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Tracking senescent cells: A new biomarker assay opens new avenues in senescence research:

Commentary on “Robust, universal biomarker assay to detect senescent cells in biological specimens”

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Cells can undergo senescence in both pathological and physiological conditions, acquiring a state of permanent cell cycle arrest that is associated with resistance to apoptosis and alterations in gene expression, metabolic activity and cell-cell communication (Campisi and d’Adda di Fagagna, 2007). This process naturally occurs during some phases of development and can also benefit the organism by enhancing wound healing and as a tumor suppression mechanism (Muñoz-Espín *et al.*, 2013; Demaria *et al.*, 2014; Campisi, 2001). However, senescent-cell accumulation with age is thought to contribute to aging-associated health decline and limits lifespan (Baker *et al.*, 2011; Baker *et al.*, 2016).

Senescent cells display some particular characteristics that allow their identification and quantification, but these are not exempt of some flaws that abridge their use (Sharpless *et al.*, 2015). Therefore, the discovery of new biomarkers of senescence that can be used both *in vitro* and *in vivo* is a great contribution with direct applications in many fields, including aging and cancer research. In this regard, it was previously described that the histochemical Sudan-Black-B (SBB) was an efficient staining agent of lipofuscin (Georgakopoulou *et al.*, 2013), a pigment granule that is accumulated in an age-dependent fashion as a result of the clustering of oxidized proteins and lipids (Jolly *et al.*, 2002). Furthermore, lipofuscin was found to accumulate in senescent cells, co-localizing with the most commonly used senescence marker, the senescence-associated beta-galactosidase activity (SA- β -gal) and consequently, becoming an interesting marker of senescent cells (Georgakopoulou *et al.*, 2013).

The SBB staining is a fast technique that can be applied even in formalin-fixed and paraffin-embedded samples, inaccessible to SA- β -gal that requires enzymatic activity and therefore, the use of fresh samples. Nevertheless, SBB staining is not exempt of some difficulties, as background staining and the granule size can encumber the correct evaluation of the samples (Evangelou *et al.*, 2017). To further broaden the use of lipofuscin as a senescence marker,

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Evangelou *et al* recently published the synthesis and evaluation of a new compound that solves these issues.

The authors set out by synthesizing *de novo* different compounds structurally similar to SBB that would later be linked with biotin. These compounds were obtained in a very high yield, avoiding possible noise during the staining produced by unwanted molecules, and their performance at detecting lipofuscin was tested in comparison with SBB. Results showed that one of them, GL13, was able to bind lipofuscin as efficiently as SBB but with a decreased background signal. Furthermore, as GL13 presents a biotin, the posterior addition of anti-biotin antibodies would allow to further amplify the signal if desired, facilitating the recognition of the lipofuscin aggregates. By doing this, Evangelou *et al* have designed a molecule that not only includes the benefits that using the SBB staining presents over SA- β -gal, but goes beyond SBB by decreasing the background and adding a biotin to the staining molecule that allows its easy immunolabeling and detection.

Considering the relevance of senescent cells in health and disease and the recent discoveries pointing at senescent cells as direct effectors of aging itself, to be able to detect these cells in an easy and efficient manner is crucial for the fast progress of this research. As SBB, GL13 can be applied to fresh, frozen, formalin-fixed and paraffin-embedded samples, hence allowing to study senescence in stored clinical and research samples. Finally, the technical difficulties and expertise required for evaluating SBB staining is bypassed as the signal can be further amplified by following immunohistochemistry and immunofluorescence approaches. The improved methodology for detecting senescent cells in a variety of specimen will pave the way for a more comprehensive assessment of senescence in biology and in human disease.

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