein kinases C (PKCs), and the p21 ^{CIP1}/Ras/phosphatidylinositol 3-kinase (PI3K)/Akt pathways are simultaneously involved in the steps linking the Ca^{2+} signal to NF-κB activity. Of note, there are studies that found NFAT and NF-κB transcription factors or their upstream activators acting as tumor suppressors and hence inhibit cellular proliferative rate [19,21]. Therefore, Ca^{2+}-dependent signaling pathways realized through NFAT and NF-κB provide a peculiar tool for balancing between proliferation and cell cycle arrest (Fig. 1; point 2.1).

2.2. Apoptosis

The involvement of Ca^{2+} and Ca^{2+} channels in extrinsic and intrinsic apoptosis is a widely investigated topic (for review, see [22]). Both during extrinsic and intrinsic apoptosis induction, the mitochondria eventually become overloaded with Ca^{2+}, causing mitochondrial depolarization and the mitochondrial outer membrane permeabilization with the opening of the mitochondrial permeability transition pore (mPTP) and the Voltage-Dependent Anion Channel (VDAC). This leads to the release of the cytochrome C in the cytosol, allowing apoptosome formation and the well-described caspase cascade.

The paradigm of apoptosis regulation by calcium resides in B-cell lymphoma-2 (Bcl-2) activity. First, Bcl-2 ensures a balance between Bcl-2 pro-apoptotic family members at the mitochondrion for the BAK/BAX (Bcl-2 homologous antagonist killer/Bcl-2 associated X protein) pore formation [23]. Additionally, these Bcl-2 protein members were proposed to affect different Ca^{2+}-handling proteins at the ER (inactivation of Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase (SERCA), interaction with the Inositol 1,4,5-Trisphosphate Receptor (IP3R)), leading to Ca^{2+} release from the ER, mitochondrial calcium uptake and cytosolic calcium elevation.

Of note, increased levels of IP3R are usually associated with promotion of cell death, whereas reduction of SERCA levels is a common feature of cancer cells that avoid apoptosis [24,25]. Overall, Ca^{2+} movement mechanism from the ER to the mitochondrion seems to be complex, and elucidation of molecular complexes located in ER/mitochondrion juxtaposition contacts is of primordial interest. In parallel, intracellular Ca^{2+} elevation causes calcineurin-dependent dephosphorylation and activation of Bcl-2 associated death promoter (Bad), a pro-apoptotic Bcl-2 family member, as well as the activation of the Ca^{2+}-dependent cysteine protease calpain. This latter mediates the cleavage of several members of Bcl-2 family and activates caspase-12, promoting apoptotic cell death induced by oxygen and glucose deprivation [26].

A recent study conducted in neuronal cells highlights the participation of the Ca^{2+} sensor Downstream Regulatory Element Antagonist Modulator/potassium channel interacting protein (DREAM/KChIP)-3 in the apoptosis regulation. Besides its transcriptional repressor activity [27], DREAM/KChIP was demonstrated to bind to the mitochondrial protein hexokinase I (HKI) and consequently could prevent apoptosis by modulating VDAC conductance and cytochrome C release [28] (Fig. 1, 2.2).

2.3. Autophagy

Autophagy is a self-digestive process that provides breakdown and recycling of damaged or unused cellular components. In this way the cell preserves its energy levels and at the same time provides additional self-repairing mechanism. Hence, increased autophagy might be triggered by various stress-factors, such as rise in free cytosolic Ca^{2+}.

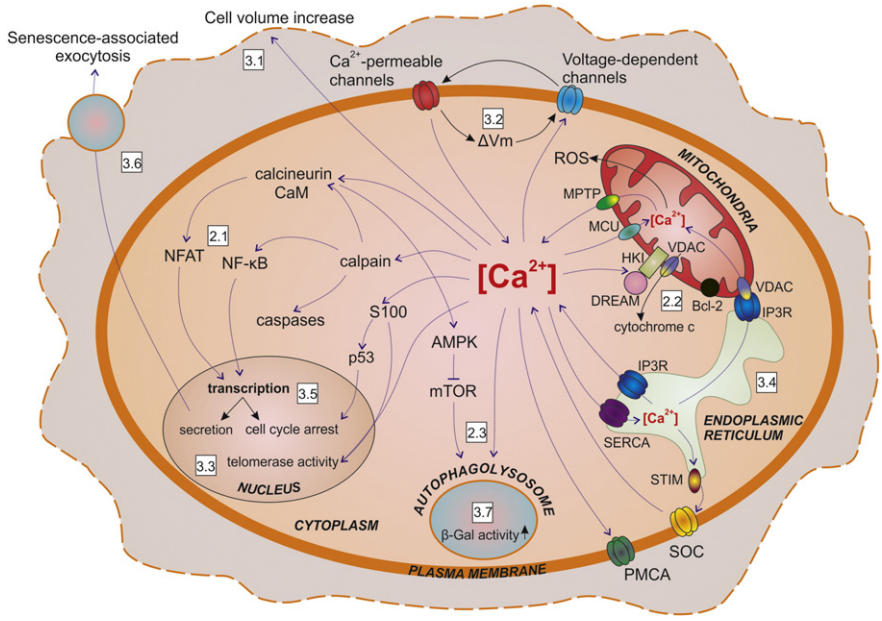

Fig. 1. Senescence may be regulated through different Ca^{2+}-signaling mechanisms provided via several pathways: 2.1 — Transcription factors, including NFAT and NF-κB pathways, for balancing between proliferation and cell cycle arrest; 2.2 — Regulation of apoptotic pathways; 2.3 — Regulation of autophagy; 3.1 — Regulatory changes in cell volume; 3.2 — Changes in membrane potential due to remodeling of voltage-dependent channels; 3.3 — Altered telomerase activity; 3.4 — Subcellular localization of Ca^{2+}-permeable channels, and their mitochondria–endoplasmic reticulum associations in particular; 3.5 — Transcriptional reprogramming; 3.6 — SASP; 3.7 — Increased autophagy. Numbers refer to the sections in the text.

However, depending on its spatial and temporal organization, Ca^{2+} has been demonstrated to both stimulate and prevent autophagy [29].

Interestingly, most studies report ER-residing IP3R as a prominent inhibitor of autophagy [30]. However, in starvation-induced autophagy IP3R has also been demonstrated as a stimulator [31]. There is some evidence on the involvement of TRPV1 and L-type calcium channels in autophagy regulation [32,33]. However, their direct implication is further to be confirmed by using more specific effectors. Interestingly, autophagolysosome formation is much facilitated due to luminal Ca^{2+} release performed via TRPML1, also known as mucolipin-1. Moreover, expressions of TRPML3 also localized in endo-lysosome compartments, directly correlate with autophagy induction [34].

Channels expressed in the mitochondria have been also demonstrated to regulate autophagy. Among them are Mitochondrial Calcium Uniporter (MCU) and its regulator MCUR1, proteins responsible for mitochondrial Ca^{2+} uptake, which have been shown to induce autophagy when in downregulated state [35].

3. Ca^{2+} and the senescent phenotype

3.1. Increase of cell volume

Modification of the cell volume is one of the most prominent features of senescent cells. In many cells, the volume increase is rapidly counteracted by the so-called regulatory volume decrease (RVD) as a consequence of Ca^{2+} entry through nonselective cation channels and extrusion of ions like K^+ and Cl^- followed by water [36].

A possible participant in RVD is the non-selective cation channel Transient Receptor Potential Vanilloid 4 (TRPV4), a member of the Transient Receptor Potential (TRP) superfamily that has been shown to react to hypotonic stimuli with a conductance for Ca^{2+}. Indeed, cells lacking TRPV4 are not able to undergo RVD, a property that is re-established in TRPV4-transfected cells [37].

The authors proposed that membrane stretching due to volume increase can activate TRPV4. In consequence, TRPV4 mediates the influx of Ca^{2+} from the extracellular space, leading to the activation of intracellular signaling cascades responsible for the regulation of RVD.

In addition to TRPV4, TRPM7 is also proposed to be activated by osmotic swelling and its knockdown attenuates RVD [38]. Piezo proteins have recently been identified as nonselective cation channels mediating mechanosensory transduction in mammalian cells. A recent paper shed new light on the role of Piezo-1 in the regulation and maintenance of cell volume homeostasis, in that its knockdown leads to zebrafish erythrocyte swelling [39].

In addition, inhibition of Ca^{2+}/calmodulin-dependent kinases has been shown to inhibit RVD or activation of cell volume regulatory ion channels [40], and it has been concluded that calmodulin/Ca^{2+} complexes are important for activation of RVD.

In senescent cells, this control mechanism is apparently modified because after senescence stimulus, cells increase their volume that remains unchanged with time. It is thus conceivable that senescent cells regulate differentially calcium-permeable channels and the associated downstream signaling pathways to trigger and maintain the specific morphological features (Fig. 1, 3.1).

3.2. Changes in membrane potential

Membrane potential (V_m), is the voltage across the plasma membrane provided due to differences in ion (e.g. Na^+, K^+, Ca^{2+}) concentrations between extra- and intra-cellular regions. Accordingly, activity of ion permeable channels/transporters provokes changes in V_m. Typically, in excitable cells, changes in V_m are required for propagation of action potential, driving force of cell-to-cell communication, whereas in other types of cells, V_m is a biophysical activator of intracellular signaling. Indeed, V_m has been shown to play key roles in multiple cellular activities such as proliferation and differentiation [9]. Recently, Lallet-Daher and colleagues have suggested the association of V_m with another feature of cellular physiology in senescence [41]. They have demonstrated that oncogenic stress triggers an increase of potassium channel KCNA1 expression and its relocation from the cytoplasm to the membrane responsible for changes in V_m and leading to cellular senescence. In similar manner, Ca^{2+} channels have been known to modulate V_m, however no direct evidence on their involvement in senescence initiation is yet known. In addition, V_m regulates activity of voltage-gated channels that provide another significant trail of

Ca^{2+} influx affecting subsequent downstream signaling [42]. Nevertheless, this aspect has not been investigated in the work of Lallet-Daher. Therefore, since changes in V_m have been implemented in oncogene-induced senescence, it would be reasonable to investigate whether similar effects could be potentiated by remodeling of Ca^{2+}-channels as wells as could affect downstream Ca^{2+}-signaling (Fig. 1, 3.2).

3.3. Inhibition of telomerase activity

Expression of telomerase is the most common mechanism by which cancer cells stabilize their telomeres and hence avoid replicative senescence [43]. Telomerase activity can be modulated by Ca^{2+} homeostasis. In HaCaT human epidermal keratinocytes, the levels of telomerase activity were reduced in response to ER Ca^{2+} release even without the downregulation of the human Telomerase Reverse Transcriptase (hTERT) expression, suggesting that Ca^{2+} release can directly modulate the activity of the telomerase complex [44]. This modulation was postulated to occur via the S100A8 Ca^{2+}-binding protein, which inhibits the telomerase complex (Fig. 1, 3.2). Ca^{2+} can also exert the opposite effect, in that elevated extracellular Ca^{2+} levels can enhance telomerase activity likely by L-type voltage-operated Ca^{2+} channels [45].

3.4. Mitochondria–endoplasmic reticulum interactions

The mitochondria and ER are well-recognized nodes where significant remodeling of Ca^{2+} signaling occurs in cancer cells to sustain proliferation and avoid cell death [46]. In a recent study conducted with a loss of function approach, ER-to-mitochondria Ca^{2+} transmission is proposed as a potent senescence-inducing signal. Indeed, senescent cells exhibit an altered calcium homeostasis. During oncogene and replicative senescence induction, the Inositol 1,4,5-Trisphosphate Receptor, Type 2 (ITPR2) triggers calcium release from the ER followed by mitochondrial calcium accumulation through the MCU channel. This Ca^{2+} remodeling causes a drop in mitochondrial membrane potential and an accumulation of oxygen species [47]. Knockdown of *MCU* or *MICU1* modulator prior to oncogenic stress application leads to a strong decrease in mitochondrial accumulation correlated to loss of mitochondrial depolarization and senescence escape. Although the precise mechanism underlying ER-to-mitochondria Ca^{2+} transfer during senescence is still unclear, it is tempting to speculate a link between mitochondrial Ca^{2+} modulation, mitochondrial respiration and eventually metabolic crisis such as autophagy [22,48] (Fig. 1, 3.4). Mitochondria and ER networks are interconnected in punctuate microdomains called the Mitochondria–Associated Endoplasmic Reticulum Membrane (MAM) sharing structural and functional interactions essential for Ca^{2+} signaling, lipid transport, energy metabolism, and cell death [49]. In mammals, these MAMs are composed of VDAC located at the outer mitochondrial membrane interacting with the IP3R at the ER membrane through the molecular chaperone glucose-regulated protein 75 (Grp75), allowing Ca^{2+} transfer from the ER to the mitochondria [50] (Fig. 1, 3.4). These 'hotspots' of calcium transfer are now considered as crosstalk platforms in response to various stresses associated with specific protein recruitment. Altogether, it appears that Ca^{2+} signals regulating cell death, autophagy and metabolism depend on spatially and dynamically restricted domains. Under this perspective, the subcellular localization of all calcium permeable channels appears to be of great significance.

3.5. Transcriptional reprogramming

In Section 2.1 we reported some of the potential effects of Ca^{2+} in the regulation of transcription factors responsible for cell proliferation. As a key regulator of senescence, p53 is the first that should be taken into account. A direct link between Ca^{2+} and p53 activity was provided by Lu et al., showing that siRNA-mediated silencing of Cav3.1 channels expressed in certain esophageal carcinoma cell lines can reduce cell proliferation via the p53 tumor-suppressing transcription factor-dependent pathway, leading to the upregulation of cell-cycle arrest protein $p21^{CIP1}$ [51]. Moreover, calcium can indirectly modulate the expression and function of p53 by regulating the activity of the large conductance Ca^{2+}-dependent K^+ channel (BKCa). Indeed, the pharmacological blockade of BKCa channels in human HeLa cervical and A2780 ovarian cancer cell lines has been reported to induce the increased expression of p53 and cell-cycle arrest in the G1 phase [52], suggesting that BKCa channel activity is required to keep p53 under negative control to prevent its antitumor actions. Additionally, Ca^{2+} can regulate the ability of partner proteins to bind and modulate p53 activity. Mueller and colleagues have in fact demonstrated that the S100A2, a member of the subfamily of S100 Ca^{2+}-binding proteins, is able to interact with p53 in a Ca^{2+}-dependent manner, and that this interaction facilitates binding of p53 to its responsive element [53]. By contrast, the S100B Ca^{2+}-binding protein exerts the opposite role, by decreasing p53 DNA binding and transcriptional activity [54]. Moreover, Delphin et al. demonstrated that in vitro, Ca^{2+}-dependent PKC is able to phosphorylate recombinant murine p53 protein on several residues contained within a conserved basic region of 25 amino acids, located in the C-terminal part of the protein, thus increasing the stability of p53 protein tetramers and oligomers [55].

Ca^{2+} is the major regulator of NFAT, however no data are available on its role in cancer-related senescence. However, dysregulation of NFAT is now known to be associated with malignant transformation and the development of cancer. For example, it is expressed and transcriptionally active in human metastatic melanoma cell lines, in which it is activated by oncogenic $B\text{-}Raf^{V600E}$ via the canonical MEK/ERK pathway [56]. Moreover, this specific pathway is also known to induce senescent-like cycle arrest of human naevi, suggesting that the additional NFAT activation is required for cells to become tumorigenic. As a matter of fact, recently Umemura and colleagues [57] showed that STIM1 and Orai1 are expressed in metastatic human melanoma cell lines, while the melanocyte cell line, HEMA-LP, displayed only a low level of Orai1 expression that could explain partially the increased activation of NFAT in malignant cells. It is thus conceivable that the activation of NFAT occurs in part as the result of changes in Ca^{2+} homeostasis due to channels expression and function remodeling that can occur in cells during the tumorigenic process, allowing cells to pass from a senescent-like to a more aggressive phenotype.

Little is known about the role of NF-κB cancer-associated senescence. It has been shown that chronic NF-κB activation delays $H\text{-}Ras^{V12}$-induced premature senescence in IMR-90 normal human diploid fibroblasts [58]. Accordingly, inhibition of p53 or p65 expression alone had no overt effect on the growth arrest phenotype induced by oncogenic $H\text{-}Ras^{V12}$ in IMR-90 cells, but inhibiting both p53 and p65 simultaneously promoted cell growth and reversed the senescent phenotype [59]. In the same paper, the authors found that NF-κB acts as a master regulator of the Senescence-associated secretory phenotype (SASP), influencing the expression of several genes, including interleukin 6 (IL-6), IL-8, CXCL1 chemokine, InterCellular Adhesion Molecule 1(ICAM1), matrix metalloproteinases and noninflammatory response genes. Indeed, oncogene-induced senescence has been associated with secretion of multiple CXCR2-binding chemokines in NF-κB-dependent manner [60] (Fig. 1, 3.5).

3.6. Senescence-associated secretory phenotype (SASP)

It is well known that senescent cells are able to secrete a large number of soluble factors (the so-called SASP) like growth factors, cytokines, extracellular matrix, and degradative enzymes, all of which can alter tissue microenvironments and affect nearby epithelial cells [61–63]. Calcium channels are well known regulators of secretion. Indeed, Cav3.2 T-type calcium channels were found to be involved in calcium-dependent secretion of neuroendocrine prostate cancer cells probably by stimulating intracellular trafficking and exocytosis [64]. Moreover,

Ca^{2+} influx via store-dependent TRPs (TRPC1, TRPC4), aids the enhanced secretion of angiogenic factors and TRPM8 is in part responsible for the secretion of mitogenic factors in prostate cancer cells [9]. Indeed, modulation of the expression of calcium channels in senescent cells could contribute to regulate the secretory phenotype acting either directly on the exocytic pathway or indirectly by promoting the activity of transcription factors such as NF-κB (Fig. 1, 3.6).

3.7. Autophagic flux

Autophagy has been linked with aging and indeed found in senescence transition phase (reviewed in [65]). Actually, the significant switch of protein degradation from proteosomal into autophagosomal pathway has been reported to take place during senescence [66]. Indeed, autophagy has been suggested to mediate acquisition of oncogene-induced senescence, whereas its inhibition delays senescence-associated processes, including specific secretion phenotype [67]. In fact, the main result of autophagy functioning is the production of particular vesicles that assist in the degradation of a required material after their fusion with lysosomes. Indeed, previously it has been shown that lysosomal membrane permeabilization induces autophagy and triggers stress-induced premature senescence, which is reversible by autophagy inhibition [68]. In fact, one of the most spread assays for determination of senescent cells is enhanced lysosomal enzyme β-galactosidase activity. In consistence with this, during Ras-induced-senescence the kinetics of β-galactosidase activity and autophagy activation have been tightly correlated [67].

Indeed, the evidence of a profound relationship between the autophagy process and oncogene-induced senescence strengthens the potential role of Ca^{2+} signaling as one of the main actors in the acquisition of the senescent phenotype (Fig. 1, 3.7).

4. Concluding remarks

Senescence is a critical step through which “oncogene-stressed” cells pass to limit the proliferation, the consequent accumulation of genomic mutations and finally the tumor progression.

Over these last few years, the importance of Ca^{2+} channels in regulating few hallmarks of cancer is emerging. Indeed, they have been demonstrated to play important roles in 1) self-sufficiency in growth signals and aberrant cell proliferation, 2) insensitivity to antigrowth signals, 3) evasion of programmed cell death (apoptosis), and 4) tissue invasion and metastasis. Some of these channels have a specific profile of expression depending on the cancer cell phenotype thereby pointing on the possibility to use them as diagnostic/prognostic markers for cancer development.

Proliferating tumor cells show very distinct patterns of expression and function of Ca^{2+} channels if compared to their normal counterparts. It is therefore highly probable that some of the features of this “intermediate” phenotype are regulated by Ca^{2+} homeostasis, through the modulation of ion fluxes caused by differential expression and/or remodeling of channels activities. However, senescence is often bypassed and cells re-acquire features of proliferating cells allowing cancer to arise and progress but little is known about the molecular mechanisms involved in this phenomenon. Thus, unveiling their role in senescence could be of help for the development of new more conscious therapeutic strategies targeting Ca^{2+} channels, or to identify new early diagnostic markers.

Transparency document

The Transparency document associated with this article can be found, in the online version.

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