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Nadine Martin, Olivier Soriani, David Bernard

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3 **Cardiac Glycosides as senolytic compounds**

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6 Nadine MARTIN<sup>1,\*</sup>, Olivier SORIANI<sup>2</sup>, David BERNARD<sup>1,\*</sup>

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8 <sup>1</sup>Centre de Recherche en Cancérologie de Lyon, Inserm U1052, CNRS UMR 5286, Université  
9 de Lyon, Centre Léon Bérard, Lyon, France.

10  
11 <sup>2</sup>Inserm, CNRS, iBV, Université Côte d'Azur, Nice, France.

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13  
14 \*Correspondence: nadine.martin@lyon.unicancer.fr (N.Martin);  
15 david.bernard@lyon.unicancer.fr (D.Bernard)

21 **ABSTRACT**

22 The identification of senolytics, compounds able to eliminate senescent cells, is presently a key  
23 challenge due to their therapeutic promises for cancer and aging-associated diseases. Two  
24 recent papers by Triana-Martínez et al and Guerrero et al report the senolytic activity of cardiac  
25 glycosides and their efficacy in these physiopathological contexts.

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27 **KEYWORDS**

28 Cellular senescence, Senolytics, Cardiac glycosides, Cancer, Aging

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33 **MAIN TEXT**

34 Cellular senescence can be induced by many stresses or during normal development. Upon  
35 senescence induction, cell cycle is stably arrested and cells secrete multiple proteins including  
36 pro-inflammatory cytokines and chemokines, growth factors and proteases. These two  
37 hallmarks of senescent cells are associated with changes in their metabolism, chromatin  
38 organization and gene expression. The senescence-associated secretory phenotype (SASP)  
39 reinforces senescence through autocrine activity and influences the microenvironment in a  
40 paracrine manner impacting tissue structure and function [1].

41 Cellular senescence can have a critical impact on the organism. It is mostly beneficial at a young  
42 age as it plays a key role in morphogenesis during normal embryonic development and it  
43 promotes tissue repair. Senescence is also beneficial, at least initially, in the context of pro-  
44 tumoral stresses, as senescence activation is a barrier against tumorigenesis, by stopping the  
45 proliferation of pre-malignant cells and promoting their clearance by the immune system  
46 through the SASP [1].

47 However, during aging and/or upon exposure to chronic stress, persistent damage and defects  
48 in immune clearance lead to the accumulation of senescent cells which are expected to limit  
49 tissue renewal, create a chronic pro-inflammatory/pro-fibrotic microenvironment and impair  
50 tissue function. Recently, it was reported that accumulation of senescent cells promotes aging  
51 and many age-related disorders including cancer relapse and progression, cardiovascular  
52 diseases, obesity, type 2 diabetes, sarcopenia, lung fibrosis, osteoarthritis, atherosclerosis and  
53 neurological disorders. Indeed, genetically-engineered mouse models allowing the clearance of  
54 senescent cells show improved healthy aging, extended lifespan and delays or attenuation in  
55 many aging-associated pathologies during normal aging or after disease-inducing challenges  
56 [1].

57 These spectacular results prompted many laboratories and more recently biotechnology  
58 companies to search for compounds able to selectively kill senescent cells, named senolytics,  
59 to delay or improve age-related diseases. A potential difficulty to find such compounds is the  
60 intrinsic cell death resistance of senescent cells. Several potential senolytics have been  
61 identified in the last few years and some are already in clinical trials, mostly in a repositioning  
62 context. Others have notable side effects, preventing their use in the context of chronic diseases  
63 [2]. There is need for new senolytics for potential clinical translation as well as for improving  
64 our understanding of the mechanisms involved in the death resistance of senescent cells.  
65 Recently, publications by Triana-Martínez et al. [3] and Guerrero et al. [4] reported the  
66 identification of cardiac glycosides (CG) as a novel class of broad-spectrum senolytics after  
67 performing drug screens in models of oncogene-induced senescence in normal cells and  
68 therapy-induced senescence in normal and cancer cells, using libraries of mainly FDA/EMA  
69 approved drugs (Prestwick library), pharmacologically active compounds (LOPAC library) and  
70 natural compounds (GPNCL and SCREEN-WELL libraries).

71 The senolytic activity of CG was then assessed *in vivo* and *ex vivo* both in the context of cancer  
72 and aging-associated disorders. Ouabain, a natural compound obtained from the seeds of  
73 *Strophanthus gratus* and other plants of the *Apocynaceae*, killed senescent pre-neoplastic cells  
74 in a model of tumor initiation in liver and in adamantinomatous craniopharyngioma. In addition,  
75 in several models, digoxin, a CG obtained from the *Digitalis* plant and already used for treating  
76 heart failure and atrial fibrillation, and ouabain were highly efficient senolytics when used after  
77 senescence-inducing drugs (senogenics), a two-step therapeutic strategy proposed for tumour  
78 cells. Ouabain also diminished the number of bystander senescent cells which accumulate after  
79 irradiation and are responsible for some side effects. When administered in old mice, ouabain  
80 reduced the number of senescent cells, diminished the level of local inflammation and immune  
81 infiltration and improved some metabolic and physical fitness parameters that decline with

82 aging. Digoxin, through its senolytic activity, also reduced lung fibrosis. Based on these  
83 findings, the authors proposed the potential use of CG as senolytics for therapeutic applications.  
84 Of note, digitoxin, a CG similar to digoxin, displays senolytic activity at concentrations similar  
85 to the ones measured in the plasma of patients treated with this compound for heart failure and  
86 atrial fibrillation. The description of the senolytic activity of CG also suggests they can act  
87 through this activity to exert previously described beneficial effects in the context of  
88 atherosclerosis, pulmonary fibrosis and anti-cancer treatment [5-7]. However, ouabain is able  
89 to induce premature senescence in immortalized human mammary epithelial cells [8],  
90 suggesting a cytostatic effect of CG in some proliferating cells and thus potential long-term  
91 deleterious effects.

92 CG are inhibitors of the  $\text{Na}^+/\text{K}^+$  ATPase, a plasma membrane (PM) enzyme pumping sodium  
93 out of and potassium into the cells against their respective concentration gradients and thereby  
94 regulating PM potential. Both publications provide data indicating that the senolytic activity of  
95 CG relies on the inhibition of the alpha subunit ATP1A1 of the  $\text{Na}^+/\text{K}^+$  ATPase. For example,  
96 inhibiting the  $\text{Na}^+/\text{K}^+$  ATPase by KCl treatment, since  $\text{K}^+$  has a well-known antagonistic effect  
97 at CG binding sites [9], reverses the senolytic activity of CG.

98 Triana-Martínez et al further observed that senescent cells are slightly depolarized, which is in  
99 accordance with previous findings [8,10,11], and that digoxin, by inhibiting the  $\text{Na}^+/\text{K}^+$   
100 ATPase, enhances PM depolarization of senescent cells. Triana-Martínez et al thus propose that  
101 enhancing PM depolarization contributes to the senolytic effect of CG. However, cell exposure  
102 to KCl is commonly used to trigger PM depolarization (as a direct consequence of the  $\text{K}^+$   
103 equilibrium potential shift to more positive values according to Nernst's equation) and previous  
104 works showed that KCl-induced depolarization by itself triggers cell senescence [8,11],  
105 hampering the evaluation of the contribution of PM depolarization to the senolytic activity of  
106 CG. Triana-Martínez et al also suggest that the senolytic activity of CG might result from

107 intracellular acidification, as increased  $\text{Na}^+$  accumulation reduces  $\text{Na}^+/\text{H}^+$  exchanger activity,  
108 resulting in intracellular  $\text{H}^+$  accumulation.

109 Transcriptomic analysis performed by Guerrero et al revealed that ouabain and digoxin activate  
110 the expression of genes of the pro-apoptotic Bcl-2 family. Among those, NOXA is induced by  
111 the JNK, GSK3- $\beta$  and p38 pathways and partially mediates the senolytic effect of CG. How  
112 CG lead to the activation of these pathways also remains to be determined.

113 In conclusion, the identification of CG as senolytics sheds light on new molecular players  
114 regulating cellular senescence and new vulnerabilities of senescent cells. The two publications  
115 describe the first pieces of a complex puzzle to explain how CG induce cell death. Signaling  
116 pathways directly regulated by the  $\text{Na}^+/\text{K}^+$  ATPase, which involve SRC, ITPR and AKT among  
117 others and are modulated by CG, could also play a role in the senolytic activity of these  
118 compounds. Moreover, although the broad spectrum of action of CGs and their possible side  
119 effects reduce the overall benefit of using these compounds for treatment, CG appear to be  
120 promising candidates for therapeutic applications exploiting their senolytic effect in the context  
121 of cancer and aging (Figure 1). A number of clinical trials with CGs are currently conducted,  
122 especially combining CGs with chemotherapies for cancer treatment, and novel ones will need  
123 to be setup to assess their benefit against aging-related chronic diseases such as arthritis or  
124 pulmonary fibrosis.

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130 **CONFLICT OF INTEREST**

131 None declared.

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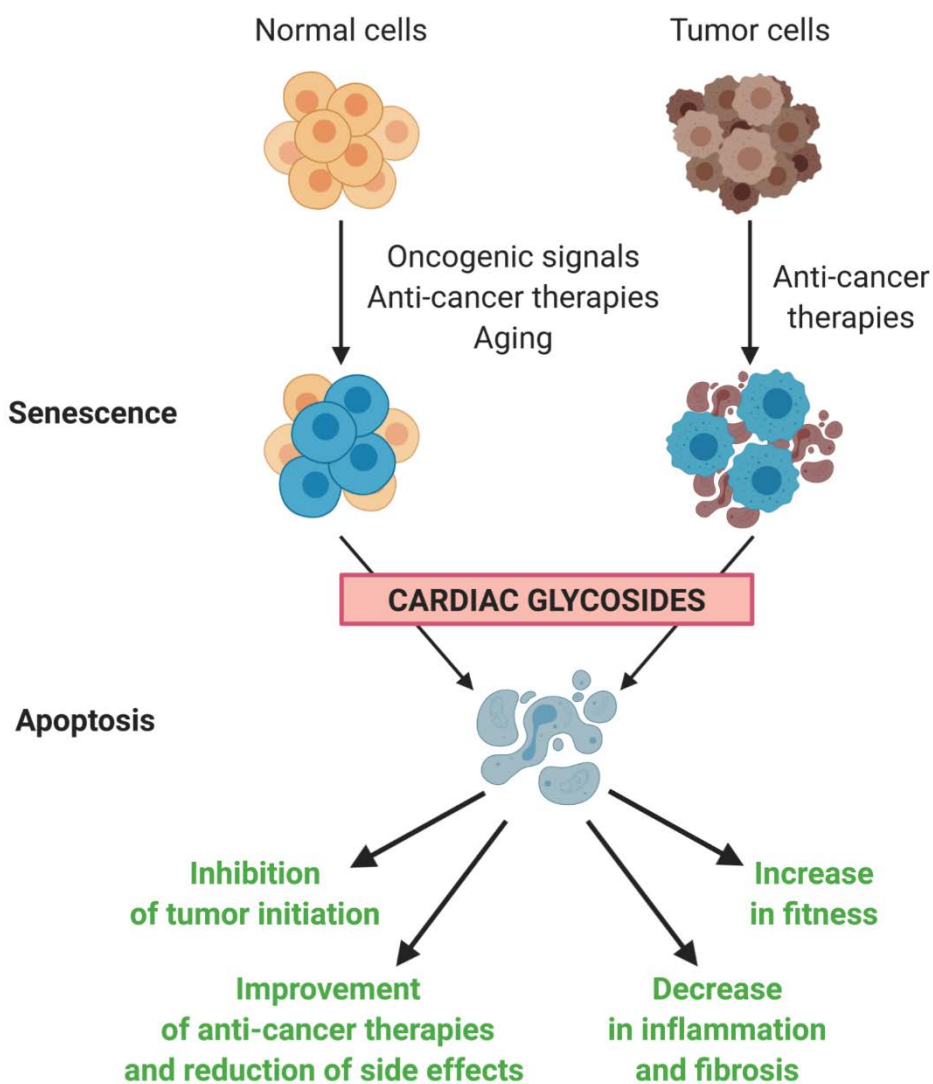
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163 **FIGURE LEGENDS**

164 **Figure 1. Potential therapeutic applications of cardiac glycosides as senolytics.** Selective  
165 elimination of senescent cells (blue) by cardiac glycosides in different contexts (senescent  
166 preneoplastic lesions, tumors treated with senescence-inducing therapies, lung fibrosis, old  
167 organism) revealed several therapeutic benefits (green) for cancer, aging and its associated  
168 diseases.

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