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COMMENTARY

Tumor dormancy and disease recurrence

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The fascinating reviews in this series highlight the importance of dormant tumor cells in predisposing to eventual recurrence, local spread, and metastasis of cancer cells that are aggressive and resistant to multiple therapeutic modalities. They highlight potential links and similarities between dormant cancer cells and cancer-harboring senescent cells. These findings raise a number of questions, suggest research directions, and indicate possible novel approaches for cancer treatment with potential for developing treatments to delay or even fully prevent cancer recurrence. As suggested from accumulating evidence, methods need to be developed for eliminating all remaining cancer-harboring cells. Perhaps only one or a few such cells remaining after primary tumor treatment is sufficient for cancer to relapse. Research in this currently underfunded area needs to be supported and initiated urgently.

Cellular senescence is a cell fate that can occur in response to repeated replication, oncogene expression, cancerous mutations, radiation, chemotherapy and other cytotoxic drugs and bioreactive molecules, inflammatory factors, cellular debris and other tissue damage signals, mechanical or shear stress, pathogens, and other injuries. Senescent cells can appear at any point during life, as for example, Down syndrome related to aged oocytes appears linked to cellular senescence. Senescence can occur across the vertebrates and possibly in some invertebrates. Most types of dividing cells as well as non-dividing cells as can enter into a senescent-like state with expression of markers of cellular senescence. In the case of dividing cells, senescence entails replicative arrest. This may protect against cancer growth [1]. However, potentially cancer harboring senescent cells can escape this replicative arrest and give rise to recurrent malignancies. Furthermore, persistent senescent cells near developing cancers may promote tumor growth because of factors they release and microenvironmental changes they induce (Fig. 1).

Many or most senescent cells can develop a senescence-associated secretory phenotype (SASP) entailing secretion of proteins, peptides, bioactive metabolites and lipids, and non-coding nucleotides such as microRNA's and mitochondrial DNA. In 30 to 70% of senescent cells, this SASP can be pro-apoptotic, pro-inflammatory, and tissue-damaging, while in other senescent cells, it can involve release of growth factors and be non-inflammatory. The nature of the senescent cell SASP depends on the type of cell that became senescent, the inducer of senescence, how long the cell has been senescent, and the microenvironment [2]. For example, SARS-CoV-2 virus can amplify release of pro-apoptotic, tissue-destructive SASP factors. Over time, senescent cells can become more genetically unstable, in part due to Line-1 retro-transpositional events ("jumping genes"), point mutations, and epigenetic changes. These changes tend to amplify the pro-inflammatory aspect of the SASP and might be an underlying contributor to escape of cells from the senescent state, including senescent cells harboring cancerous mutations and chemotherapy/radiation–induced senescent cells (therapy-induced senescent cells or TIS).

Senescent cells are resistant to apoptosis. Senescent cells are generally cleared by the innate immune system, but under some conditions can evade immune clearance and persist [3]. Some senescent cells, for example most senescent mesenchymal cell types, continue to resist apoptosis even after treatments that target individual anti-apoptotic mechanisms, such as BCL-xL inhibitors like Navitoclax, A1331852, or A1155463. This appears to be because multiple distinct anti-apoptotic mechanisms can protect certain types of tissue-destructive senescent cells from their own pro-apoptotic SASP [4, 5]. Many or perhaps all cancers may arise from cells with oncogenic mutations and oncogene expression

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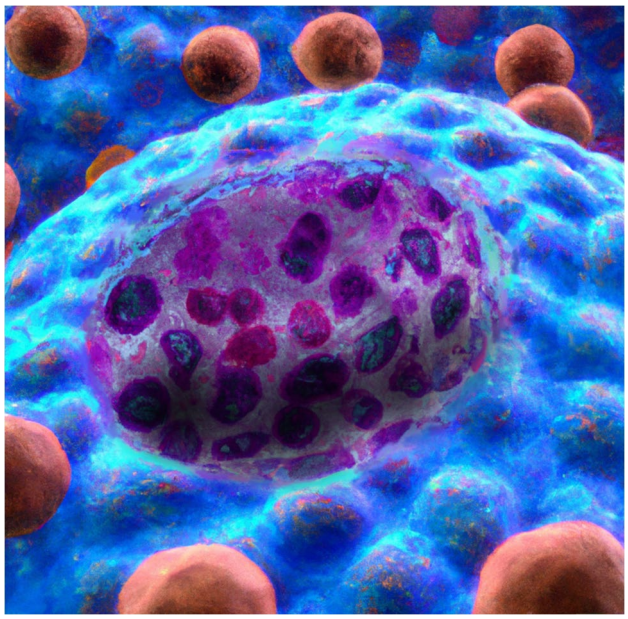

Fig. 1 Tumor cells are depicted in purple in the middle of the image. Their color is dark to indicate that they are dormant. The shadows show that these cancer-harboring senescent/dormant tumor cells are shielded from immune cells. Some of the previously senescent/dormant cancer-harboring cells are starting to show activity, making cancer recurrence and/or metastasis imminent (brighter red cells). Designed by Larissa Langhi Prata, Ph.D. using Dall-E and Microsoft Photoshop

that have entered and passed through a phase of cellular senescence. For most cellular senescence–related disorders and diseases, it is becoming apparent that eliminating that subset of senescent cells that are tissue-damaging is sufficient to alleviate dysfunction [4]. Indeed, for some disorders, it could even be best to eliminate such pro-apoptotic, tissue-destructive senescent cells while not removing those senescent cells that produce growth factors but not pro-apoptotic substances. However, in the cases of cancer-harboring senescent cells, TIS, and dormant cancer cells, all cells harboring cancerous mutations may have to be removed to guard against tumor development and relapse.

Senescent cells can attract, activate, and anchor immune cells through SASP chemokines and other SASP components [3, 4]. However, interferon-related IL-17 produced by senescent cells may influence IL-23 in T lymphocyte subsets and thereby contribute to decreased effectiveness of tumor antigen-specific immune surveillance or clearance of cancer cells. Furthermore, IL-17, IL-21, IL-22, and IL-23 may be useful blood or tissue gerodiagnostic indicators of senescent cell abundance, especially in the context of TIS and risk of cancer development or recurrence. Senescent cells can modulate the extracellular matrix through senescence-associated matrix metalloproteinases and pro-fibrotic mediators, such as TGF-β and activin-A. Potentially, the impact of TIS/dormant cancer cells on the extracellular matrix (ECM) may shield them and any proliferating cancer cells emerging from them against immune system surveillance and clearance. A great deal of research is needed elucidate the potential involvement of the ECM to determine if interventions based on targeting these ECM changes and responsible mechanisms could help attenuate cancer recurrence and metastasis.

"Cancer stem cells," "tumor-initiating cells," "disseminating tumor cells," "drug-tolerant persisters," "dormant cancer cells," "cancer-harboring senescent cells," and "therapy-induced senescent cells (TIS)" may all be closely related or even the same. Cancer harboring TIS and dormant cancer cells appear to be able to re-emerge as proliferating, multi-drug-resistant, locally invasive or metastatic cancers after months to years and may account for most cancer deaths, rather than the primary tumors. While it may be useful to distinguish cancerous mutation-harboring senescent cells from dormant cancer cells, there may be a range of intermediate cell types between these states, considerable overlap between them, or they might even be essentially identical. The bottom line is that all cancer-harboring (transiently) non-dividing cells may need to be eliminated to delay or prevent cancer recurrence, regardless of what we call them. Also, a better understanding is needed about pre-senescent cancer-harboring cells with features of senescent cells, but some limited remaining replicative potential.

Gerodiagnostic modalities are needed that can aid in detecting residual TIS and/or dormant cancer cells. Research into developing sensitive and specific screens for these cells in tissue sections, peripheral blood mononuclear cell fractions, and/or body fluids is particularly necessary, as is research to determine the extent to which factors produced by classically senescent cells are related to those potentially secreted by dormant cancer cells of various types. With respect to gerodiagnostic factors related to cellular senescence and other fundamental aging mechanisms, analyses are being conducted across multiple current clinical trials for a number of conditions [4] ranging from cancer survivors to frailty in the elderly to post-viral syndromes and dementias by the Translational Geroscience Network (R33AG 61,456). These assays include such cellular senescence-associated secretory phenotype (SASP) factors as senescence- and aging-linked proteins, peptides, non-coding nucleotides (miRNA's, circular DNA's, mtDNA), reactive metabolites (increased ROS, decreased NAD, increases in particular bradykines, ceramides, prostanoids, etc.), exosomes/ microsomes/ mitosomes; immune system signals potentially related to interactions with the SASP of TIS and/or dormant cancer cells (e.g., IFNγ/IL-17/IL-23/T lymphocyte subset axis; "don't eat me" signals; "don't find me" signals; ECM modifications caused by senescent cells related to MMPs, fibrosis/TGFβ/activin A, etc.); hemostatic/

pro-thrombotic signals emanating from TIS/dormant cancer cells (e.g., serpines, non-coding nucleotides, aggregated and misfolded proteins such as amyloid isoforms); and decreases in "geroprotective" factors such as α-Klotho.

As with identifying senescent cells, no single gerodiagnostic factor that is pathognomic of senescent cells and senescent cell burden has been generally agreed upon so far. Composite scores of SASP factors are being developed by multiple groups. The first such composite score of blood SASP factors that tracks decreases in senescent cells following treatment with senolytics, agents that selectively eliminate senescent cells [5], was published in 2019 [6]. Composite scores for quantifying senescent cell burden in tissue biopsies have also been developed [7] that may help to indicate senescent cell burden in and near cancers. Research into developing SASP factor composite scores that are sensitive enough to detect TIS and perhaps all dormant cancer cells need to be supported to facilitate clinical trials of interventions to eradicate TIS/dormant cancer cells and prevent recurrence and metastasis.

Hence, it may be feasible to use gerodiagnostic modalities to detect residual TIS/dormant cancer cells in body fluids (blood, PBMCs, urine, saliva, sputum, CSF, feces, pulmonary lavage, gastric washings, etc.), tissue biopsies (skin, subcutaneous adipose tissue, bone marrow, lymph nodes, etc. for immunohistochemical staining, RNAish, CyTOF, single cell epigenome, genome, transcriptome, proteome), imaging (PET tracers, ultrasound with tracers or for testing microfibrosis, high resolution MRI/CAT to detect suggestive but subtle changes potentially related to TIS/dormant cancer cells such as microcalcifications, skin fluorescence/reflectance, etc.), and composite scores across modalities. Furthermore, gerodiagnostic assays of other fundamental aging processes may add value to SASP factors and senescent cell–linked non-coding nucleotides or exosomes/microsomes in predicting senescent cell burden, including cancer-harboring senescent cells/dormant cancer cells. The "Unitary Hypothesis of Fundamental Aging Processes" posits that if any one aging mechanism, such as cellular senescence, is increased, many or all of the rest might be. For example, senescent cells directly or indirectly decrease geroprotective factors such as α-Klotho or NAD^+ and, conversely, decreased NAD^+ is associated with increased production of ROS that can drive cellular senescence [8].

At this juncture, there are no single, universally agreed upon fully sensitive and specific markers of all types of senescent cells. Hence, it appears unlikely that drugs targeting any single such marker will be universally senolytic. Approaches for eliminating all cancer-harboring TIS/dormant cancer cells may therefore involve using combinations of drugs or "dirty" agents to reduce or prevent cancer recurrence or metastasis.

TIS may contribute to delayed complications of cancer treatments such as the accelerated aging-like state in some childhood cancer survivors. Some patients who received chemotherapy or radiation for cancers as children develop frailty, sarcopenia, early onset of multiple age-related diseases such as Alzheimer's disease, diabetes, osteoporosis, and second, unrelated cancers and have greatly decreased lifespan. Development of this syndrome is associated with senescent cell abundance in skin biopsies. A clinical trial of senolytics for such childhood cancer survivors is currently underway (clinicaltrials.gov identifier NCT040733534), as is a trial for older bone marrow transplant recipients (clinicaltrials.gov identifier NCT02652062), and a trial in adult cancer survivors is about to begin. The impact of senolytic interventions on cancer recurrence will be important to study in subjects in these trials.

To maximize dormant tumor cell/TIS elimination throughout the body, there is a need for preclinical human tumor cell culture, tissue explant, and tissue section studies and animal models to test the impact of sequencing rounds of chemotherapy/radiation with senolytics [9]. There are already preclinical indications that sequencing chemotherapy/radiation with senolytics might enhance elimination of cancer-harboring cells in glioblastoma [10]. Such studies could answer questions about the impact of spacing of different types of interventions within each such round, the spacing between rounds, varying the forms of chemotherapy/radiation used among successive rounds, and determining the impact of using "waves" of different senolytics within each round. As highlighted by the reviews in this series, more research is needed about the possible role of myeloid derived suppressor cells (MDSC) in escape of cancer harboring senescent cells/dormant tumor cells from innate and adaptive immune responses that would otherwise guard against cancer recurrence and metastasis. Also, more research is needed relating to KISS1 and other cancer suppressive genes that may act by maintaining cancer harboring TIS/dormant cancer cells in a non-proliferative state or even clear such cells by effectively acting as senolytics.

The articles in this series all point to a need to support research focussed on the characteristics and consequences of senolytic-resistant TIS/dormant cancer cells. Indeed, directed funding and support from the National Cancer Institute, other NIH and governmental components, public and private foundations, and universities around the world is urgently needed.

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Declarations

Conflict of interest James L. Kirkland has a financial interest related to this research, including patents and pending patents covering senolytic drugs and their uses that are held by Mayo Clinic. This research has been reviewed by the Mayo Clinic Conflict of Interest Review Board and was conducted in compliance with Mayo Clinic conflict of interest policies.

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