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Revisiting sensitivity of senescent cells to BH3 mimetics

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ABSTRACT

BH3 mimetics were reported to selectively kill senescent cells and improve age-related diseases. Defining why these cells show increased sensitivity to these molecules will help to identify new pharmacological compounds with senolytic activity. Here, we discuss how recent research findings provide new clues to understand this vulnerability.

Cellular senescence is a cellular state mainly characterized by a stable proliferation arrest and the production of a senescence-associated secretory phenotype (SASP). This **secretome** (see Glossary) modulates the microenvironment of senescent cells and triggers their clearance by the immune system. When timely and spatially controlled, senescence can have beneficial effects, playing a key role in development, wound healing and tumor suppression [1]. However, senescence is activated by many pro-aging signals, either endogenous (e.g., short telomeres, reactive oxygen species) or exogenous (e.g., UV, tobacco, alcohol, **western food**) and chronic exposure to these stresses, combined with the decline over time of the efficacy of the immune system, leads to the accumulation of senescent cells in the organism, with detrimental consequences for tissue architecture and function. After the initial discovery that eliminating p16-positive cells (presumably senescent cells) improves many age-associated diseases and promotes healthy aging [2, 3], a race started to discover molecules exerting **senolytic activity** (ability to selectively induce the death of senescent cells) in order to use them as new intervention strategies against age-related diseases, therefore favoring healthy aging.

Senolytic activity of BH3 mimetics

BCL2 homology domain 3 (BH3) mimetics are small molecules acting as competitive inhibitors of the anti-apoptotic members of the B-Cell CLL/Lymphoma 2 (BCL2) family, which critically regulates apoptotic cell death (Figure 1). The senolytic activity of these compounds was independently identified by 2 groups in 2016. In transcriptomic data, Kirkland and colleagues observed increased mRNA levels of some anti-apoptotic BCL2 proteins in senescent cells. They next demonstrated the efficacy of targeting these proteins using the ABT-263 BH3 mimetic, which selectively induced the death of senescent cells [4, 5]. In an alternative approach, Zhou and colleagues screened a collection of compounds and also identified ABT-263 as a preferential inducer of senescent cell death [6]. Since these pioneer papers, the senolytic effects

of BH3 mimetics, and not only ABT-263, have been confirmed and proven to delay many age-related marks and disorders, for instance hematopoietic stem cell ageing, atherosclerosis, infection severity, neurodegenerative disorders or cancer formation in mouse pre-clinical models [3, 7-10]. Many other senolytic compounds have also been discovered (see the review by Chaib et al [3] for an extensive list of proposed senolytics).

The initial observation by Kirkland and colleagues that the expression of BCL2 anti-apoptotic factors is upregulated in senescent cells has been confirmed in numerous studies, demonstrating a particular increase in BCL2, BCL2L1 (encoding BCL-XL), BCL2L2 and/or MCL1 [4-6, 11]. These observations led to the general belief that the upregulation of these anti-apoptotic BCL2 factors underlies the broad resistance of senescent cells to cell death, a well-known hallmark of senescent cells, and also their increased sensitivity to BH3 mimetics.

Senescent cells display some permeabilized mitochondria

Anti-apoptotic BCL2 factors mainly exert their anti-apoptotic activity by preventing the formation of BAX/BAK protein pores at the mitochondrial outer membrane, preventing mitochondrial outer membrane permeabilization (MOMP) and release of cytotoxic proteins such as cytochrome C and Smac proteins in the cytosol (Figure 1) [12]. Senescent cells, considered to express high levels of anti-apoptotic BCL2 factors, are thus expected to display no permeabilized mitochondria. Unexpectedly, Passos and colleagues recently reported the presence of permeabilized mitochondria in senescent cells [13]. They showed that only a few mitochondria are permeabilized per senescent cell, a phenotype called minority MOMP or miMOMP, insufficient to induce apoptosis, but potentially inducive of different cell phenotypes and behaviors [14]. In the context of cellular senescence, these miMOMP lead to the release of some mitochondrial DNA into the cytosol. The cGAS-STING pathway is activated by this

cytosolic mitochondrial DNA and triggers the upregulation of the pro-inflammatory senescence-associated secretory phenotype (SASP). Blocking miMOMP using BAX inhibitors strongly decreases the SASP *in vitro* and *in vivo* and leads to improving some age-related alterations in old mice [13]. Observations and conclusions by Passos and colleagues (i) support that high expression of anti-apoptotic BCL2 proteins in senescent cells prevents miMOMP to further increase and induce cell death and (ii) challenge the current view that increased sensitivity of senescent cells to BH3 mimetics is due to increased expression of anti-apoptotic BCL2 proteins, as the presence of miMOMP in senescent cells suggests decreased activity of these proteins.

Increased expression of pro-apoptotic BCL2 proteins may lead to miMOMP in senescent cells

A possible explanation to reconcile this apparent discrepancy could be that increased expression of anti-apoptotic BCL2 proteins observed in senescent cells is counterbalanced by an increased expression, so far unreported, of pro-apoptotic BCL2 proteins. In line with this hypothesis, some transcriptomic datasets of senescent cells provide some clues. For instance, transcriptomic datasets of Kirkland and colleagues not only show an increase in anti-apoptotic BCL2 factors (e.g., BCL2, BCL2L1, BCL2L2) in senescent preadipocytes but also significant increase in pro-apoptotic BCL2 proteins (e.g., BAX, BNIP3, BNIP3L) [5]. Similarly, other transcriptomes reveal that MCL1 and BCL2A1 anti-apoptotic proteins and BAK1, BNIP3 and BNIP3L pro-apoptotic proteins significantly increase in RAS-induced senescent IMR90 fibroblasts [15] or that BCL2L1 anti-apoptotic and BAX, BID, BNIP3L, BAD and HRK pro-apoptotic factors increase in etoposide-induced senescent WI38 fibroblasts [16]. Although these transcriptomic data analyses would need further investigation, as for instance BCL2L1 can generate anti-apoptotic BCL-XL and/or pro-apoptotic BCL-XS, they strongly emphasize a very complex

picture of the expression of these anti- and pro-apoptotic BCL2 factors in senescent cells, which was not anticipated. They not only show that BCL2 pro-apoptotic factors can be induced in senescent cells, but they also support that their upregulation could be higher or at least equivalent than the one observed for anti-apoptotic BCL2 factors. This would be in line with the appearance of miMOMP in senescent cells reported by Passos and colleagues [13]. Interestingly, increased expression of pro- and anti-apoptotic factors of the BCL2 family is also observed in cancer cells and in this context most of the anti-apoptotic proteins are already loaded with pro-apoptotic proteins, making cancer cells more sensitive to BH3 mimetics than normal cells [17], supporting that senescent and cancer cells could share this feature.

Concluding remarks

The expression profile of the BCL2 family at the mRNA level in senescent cells is far more complex than initially described and analysis, in this context, of the protein levels of each of its members is still missing. Future studies will thus be needed not only to provide a more complete picture of the relative abundance of anti- and pro-apoptotic factors of this family in senescent cells but also to describe the dynamic interactions between these two groups of proteins, which are in part dictated by mitochondrial membrane-dependent conformational changes, as well as their interactions with other proteins and their regulation by different signals, since they are all key for determining cell fate outcome [12, 18]. Nevertheless, the description of miMOMP in these cells by Passos and colleagues and the observations made by revisiting some transcriptomic data as described above already support the need to revisit old concepts of increased sensitivity of senescent cells to BH3 mimetics. We can speculate that senescent cells, even if they may be more resistant to cell death in some contexts, especially DNA damaging drugs as they do not divide, could be more sensitive to BH3 mimetics as they already display

miMOMP, and as a result, compounds specifically and directly favoring additional mitochondrial permeabilization more efficiently induce cell death in these cells (Figure 2). The occurrence of miMOMP could be seen as an Achille heel of senescent cells and its discovery could pave the way for future studies aiming to assess the senolytic activity of compounds promoting mitochondrial permeabilization, ideally amplifying miMOMP, to reach the threshold of permeabilized mitochondria potentially triggering cell death, without inducing it.

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DECLARATION OF INTERESTS

The authors declare no conflicts of interest.

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GLOSSARY

Secretome: set of molecules secreted by cells in their microenvironment

Senolytic activity: ability to selectively induce the death of senescent cells

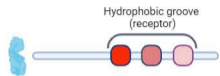
Western food: regimen rich in sugar and fat that can induce obesity and long-term diabetes and cardiovascular diseases

FIGURE LEGENDS

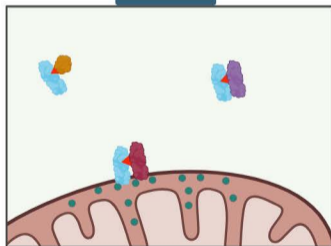
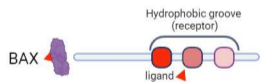
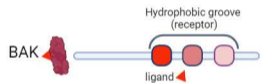
Figure 1. The BCL2 family: members and mode of action. The BCL2 family of proteins is composed of anti-apoptotic and pro-apoptotic members. In normal non-permeabilized mitochondria, anti-apoptotic proteins (BCL2, BCL2L1, MCL1, etc.) interact with and inhibit pro-apoptotic proteins BAX and BAK. However, in the case of overexpression of BAX/BAK and/or BH3-only pro-apoptotic BCL2 family members (BAD, BID, BNIP3L etc.), BAX and BAK can interact, oligomerize and form pores in the mitochondrial membrane, permeabilizing it, leading to the release of cytochrome C and Smac (shown as green dots) from the mitochondria, and usually, to apoptosis. The figure has been created with BioRender.com.

Figure 2. Molecular and cellular bases underlying the sensitivity of senescent cells to BH3 mimetics. To date, multiple studies have shown an increased expression of anti-apoptotic BCL2 family members in senescent cells. This reinforced the assumption that the increased sensitivity of these senescent cells to BH3 mimetics - small antagonist molecules of the anti-apoptotic BCL2 members - was associated with the above observations. More recently, it was reported that senescent cells can have a few permeabilized mitochondria, a phenomenon known as minority mitochondrial outer membrane permeabilization (miMOMP). This finding in combination with the observed upregulated expression of pro-apoptotic BCL2 family members during senescence, may better explain the increased sensitivity of senescent cells to BH3 mimetics, which promote the permeabilization of additional mitochondria, thus reaching a point-of-no-return leading to apoptosis. The figure has been created with BioRender.com.

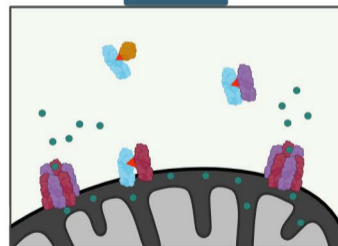
Anti-apoptotic BCL2 family proteins



Pro-apoptotic BCL2 family proteins



Non-permeabilized mitochondria



Permeabilized mitochondria

