

BCL-2 proteins in senescence: beyond a simple target for senolysis?

Nadine Martin^{1,2*}, Nikolay Popgeorgiev^{1,3}, Gabriel Ichim¹ and David Bernard^{1,2*}

¹Centre de Recherche en Cancérologie de Lyon, Inserm U1052, CNRS UMR 5286, Centre Léon Bérard, Université de Lyon, Lyon, France

²Equipe Labellisée la Ligue Contre le Cancer, Lyon, France

³Institut Universitaire de France (IUF), F-75013 Paris, France

*Corresponding authors: Nadine Martin: nadine.martin@lyon.unicancer.fr, David Bernard: david.bernard@lyon.unicancer.fr

Standfirst

BCL-2 and related proteins are major regulators of apoptosis that have emerged as promising targets for senescent cell elimination (senolysis) to improve age-related diseases and promote healthy ageing. However, seminal observations and recent findings shed light on non-apoptotic roles of BCL-2 proteins in the regulation of cellular senescence.

Main text

Hallmarks of senescence

Cellular senescence is activated by many stresses such as telomere shortening, oncogenes or exposure to alcohol, tobacco or anti-cancer agents including chemotherapies and ionizing radiation. Senescent cells are characterized by stable cell cycle arrest, which occurs mainly in the G1 phase and results from activity of a network of important cell cycle regulators comprising p21 and p16 cyclin-dependent kinase inhibitors (CDKIs), p53 and retinoblastoma protein (Rb). Another hallmark of senescent cells is the secretion of a complex set of soluble factors, constituting the so-called senescence-associated secretory phenotype (SASP). SASP includes a plethora of cytokines, chemokines, growth factors and metalloproteases among others, which have a major impact on tissue organization and function. The SASP is heterogeneous, it can evolve during the senescence time course and is regulated at multiple levels by NOTCH, mTOR and cGAS–STING pathways as well as NF- κ B and CEBP- β transcription factors. Cellular senescence acts as a two-edged sword, displaying transient beneficial effects in embryonic development, wound healing, stemness and anti-tumour response, but having largely detrimental effects during ageing by inhibiting tissue renewal, disrupting tissue architecture and function and establishing a pro-inflammatory and pro-fibrotic microenvironment¹.

Eliminating senescent cells improves healthy ageing

Senescent cells accumulate during ageing or during chronic exposure to toxic molecules. However, whether they actively participate in age-related diseases and in overall functional decline associated with aging remained unknown until recently. This knowledge gap was closed by the recent development of novel genetic tools to eliminate senescent cells (senolysis), based mainly on the expression of inducible toxic proteins under the control of the *CDKN2A* or *CDKN1A* promoters. These studies demonstrated that senolysis improved murine health and longevity^{2,3}, raising great attention from scientists, clinicians and investors to develop specific and efficient senolytic drugs to kill senescent cells with the hope for enabling healthier ageing in the near future¹.

BCL-2 anti-apoptotic proteins as senolytic targets

To extend this initial proof-of-concept that senolysis could alleviate ageing, several teams then went on a quest to identify druggable factors in senescent cells to promote their removal. It is interesting to note that although common stresses and intracellular effectors can lead to both senescence or apoptosis, these two fates are strongly divergent with senescent cells being notoriously resistant to apoptosis (see Supplementary Box 1 for description of common and different features of senescent and apoptotic cells). Apoptosis resistance in senescent cells can occur through their increased expression of BCL-2 (B-cell lymphoma 2 gene) anti-apoptotic proteins, mainly at the level of transcription⁴. The different members of the BCL-2 family are major regulators of apoptosis, as they control mitochondrial outer membrane permeabilization (MOMP), a critical step in apoptosis. Indeed, pro-apoptotic proteins BAX and BAK form pores at the mitochondrial outer-membrane (MOM), leading to the release of cytotoxic molecules

into the cytosol and downstream caspase activation, whereas BCL-2 and related anti-apoptotic proteins prevent MOMP by antagonizing BAX/BAK oligomerization. This occurs through conserved molecular interactions between the hydrophobic pocket of BCL-2 anti-apoptotic proteins and the BH3 motif of BAX and BAK⁵. Interestingly, recent advances led to the development of molecules mimicking BH3 motifs of pro-apoptotic proteins, called BH3-mimetics. Such molecules, including Navitoclax (ABT-263), specifically bind to the hydrophobic pockets of BCL-2, BCL-xL and BCL-w to block their activity and were recently repurposed to eliminate senescent cells⁴. In essence, BH3 mimetics delay and improve several age-related alterations and diseases, including haematopoietic stem cell ageing, atherosclerosis, neurodegenerative disorders or cancer in mouse pre-clinical models and some are currently under clinical trial¹. This rapid transfer of basic research to the clinic could encounter issues linked to the many roles of BCL-2 proteins in senescent cells, aside from their well-known anti-apoptotic function. Indeed, since the discovery of the *BCL-2* gene in the mid-80s, it was suggested that this family of proteins also regulates cell cycle arrest and controls the SASP, as discussed below.

BCL-2 anti-apoptotic proteins could participate in proliferation arrest of senescent cells

The first evidence that BCL-2 could participate in cell cycle control came from observations that its overexpression in mitogen-stimulated B and T-cells or quiescent NIH-3T3 fibroblasts significantly delayed cell cycle entry. This was not restricted to BCL-2, as overexpression of BCL-xL, BCL-w or the viral BCL-2 homolog E1B19kDa had similar effects⁶. Conversely, *BCL-2*-deficient T cells presented accelerated cell cycle progression⁷. Intriguingly, a mutation of a conserved tyrosine (Y28) in the N-terminus BH4 motif or in the hydrophobic pocket interfered with the ability of BCL-2 to restrain entry into cell cycle⁶. Even more intriguing, transgenesis of the pro-apoptotic BCL-2 family member BAX in T cells led to an increased

number of cycling thymocytes. In this case BAX overexpression in mature T cells was correlated with lower levels of CDKI p27Kip1 and faster entrance into S phase compared to wild-type counterparts in response to interleukin-2 stimulation⁸. These results suggest that the increase in the expression of anti-apoptotic BCL-2 factors could contribute to the stable proliferation arrest observed in senescent cells. Albeit, these findings remain largely underexplored, likely because these effects on cell proliferation can be only seen in specific experimental settings. We reason that the primary effect of BCL-2 proteins on apoptosis could hide their effects on cell proliferation in most contexts.

BCL-2 family proteins control the secretome of senescent cells

MOMP represents a central event in apoptosis execution and was considered for many years as a point of no-return in the path towards cell death. However, this process is more subtle than expected since MOMP was also described to occur in a minority of mitochondria (minority MOMP). Although able to locally release cytochrome *c*, minority MOMP is insufficient to trigger wide-spread apoptosis⁹. In a recent pre-print, Passos et al. reported that minority MOMP and sub-lethal release of cytochrome *c* also occurs in senescent cells. Within cells with few permeabilized mitochondria, pores formed by BAX and BAK mediate the release of mitochondrial DNA (mtDNA) into the cytosol. This leads to cGAS–STING pathway activation and enhanced SASP, probably through the activity of NF-κB transcription factors. These in vitro observations have been extended to old mice, where BAX inhibition was shown to increase healthspan, probably by decreasing SASP¹⁰. These data suggest that the increased expression of anti-apoptotic BCL-2 proteins observed in senescent cells is sufficient to prevent BAX/BAK-induced apoptosis but inefficient to block all MOMP (favouring minority MOMP). Minority MOMP was previously described to impact several phenotypes linked to tumour

initiation and progression. The study of Passos et al. provided evidence that minority MOMP can also participate in physiological ageing by promoting the production of SASP and that inhibiting BAX/BAK can offer a strategy to improve healthspan by blocking SASP.

Concluding remarks

Together, the results summarized above highlight a potential role for BCL-2 proteins in regulating the two main hallmarks of senescent cells, namely cell cycle arrest (via increased activity of anti-apoptotic BCL-2 proteins) and the SASP (via minority MOMP caused by incomplete block of BAX/BAK activity) raising the need to better understand the biology of senescent cells prior to designing effective senotherapeutic strategies. Why senescent cells acquire miMOMP remains unclear. Equilibrium between expression of anti- and pro-apoptotic BCL-2 proteins is probably complex in senescent cells as in parallel of the well-admitted increased expression of anti-apoptotic BCL-2 proteins, increased expression of BAX/BAK has also been suggested¹¹, which could contribute to miMOMP in senescent cells.

Senolytic strategies using BH3 mimetics to block BCL-2 anti-apoptotic proteins and promote BAX/BAK-mediated MOMP have been validated to alleviate ageing¹. However, what would happen if senolysis is incomplete following treatment with BH3 mimetics? In this case, we can speculate that senescent cells resistant to BH3 mimetics could arise and eventually re-proliferate; they may then display minority MOMP leading to increased SASP and other pro-tumoral damages⁹. In line with this possibility, Passos et al. showed that inhibition of BCL-2 and BCL-xL using the ABT-737 BH3 mimetic induces premature senescence and its SASP in non-senescent cells¹⁰. If those occur, especially during long-term treatment in the context of chronic age-related diseases, this could limit the beneficial effects of senolysis induced by BH3

mimetics. Overall, we believe that senolytic strategies using BCL-2 inhibitors should be approached with caution and deeply evaluated during pre-clinical studies.

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Acknowledgements

We thank Brigitte Manship for editing the article. We sincerely apologize to the many authors whom we could not cite due to space limitations. Our work was supported by the Fondation ARC, the Ligue contre le Cancer and the INCA.

Competing interests

No conflict of interest.

Supplementary Box

Common and different features characterizing senescent and apoptotic cells

Apoptosis and senescence are induced by a broad range of similar endogenous and exogenous signals and stresses. Those include developmental signals during embryogenesis, UV, chemotherapy, radiotherapy, cigarette smoke components and oncogene hyperactivation, among others. These senescence or apoptosis inducers regulate a wide range of intracellular signals leading to both cell fates, including p53 pathway activation, reactive oxygen species (ROS) production, DNA damage, mitochondrial calcium accumulation and MOMP. These common inducers and upstream signaling factors will then diverge to drive each specific and opposite cell trajectory leading to apoptosis or apoptosis resistance (among others), for senescence. Specific effectors of cellular senescence include the p16 cyclin-dependent kinase inhibitor which is generally induced to stably block cell proliferation, the mTOR pathway and the NF- κ B transcription factors that are generally induced to promote SASP production but which also promote cell survival and the up-regulation of anti-apoptotic proteins of the BCL-2 family. One can hypothesize that lack of activation of anti-apoptotic/survival molecules (mTOR, NF- κ B, BCL-2, BCL-xL and/or MCL1) in response to aforementioned signals and stresses can result in apoptosis induction instead of senescence. Specific effectors for apoptosis include activation, at least to a certain threshold, of caspases and the subsequent chromatin fragmentation. We can also speculate, as suggested by Passos and colleagues¹⁰, that a critical parameter in the choice between these two cell fates is the kinetic and level of activation of pro-apoptotic signals, including the level of caspase activation which can depend on levels of ROS production, DNA damage accumulation, mitochondrial calcium accumulation and quantity of permeabilized mitochondria. In conclusion, many common features are shared between apoptosis and senescence, but why a cell enters into senescence or instead into apoptosis is far from being fully understood.