Implications of cellular senescence in paediatric pituitary tumours

Jose Mario Gonzalez-Meljem[a,*] and Juan Pedro Martinez-Barbera[b,**]

[a]Tecnologico de Monterrey, School of Engineering and Sciences, Mexico City, Mexico
[b]Developmental Biology and Cancer Programme, Birth Defects Research Centre, UCL Institute of Child Health, London, UK

eBioMedicine
2024;99: 104905
Published Online xxx
https://doi.org/10.1016/j.ebiom.2023.104905

Summary

The long-standing view of senescent cells as passive and dysfunctional biological remnants has recently shifted into a new paradigm where they are main players in the development of many diseases, including cancer. The senescence programme represents a first line of defence that prevents tumour cell growth but also leads to the secretion of multiple pro-inflammatory and pro-tumourigenic factors that fuel tumour initiation, growth, and progression. Here, we review the main molecular features and biological functions of senescent cells in cancer, including the outcomes of inducing or targeting senescence. We discuss evidence on the role of cellular senescence in pituitary tumours, with an emphasis on adamantinomatous craniopharyngioma (ACP) and pituitary adenomas. Although senescence has been proposed to be a tumour-preventing mechanism in pituitary adenomas, research in ACP has shown that senescent cells are tumour-promoting in both murine models and human tumours. Future studies characterizing the impact of targeting senescent cells may result in novel therapies against pituitary tumours.

Keywords: Cellular senescence; SASP; Pituitary tumours; Craniopharyngioma

Introduction

The term *cellular senescence* is widely used to describe a phenotype characterized by an irreversible (or at least long-lasting) exit from the cell cycle, mainly upon chronic exposure to molecular stress and damage. First discovered as an outcome of continuously culturing finite human cell lines, the potential implications of such a mechanism in aging and cancer, proposed by the authors, were very controversial at the time and not immediately recognized by the scientific community.[1] Today, more than 60 years after being first described, there is sufficient evidence demonstrating that senescent cells are involved in normal physiological processes on one side, while contributing to ageing and multiple pathologies on the other. This dichotomy is best appreciated in cancer, where cellular senescence can efficiently prevent tumour growth and progression, while paradoxically mediating a plethora of protumourigenic processes in several cancers.

The pituitary gland, or hypophysis, can harbour distinct types of neoplasias that are widely considered histologically benign (WHO I classification). However, some pituitary tumours can behave aggressively in the clinic, showing rapid growth and local invasive behaviour, becoming resistant to treatment, or acquiring a tendency for relapse. This is particularly relevant to Adamantinomatous Craniopharyngioma (ACP), the most common pituitary tumour in children and young adults. Clinical management of ACP can be considerably difficult, mainly due to significant post-treatment morbidity, leading to poor quality-of-life and shortening lifespan in many patients. Research over the last decade has provided unique insights into the aetiology and pathogenesis of ACP, leading to clinical trials targeting pathogenic signalling pathways. Importantly, findings in ACP murine models and human ACP have uncovered cellular senescence as a key factor in tumour initiation and progression, as well as a novel therapeutic target.

The main aim of this review article is to describe our current knowledge on the role of cellular senescence in the initiation and further development of paediatric pituitary tumours. We will focus on ACP, due to its relevance for the paediatric population, but we will also discuss research conducted in pituitary adenomas (also known as pituitary neuroendocrine tumours, PitNETs). Finally, we will describe findings from studies in other cancer models which highlight the relevance of senescence as a potential target against cancer.

Senescent cells: an overview

Senescent cells have common molecular features

A major outcome from the last two decades of research into the causes of cellular senescence is that this phenotype can be induced by chronic exposure to a growing list of cellular and molecular damaging factors.[2]

*Corresponding author.
**Corresponding author.
E-mail addresses: jmgonzalezmeljem@tec.mx (J.M. Gonzalez-Meljem), j.martinez-barbera@ucl.ac.uk (J.P. Martinez-Barbera).

Among the most studied senescence inducers are the attrition of telomeres, oncogene signalling (or the loss of tumour-suppressor genes), chronic DNA damage caused by both physical and chemical agents (including radiation and chemotherapeutics), in addition to metabolic stress caused by oxidative species and mitochondrial dysfunction.[3–5] On the other hand, a growing body of evidence has also shown that senescent cells can arise through other mechanisms not directly related to chronic stress or damaging stimuli, as senescent cells have been shown to exist transiently during embryonic development in mice, zebrafish, amphibians, birds, naked mole rats and humans (known as developmentally programmed cellular senescence).[6–12]

The senescent phenotype varies widely at the molecular level, differing even among individual cells within the same population. Major determinants include the cell-type induced to senesce, the type of damaging stimulus, time after stimulation, the cellular microenvironment (e.g., differential responses between *in vitro* and *in vivo*, or between 2D and 3D systems) and interactions with the immune system.[13,14] There are, however, some features that can be considered common across senescent and "senescent-like" states.[15,16] First, most senescent cells appear to have an expanded lysosomal compartment, which is related to their amplified metabolism. This fact allowed the establishment of the Senescence-Associated Beta-Galactosidase (SA-β-Gal) assay,[17] a straightforward technique based on labelling the over-accumulation of Lysosomal β-Galactosidase (encoded by the GLB1 gene) in senescent cells.[18,19]

The most elemental features in the definition of a senescent cell are a stable cell cycle arrest and the resulting long-term absence of cell proliferation, even in the presence of growth-inducing conditions.[20] However, *in vivo* and *in vitro* experimental demonstration of these features is often overlooked in senescence characterization studies, often in favour of more convenient, albeit non-specific, molecular markers such as SA-β-Gal. Assessing a lack of cell division can be conducted through proliferation assays in culture, while the cell-cycle arrest should be corroborated through the absence of DNA nucleotide analogue incorporation during the DNA replication phase (e.g., EdU), or through the lack of cell cycle marker expression such as Ki67, phosphorylated Histone H3 or phosphorylated retinoblastoma protein (pRB).[21]

In senescence characterization studies, it is now standard practice to assess the robust expression of the two main cell cycle-inhibiting pathways p53/p21$^{WAF/CIP1}$ and p16^{INK4a}/RB, as both (or at least one) of these pathways are crucial for the initiation and establishment of most reported senescent cell states.[22] Caution should still be taken as the expression of p21 (encoded by the *CDKN1A* gene) is can also be found in quiescent cells. Although the expression of p16 (encoded by the *CDKN2A* gene) is generally considered to be a more robust marker of a well-established senescent phenotype, it can also be expressed in proliferating tissues and cells (e.g., cancer cells with RB loss of function).[23] As both examples suggest, the use of only a single marker could be misleading during the characterization of senescent cells.

Another hallmark of cellular senescence is the unresolved accumulation of DNA damage (e.g., double-strand breaks), which leads to the long-term activation of the DNA-Damage Response pathway (DDR). DNA damage and the DDR can be assessed by the expression of markers such as γH2A.X, pATM or pATR.[24] Other senescence and DNA damage associated features include nuclear lamina disruption (evaluated by loss of Lamin B1) and the presence of Senescence-Associated Heterochromatin Foci (SAHF), which can be detected by DAPI puncta and increased localized expression of the histone variants H3K9Me2/3, MacroH2A, or HP1γ.[25] Furthermore, DDR signalling will reinforce the cell cycle arrest through the p53/p21$^{WAF/CIP1}$ and p16^{INK4a}/RB pathways and lead to overexpression of BCL-2 family members such as BCL-XL and BCL-W, which can be measured to show resistance to apoptosis.[26,27] The senescent phenotype is thus a highly multifaceted, dynamic, and context-dependent cellular state. Although the SA-β-Gal assay is the most widely used method for identifying senescence, detailed molecular and cellular studies should always be conducted afterwards, as SA-β-Gal can be present in some proliferating or quiescent cells.[28] Thus, unequivocal identification of senescent cells requires characterization of multiple markers belonging to the diverse hallmark molecular pathways and cellular processes that characterise senescence.

Senescent cells are paracrine signalling powerhouses

Despite having a name suggesting a deteriorated function, senescent cells are characterized by a viable and overcharged metabolism which allows them to synthesize and secrete large amounts of cytokines, chemokines, growth factors, extracellular matrix (ECM) components and remodelling factors, as well as other pro-inflammatory metabolites and extracellular vesicles.[5] This signalling arsenal is known as the Senescence-Associated Secretory Phenotype (SASP), and it is a hallmark senescence feature in addition to the ones previously described. SASP gene expression is driven by the proinflammatory master transcription factors NF-κB and C/EBPβ, which lie downstream of the DDR. In addition, the onset and maintenance of the SASP depend on different pathways involved in cell growth, metabolism and molecular stress response regulation including pI3K/AKT/mTOR, p38-MAPK, JNK/ERK, inhibition of selective GATA4 autophagy, cGAs-STING, the NLRP3 inflammasome and Notch, among others.[5]

The majority of reported senescent phenotypes produce a SASP and share some canonical members such as IL6, IL8, IL1A, CCL2, CXCL1 and CXCL2, whose expression can be measured as part of senescence characterization studies. The exact list of secreted components (and their amounts) can widely vary across biological conditions and time, and even differ among individual cells within the same senescent population.[29] Some major determinants of SASP diversity that should be considered are the senescence inducer (e.g., developmental programming, chemotherapeutics, radiation, oncogenes, etc.), the induced cell type, time during and after exposure, the number of initial senescent cells, and the status of certain senescence-related pathways (e.g., TP53 mutations).[4]

One of the best studied consequences of paracrine SASP signalling is the induction of senescence in neighbouring proliferating cells (known as bystander effect or paracrine senescence) and the reinforcement of pre-senescent phenotypes.[5] Nevertheless, the SASP can significantly impact microenvironmental and systemic functions on both pathological and physiological contexts. For example, current evidence suggests that developmentally programmed senescent cells (and their SASP) are required for the proper formation of some organs including the mesonephros, otic vesicles, endolymphatic sacs and developing limbs.[7,9–12,30–32] Moreover, senescent cells been shown to mediate tissue repair through control of partial cell reprogramming or differentiation in the liver, muscle, and skin.[6,33–35] In summary, current evidence supports the notion that senescence can have beneficial roles in wound healing and regeneration when their presence and SASP signalling effects are acute (which follows their removal), while detrimental roles of senescent cells are widely observed in chronic wounding scenarios.

Senescent cells and their SASP can be targeted in disease contexts

The burden of cellular senescence in organs and tissues represents a strong predictor of both health-span and lifespan in multiple species.[36] Two breakthrough studies showed that elimination of senescent cells can lead to significant improvements in organ function during ageing, in addition to increased life expectancies.[37,38] Importantly, small molecule-guided elimination of senescent cells propelled further research implicating them in the development and/or progression of multiple pathologies including diabetes, atherosclerosis, rheumatoid arthritis, metabolic dysfunction, fibrosis (including in lungs, heart, and kidneys) and neurodegenerative disease.[39,40] Such molecules able to exclusively (or preferentially) target senescent cells and their SASP are collectively known as senotherapeutics.

Senotherapeutics can be further classified according to their specific mode of action. Most studies have made use of senolytics (which kill senescent cells), including inhibitors of BCL-2 antiapoptotic pathway members, the Heat Shock Protein 90 chaperone family (HSP90), several tyrosine kinases and some cardiac glycosides, among others. Alternatively, compounds able to specifically target the SASP and its paracrine effects have also been investigated. These are known as senomorphics and some of the most successful compounds within this category include rapamycin (an inhibitor of the mTOR pathway, commonly known as sirolimus) and metformin (which targets the NF-κB pathway). Importantly, a growing number of these senotherapeutics are under phase I and II clinical testing for their safety and effectiveness in age-related pathologies. We refer the reader to comprehensive reviews on these and newer senotherapeutic compounds.[41,42]

Senescent cells have dichotomous roles in cancer and tumourigenesis

A wealth of evidence indicates that cellular senescence can act as a potent anti-tumour growth mechanism by arresting the proliferation of cancerous cells in response to oncogenic signalling, a process known as Oncogene-Induced Senescence (OIS). Notably, the chronic induction of DNA-damage caused by chemotherapy and ionizing-radiation can lead to the onset of Therapy-Induced Senescence (TIS) in tumours. Moreover, TIS is accompanied by a SASP capable of inducing bystander senescence in cancerous (and normal) neighbouring cells, and mediate activation of the immune system for tumour cell clearance.[4,5]

Despite its tumour suppressor activities, senescence and the SASP can drive serious adverse effects during cancer pathogenesis. For example, an elegant study in models of acute lymphoblastic and acute myeloid leukaemias showed that senescence can lead to cell autonomous acquisition of stemness phenotypes (and thus increased malignancy) after senescent cells successfully escape their cell cycle arrest.[43] Paracrinally, the SASP can drive cancer cell proliferation, malignant progression (such as promoting the acquisition of novel mutations and the induction of invasive phenotypes), tumour immune evasion, resistance to therapy-induced cell death, and angiogenesis, in addition to the formation or maintenance of metastatic niches.[44] Notably, the SASP can also promote the acquisition of mutations in cells lacking the original driver oncogenic mutation and thus leading to the growth of paracrinally induced tumours. We have named this process as senescence-induced tumourigenesis and refer the reader to a more thorough review on its implications.[45]

There is growing evidence showing that altering the tumour microenvironment (TME) can be a major consequence of SASP signalling. In this regard, significant advances have been made by studies showing their influence during chronic wounding, fibrosis and inflammation, all of which are processes that can strongly

impact tumour development and progression.[46–48] For example, researchers showed that specific targeting of senescent cells in mouse models of pulmonary fibrosis leads to a reduction on abnormal collagen deposition and restoration of pulmonary function.[49] This, and evidence derived from other studies in mouse models and human clinical trials, support the notion that senescent cells and the SASP are significant players in the development and progression of pulmonary fibrosis.[50–52] From this we expect future studies to arise addressing the impact of senescent cells, and the SASP, in cancer predisposition enabled by chronic wounding, fibrosis and inflammation.

Altogether, the evidence suggests senescent cells represent a mixed blessing in cancer pathogenesis, prompting support for the advancement of one-two punch sequential therapies wherein tumour growth can first be stopped by traditional senescence-inducing approaches (e.g., radiotherapy, chemotherapy, or targeted therapies), followed by senotherapeutic clearance (e.g., senolytics).[53–55]

Evidence of cellular senescence and their impact in paediatric pituitary tumours

Pituitary tumours represent 20% of all intracranial tumours in children and despite their mostly benign histopathological features, they can display aggressive clinical behaviour due to compression and/or invasion of surrounding brain structures such as the hypothalamus, cranial nerves, and optic tracts. Although some tumours can be managed medically (e.g., reducing the effects of hormone secretion in pituitary adenomas using specific drugs), large and aggressive tumours are mainly treated with surgery and/or radiotherapy. These non-curative treatments result in high recurrence frequency and significant morbidity including panhypopituitarism, blindness and hypothalamic syndrome, leading to poor quality of life for many patients or even premature death.[56]

Two major types of tumours can arise in the pituitary region at this life stage: craniopharyngiomas and pituitary adenomas, also known as pituitary neuroendocrine tumours (PitNETs). Craniopharyngiomas (CPs) are the most common type of intracranial non-neuroepithelial neoplasm in children and represent around 80% of cases associated to the pituitary gland in this population.[57] Their clinical presentation involves symptoms mostly derived from increased intracranial pressure and disrupted pituitary hormone secretion. These include visual impairment, nausea, headaches, and pathological growth retardation which is often followed by weight gain indicative of hypothalamic obesity.[57] Although post operative long-term survival is high, mortality is around 3–5 times higher than the general population. This increased mortality has been associated to long term complications including tumour recurrence (around 25% of cases), cerebrovascular disease, chronic neuroendocrine alterations, morbid hypothalamic obesity, non-alcoholic fatty disease, metabolic syndrome, and cardiovascular disease.[57,58]

Although all CPs are thought to originate from remnants of Rathke's Pouch (the embryonic pituitary precursor), they are classified into two subvariants that can be distinguished at genetic, epidemiological, and pathological levels: papillary craniopharyngiomas (PCPs) and adamantinomatous craniopharyngiomas (ACPs). PCPs, normally affecting the elderly, carry mutations in the *BRAF* gene and are thus characterized by MAPK pathway over-activation,[59] while ACPs are diagnosed in children and adults and are characterized by mutations in the *CTNNB1* gene leading to the over-activation of the canonical WNT pathway.[57]

ACPs are the most common subvariant found in children.[60] MRI assessment of the sellar region describe masses with variable levels of solid and cystic compartments, the latter containing a characteristic oily substance known as colloid. Differential diagnosis can also be further informed by computed tomography (CT) imaging for the identification of calcified regions, another ACP hallmark.[57] Histologically, the solid neoplastic component of ACPs displays a complex arrangement of different compartments including tightly packed columnar cells arranged as a "palisaded epithelium", regions of loosely connected cells together with microcystic formations (known as "stellate reticulum"), epithelial cell whorls, anucleate cell remnants (known as "ghost cells" or "wet keratin"), calcifications and immune cell infiltrates.[61]

Adamantinomatous craniopharyngiomas harbour senescent cells with potential paracrine tumour-promoting activities

Although senescence is usually associated with aged tissues, research on ACPs has demonstrated the presence of senescent cells in mouse and human tumours at an early age and revealed a key role in the pathogenesis of these neoplasias.

Several sequencing studies have established that the vast majority of ACPs carry activating mutations in exon 3 of the β-catenin gene (*CTNNB1*). This mutation prevents the β-catenin protein from being degraded, thus accumulating in the cytoplasm, and translocating to the nucleus where it unrestrictedly activates the expression of canonical WNT pathway genes. Immunostaining in histological sections allows the observation of cells that accumulate nucleocytoplasmic β-catenin, either arranged in tight cell groups or whorls (or simply β-catenin cell clusters, as we will refer to them hereafter), or dispersed throughout the tumour as single cells. Both sequencing for *CTNNB1* or staining for nucleocytoplasmic β-catenin accumulation have thus become standard practices in the accurate differential diagnosis of ACPs from other pituitary tumour entities.[62]

Two mouse models in which a similar *Ctnnb1* mutation is targeted to the pituitary gland, either in embryonic HESX1+ precursor cells (an embryonic ACP model) or in adult SOX2+ stem cells (an inducible ACP model), develop lesions and tumours closely resembling human ACP at the molecular, cellular, and even imaging levels.[63–65] Initial molecular and cellular studies conducted in these mouse ACP models led to important insights about the mechanisms underlying ACP tumorigenesis. First, expression of oncogenic β-catenin in pituitary stem cells or embryonic progenitors was sufficient to induce tumours.[63,66] Before tumour formation, oncogenic β-catenin expression results in the formation of clustered groups of cells showing nucleocytoplasmic β-catenin accumulation and overactivation of the WNT pathway. These β-catenin accumulating cell clusters greatly resemble those found in human ACP, both at the histological and molecular levels. Second, the formation of β-catenin cell clusters precedes tumour development (with latency periods ranging from 18 weeks in the embryonic model to 6 months in the inducible one), which is interesting considering that neonatal and *in utero* ACP cases have been reported. Third, β-catenin cell clusters are paracrine signalling hubs that secrete a plethora of protumourigenic factors including developmental factors, ECM components, as well as several cytokines and chemokines. Altogether, these mouse models have been instrumental in establishing the importance of WNT pathway activation in ACP pathogenesis and revealing that *CTNNB1* mutations are oncogenic drivers in human ACP.[63,64]

Aiming to further understand the role of β-catenin accumulating cell clusters in ACP pathology, our group first showed that cluster cells are mostly devoid of proliferation markers, initially suggesting they could be akin to slow dividing cancer stem cells. Rather unexpectedly, genetic lineage tracing and sequencing experiments in both models showed that cells carrying the oncogene do not give rise to the tumours themselves, with the latter even displaying distinct *de novo* genetic alterations (i.e., lacking a *Ctnnb1* mutation). These findings thus indicate the existence of a non-cell autonomous mechanism of tumour formation in mouse ACP models.[66,67]

Extensive molecular characterization studies at RNA and protein levels later indicated that β-catenin cell clusters in both mouse ACP models, and in human ACP, do not actively divide because they are senescent. As explained in the previous section, cellular senescence is a complex phenotype that requires the evaluation of an assortment of hallmark processes. In brief, mature β-catenin cell clusters show absence of proliferation and apoptosis markers, a permanent expression of cycle inhibiting (e.g., $p21^{CIP1}$/p53) and DNA Damage-Response pathways (e.g., γH2A.X), an expanded lysosomal compartment (e.g., positive for SA-β-Gal), as well as constitutive NF-kB signalling (e.g., pIκBα). Additionally, β-catenin cell clusters were shown to possess a SASP comprised mostly of developmental factors of the WNT, FGF, BMP and SHH families,[64,66,67] along with some canon SASP members including: IL6, IL1, CXCL1, CXCL2 and CCL20[67] (Fig. 1). Importantly, other data pointing at β-catenin cell clusters as proinflammatory and protumourigenic signalling centres has been recently reported using single cell RNA sequencing (scRNA-seq) coupled to bioinformatic inference of cell–cell communication and ligand–receptor pair interactions.[68]

In ACP mouse models, senescent cell clusters and their SASP appear capable of drastically altering the pituitary microenvironment. This includes changes in ECM composition and structure, recruitment of migrating undifferentiated cells and inducing proliferation in cells directly in contact with β-catenin clusters. To show that senescence is involved in ACP tumourigenesis, two different mouse models were generated (one targeting embryonic pituitary precursors and another targeting adult stem cells) in which senescent β-catenin clusters with WNT pathway overactivation still formed, albeit with a significantly diminished SASP expression. Notably, pituitaries with attenuated-SASP clusters lack microenvironmental changes and are not capable of forming tumours, further supporting a model of senescence-mediated paracrine tumourigenesis.[67]

The cell non-autonomous contribution of senescent cells to tumour formation and progression has also been recently exemplified. This was done using a mouse model capable of labelling, isolating, and ablating senescent cells in which macrophages and their SASP were shown to be critical in the initiation and progression of lung adenocarcinomas.[69] The adoption of a similar genetic-ablation approach in mouse ACP models is warranted to provide conclusive evidence of the role of senescent β-catenin clusters in tumour formation.

Current evidence indicates that paracrine tumorigenic mechanisms are also critical for human ACP. Importantly, human β-catenin cell clusters are molecularly similar to those found in murine models by sharing a signature of senescence and SASP. Histological and imaging analyses show these clusters are commonly found at the vanguard of the tumour's invasive front, which takes the form of epithelial finger-like protrusions that infiltrate normal brain tissue (Fig. 1). Clusters found in such protrusions are observed in close contact with both brain and tumour regions displaying high cell proliferation rates, strong expression of inflammatory markers (e.g., presence of reactive glia) and overactivation of protumourigenic pathways like MAPK/ERK.[70] Notably, senescent clusters express high levels of SASP factors including several FGFs and EGF, which can activate the MAPK/ERK pathway.[70] Furthermore, the targeted inhibition of this pathway using MEK inhibitors (e.g.,

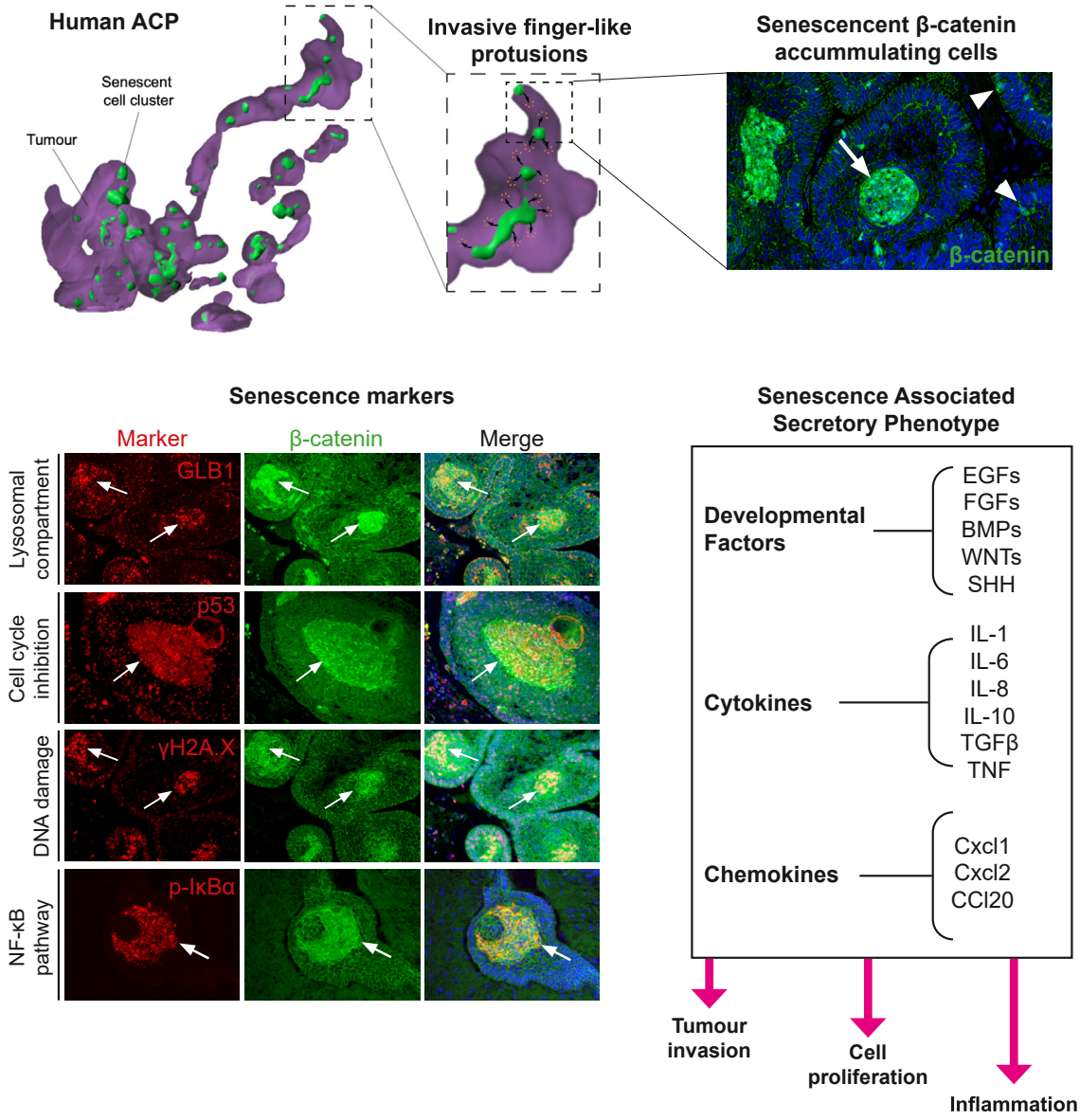

Fig. 1: **Human ACP contains β-catenin accumulating cells that are senescent and display a secretory phenotype.** A three-dimensional reconstruction of multiple micro-CT images taken from a case of adamantinomatous craniopharyngioma (ACP) shows the area occupied by the tumour itself (shown in purple) and regions occupied by epithelial whorls (shown in green). Note that epithelial whorls often lie at the boundaries of finger-like protrusions that invade surrounding brain tissue (shown in first inset). Epithelial whorls frequently contain β-catenin accumulating cells that can be detected by immunostaining (arrow, second inset). Of note, β-catenin accumulation can additionally be found in individual cells distributed throughout the tumour, including the palisaded epithelium (arrowheads in second inset). A panel of multiple senescence markers (shown in red) colocalize with β-catenin (shown in green) accumulating clusters (arrows in panel) in double immunostaining experiments, indicating a senescent phenotype in cluster cells. Additionally, senescent clusters express a SASP composed of several pro-inflammatory and protumourigenic secretory factors that mediate tumour invasion, cell proliferation and inflammation in surrounding cells. The single immunofluorescence staining in human ACP image was adapted with permission from Carreno G et al. (2016) Stem cells and their role in pituitary tumorigenesis. *Mol Cell Endocrinol* 445:27–34. The micro-CT 3D model is adapted from Apps J et al. (2016). Imaging Invasion: Micro-CT imaging of adamantinomatous craniopharyngioma highlights cell type specific spatial relationships of tissue invasion. *Acta Neuropathologica Communications*, *4*(1), 57. The double immunofluorescence panel is adapted from Gonzalez-Meljem JM et al. (2017) Stem cell senescence drives age-attenuated induction of pituitary tumours in mouse models of paediatric craniopharyngioma. *Nat Commun* 8:1819. Both are open-access articles licensed under a Creative Commons Attribution 4.0 International License.

trametinib) led to reduced proliferation and increased apoptosis in both murine and human tumours.[70] It also is possible that cluster secretions might contribute to the development of other tumour compartments. For example, many SASP factors have been detected within the tumour's cystic fluid, which is suspected to mediate neuroinflammation.[71]

In conclusion, evidence has shown that senescence is relevant in both mouse and human tumours and that SASP modulation may result in reduced tumour burden and potentially impair cystic fluid formation. Together, the data support further research on the potential of senotherapies in the management of ACP.

Evidence of senescence in pituitary adenomas

Pituitary adenomas (PAs) are currently classified according to their expression of the lineage-restricted pituitary transcription factors TPIT, PIT1 or SF-1. Detailed pathological characterization involves determining the expression (or lack thereof) of anterior pituitary hormones, cellular proliferation indexes, and P53 expression. Additional anatomical classifications derived by imaging and/or surgery involve features as tumour size, direction and symmetry of growth, treatment refractiveness, as well as infiltration level of the cavernous sinus and suprasellar structures.[56,72]

PAs comprise around 5% of pituitary tumour cases in paediatric and adolescent populations.[73] Most paediatric PAs are positive for growth hormone (GH), adrenocorticotropic hormone (ACTH) and prolactin (PRL), and similarly to their adult counterparts, they are vastly slow growing and histologically benign.[73,74]

In somatotropinomas, evidence from animal models, cell lines and human samples indicates an important role for $p53/p21^{WAF/CIP1}$ mediated senescence, and the SASP, in restricting adenoma cell growth.[75–77] Importantly, growth hormone (GH) hypersecretion appears to be part of the SASP in this context, which can induce the accumulation of DNA damage in normal pituitary cells and other organs, such as the colon.[78–81] More recently, a mechanism underlying this activity was unveiled in a study showing that GH signalling leads to WIP1-mediated suppression of the DDR pathway.[82] In the future, it would be interesting to explore the effect of either eliminating these GH-secreting senescent cells, and their SASP, or describing the effect of inhibiting their senescent program, both in somatotropinomas and other organs. Such studies would certainly provide important insights regarding the application of senotherapeutics in somatotropinoma management.

Beyond somatotropinomas, evidence of cellular senescence in other PA subtypes has been produced through a few select markers in histological sections, such as p53, p21, p16, KI67 and SA-β-Gal.[83,84] As these markers have only been observed separately, it is important that further studies build upon this evidence by showing the coexpression of these and other important markers of senescence (DNA damage, DDR, NF-κB signalling, the SASP, etc.) to accurately determine the identity and proportion of the senescent population(s) in PAs. A noteworthy example lies in a study in which multiple cellular senescence markers including SA-β-Gal, the absence of cell proliferation, presence of DNA damage, expression of cycle inhibitors p53 and p21, were used to demonstrate lactotroph senescence in an oestrogen-induced PA model in rats.[85]

The SASP, which is a hallmark of senescence, has also been scarcely described in senescence studies of PAs, except for the canonical SASP member Interleukin-6 (IL6). This potent cytokine is normally secreted by pituitary folliculostellate cells and has been shown to be able to induce senescence in adenoma cells, while it can also have protumourigenic effects in cell lines and adenoma transplant models.[86]

Unfortunately, there is a scarcity of studies addressing the presence of cellular senescence in other types of PAs and that have conducted a thorough molecular characterization of this phenotype. Such studies are nonetheless warranted to be conducted in the future, given a report of upregulated expression of genes associated to cellular senescence in different PA subtypes.[87]

In summary, cellular senescence studies in PAs have mostly focused on somatotropinomas, postulating that cellular senescence is a safeguard for tumour proliferation and malignant progression in PAs. It is also worth noting that most studies have focused on the tumour parenchyma compartment, with little emphasis on the tumour stroma (i.e., non-neoplastic cells in the TME). Notably, evidence is emerging for a role of stroma in PA pathogenesis.[88,89] Whether some of these protumourigenic stromal cells are senescent is an interesting question that merits further research.

Implications of therapy-induced senescence and senotherapeutic interventions for pituitary tumour management

An important lesson from the last decade of research into the role of senescence in cancer is that both radiation and chemotherapeutics not only cause cancer cell death in tumours, but also lead to the induction of therapy-induced senescence (TIS) in both cancerous and normal cells.[90–95] Importantly, a host of molecular anticancer therapies have been shown capable of inducing senescence, specially at lower and chronic doses. We refer the reader elsewhere for comprehensive lists and reviews on TIS inducing agents and their applications.[92,96] Although TIS has only begun to be studied in depth recently, the evidence generated so far has shed important insights into its implications in tumours and off-target organs.

Therapy-induced senescent cells have significant local and systemic detrimental effects

Similar to the endogenous OIS response, TIS has a dualistic role in tumours. In many contexts, it can have a beneficial result by slowing down or completely arresting tumour cell growth, as senescent cells are unable to divide even in the presence of oncogenic growth signals.[96–99] However, this benefit can be nullified in the long term by senescent cell-mediated protumourigenic effects by both cell autonomous and paracrine mechanisms. Cell autonomously, senescence can drive the accumulation of genomic instability, development of resistance to both radio- and chemotherapy[100–102] and the acquisition of more aggressive and cancer-stem cell phenotypes upon senescence escape.[43,103] Cell non-autonomously, the SASP can spread such effects throughout the tumour microenvironment but can additionally drive other protumourigenic activities such as tumour cell proliferation, increased invasiveness, cancer relapse, immune evasion of cancer cells and tumour angiogenesis, among others.[44,104]

More recently, attention has been brought to the repercussions of inducing TIS in off-target organs. For example, TIS and the SASP has been shown to mediate the dysfunction of salivary gland stem cells underlying the hyposalivation associated with head and neck cancer radiotherapy.[105] Importantly, they have been implicated in the induction of therapy-related myeloid neoplasms,[106,107] and shown to promote post-chemotherapeutic systemic side effects such as inflammation, diminished physical robustness, fatigue, cardiac toxicity and loss of bone density.[104,108] It will be therefore of great importance to study and understand the implications of irradiation, chemotherapeutics, and resective surgery on the off-target induction of cellular senescence during pituitary tumour management (Fig. 2).

Targeting senescent cells or their secretory phenotype can potentially improve anti-tumour therapies

Currently, several studies across various *in vitro* and *in vivo* cancer models have provided compelling evidence of the beneficial effects of senotherapies in the context of cancer treatment. These mostly include scenarios where senescent cells are endogenously generated by the OIS response, as shown for example in two independent studies in which genetic and chemical depletion of senescent macrophages significantly diminished tumour burden in murine lung adenocarcinoma models.[69,109] Additionally, another study showed that elimination of senescent cancer cells leads to increased survival in a murine glioblastoma model.[110] On the other side, targeting the TIS response has yielded beneficial outcomes in models epithelial ovarian, non-small cell lung and pancreatic cancers, along with lymphoma.[111–115] Despite these promising results, further preclinical investigation will be required before initiating clinical testing of senotherapeutics as adjuvants in cancer treatment.

Although there are not many studies regarding the effects of targeting senescent cells in paediatric populations, there is encouraging evidence from models for ACP, pilocytic astrocytoma and diffuse intrinsic pontine glioma (DIPG).[67,116–118] Follow-up studies will certainly support future use of senotherapies in paediatric tumour management. As young tissues have very low or negligible levels of senescence, this reduces the potential risk associated from unselectively eliminating non-cancerous senescent cells and affecting their physiological functions, which represents an important safety concern in adult cancers.[119]

Conclusions and outstanding questions

Senescence is a critical player in cancer, and its significance has recently been highlighted by its inclusion as a novel hallmark of cancer.[120] The perception that senescence is an exclusive phenomenon of cancerous cells is also changing, and it is increasingly evident that senescent cells within the tumour microenvironment may also be critical in tumour development and progression.[69,109,121–124] Moreover, it is now becoming clear that senescent cells can be targeted in the context of cancer, as well as other diseases in mouse models and humans.[52,125,126]

So far, however, less emphasis has been given to the study of senescence in children. After all, the accepted idea in the field is that children are devoid of senescent cells. This statement holds true for 'healthy' children, but growing evidence shows that paediatric tumours contain functional senescent cells that can contribute to tumourigenesis.[45,127] Moreover, it is possible that senescence may be pathogenic in a variety of congenital disorders.[9,10]

In the case of ACP, further research is required to translate these findings into clinical trials, but current evidence suggests that senotherapy may prove helpful to complement the current standard of care for patients (i.e., surgery and radiotherapy). It is possible that senescent cells in other childhood tumours may also be protumourigenic, and their elimination may reveal useful as adjuvant therapies to other anti-cancer treatments. The low burden of senescent cells in normal tissues in children and young adults, relative to the elderly, may offer some advantages when testing novel senotherapies against cancer, as on-target side effects are expected to be diminished in comparison with the adult population.

We anticipate that more emphasis will be put in the future into the identification of specific senescence inducers and senolytics, tailored to specific disease contexts. Likewise, we expect that further studies aiming to understand the role of senescent cells in non-tumour cell compartments (e.g., TME cells such as fibroblasts, endothelial cells and macrophages and other immune cells) will provide key knowledge to improve current cancer therapies.

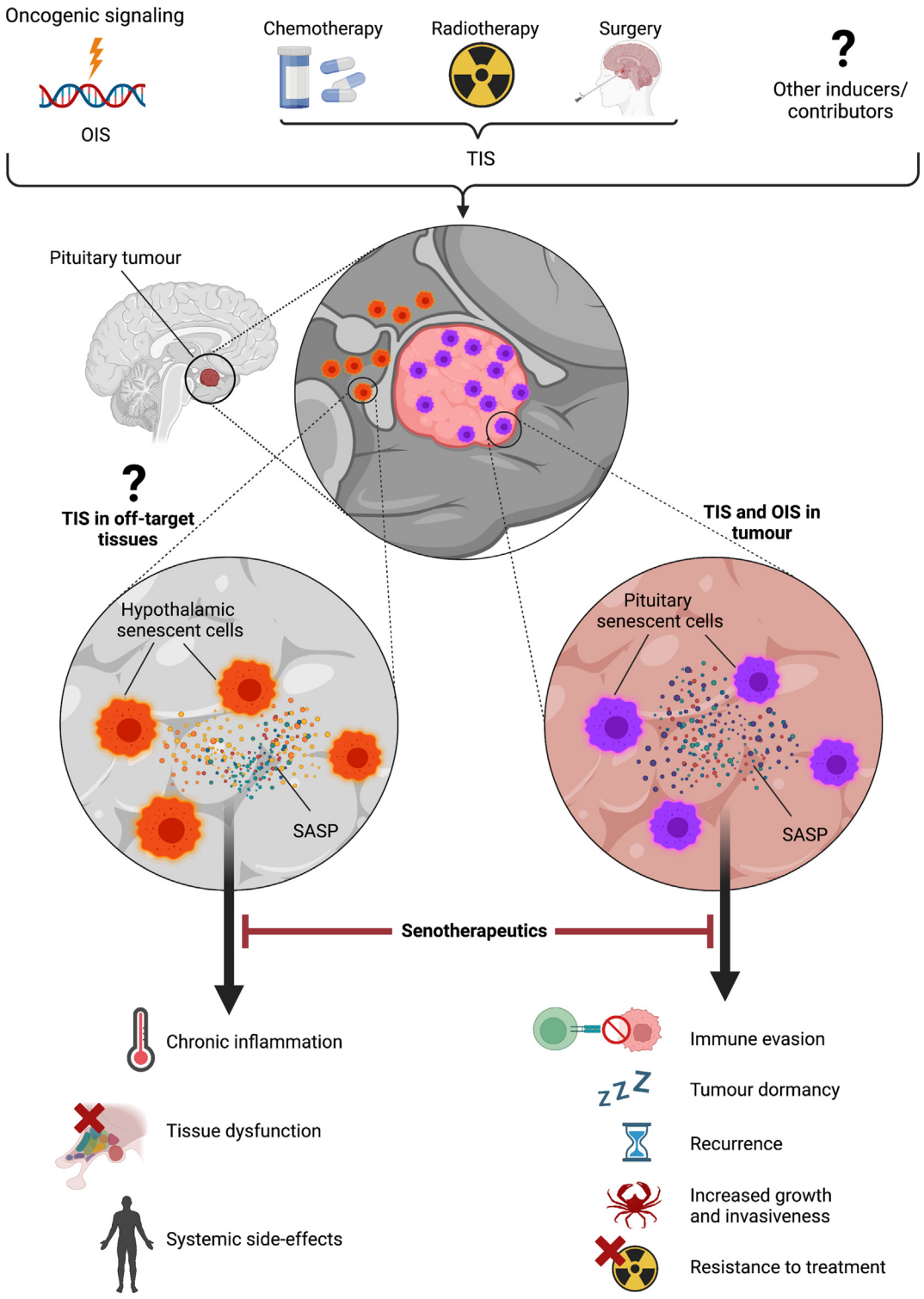

Fig. 2: **A model of the implications of OIS and TIS in pituitary tumour management.** Senescent cells can be induced within pituitary tumours and neighbouring tissues by different factors. Within tumours, the expression of oncogenes (and the loss of tumour suppressor genes) will induce Oncogene-Induced Senescence (OIS). Additionally, chemotherapy, irradiation and resective surgery all can potentially contribute to the generation of Therapy-Induced Senescence (TIS) on any remaining tumour tissue and off-target organs. Other possible contributing factors to OIS and TIS may include any that can lead to a senescence-permissive environment (e.g., in the context of impaired detection and removal of

Search strategy and selection criteria

Articles revised for this manuscript were obtained through a search using PubMed and Scopus using searches for the following terms: "cellular senescence", "senescence", "senescent cell", "oncogene-induced senescence", "therapy-induced senescence", "senolytic", "senomorphic", "senotherapeutic", "adamantinomatous craniopharyngioma" "craniopharyngioma", "pituitary paediatric tumour", "pituitary tumours", "pituitary tumour treatment". Due to a limitation in the number of references allowed for this review, we were unable to cite a considerable part of the primary literature and have thus focused our review on literature published during the last 5 years.

Despite substantial advances in the senescence field during the last two decades, we are still learning about the complexity of senescent phenotypes and their multifaceted functions, both in normal physiology and in disease. Further research is needed to better characterise senescent cells in a variety of normal and disease contexts, not just in murine models but also in humans. Deep understanding of their functions is required for the development of well-designed and rational clinical trials. In cancer, as in any other disease, this will involve the detailed dissection of which senescent cells are pathogenic and which ones are beneficial. In this regard, the use of novel single-cell and spatial transcriptomics (or multiomics) will prove critical for studying the contribution of senescence to aspects like tumour heterogeneity and cell-to-cell communication. These considerations will allow the development of specific senotherapies able to target those senescent cells that contribute to cancer growth, recurrence, therapy resistance and post-therapeutic side-effects.

Contributors

J.M.G.-M. and J.P.M.-B. contributed equally to conceptualization, literature searching, writing of the original draft, reviewing & editing. J.M.G.-M. contributed to original figure preparation. Both authors read and approved the final version of the manuscript.

Declaration of interests

The authors declare no conflict of interest.

Acknowledgements

J.M.G.-M. belongs to the Molecular and Systems Bioengineering Research Focus Group at Tecnologico de Monterrey. The following have provided funding for research conducted at the JP Martinez-Barbera lab: Cancer Research UK (C54322/A27727), the Brain Tumour Charity (EVEREST (GN- 000382) and GN-000522), CHILDREN with CANCER UK (CwCUK 15-190) and National Institute of Health Research Biomedical Research Centre at the Great Ormond Street Hospital for Children NHS Foundation Trust, and the University College London.

References

1 Hayflick L. The limited in vitro lifetime of human diploid cell strains. *Exp Cell Res*. 1965;37:614–636. https://doi.org/10.1016/0014-4827(65)90211-9.
2 Calcinotto A, Kohli J, Zagato E, Pellegrini L, Demaria M, Alimonti A. Cellular senescence: aging, cancer, and injury. *Physiol Rev*. 2019;99:1047–1078. https://doi.org/10.1152/physrev.00020.2018.
3 Wiley CD, Campisi J. The metabolic roots of senescence: mechanisms and opportunities for intervention. *Nat Metab*. 2021;3:1290–1301. https://doi.org/10.1038/s42255-021-00483-8.
4 Lee S, Schmitt CA. The dynamic nature of senescence in cancer. *Nat Cell Biol*. 2019;21:94–101. https://doi.org/10.1038/s41556-018-0249-2.
5 Faget DV, Ren Q, Stewart SA. Unmasking senescence: context-dependent effects of SASP in cancer. *Nat Rev Cancer*. 2019;19:439–453. https://doi.org/10.1038/s41568-019-0156-2.
6 Rhinn M, Ritschka B, Keyes WM. Cellular senescence in development, regeneration and disease. *Development*. 2019;146:dev151837. https://doi.org/10.1242/dev.151837.
7 Da Silva-Álvarez S, Guerra-Varela J, Sobrido-Cameán D, et al. Developmentally-programmed cellular senescence is conserved and widespread in zebrafish. *Aging*. 2020;12:17895–17901. https://doi.org/10.18632/aging.103968.
8 Zhao Y, Tyshkovskiy A, Muñoz-Espín D, et al. Naked mole rats can undergo developmental, oncogene-induced and DNA damage-induced cellular senescence. *Proc Natl Acad Sci U S A*. 2018;115:1801–1806. https://doi.org/10.1073/pnas.1721160115.
9 Klein A, Rhinn M, Keyes WM. Cellular senescence and developmental defects. *FEBS J*. 2023;290:1303–1313. https://doi.org/10.1111/FEBS.16731.
10 de Lope C, Garciá-Lucena R, Magarinõs M, et al. Dysfunction of programmed embryo senescence is linked to genetic developmental defects. *Development*. 2023;150:dev200903. https://doi.org/10.1242/DEV.200903/306250/AM/DYSFUNCTION-OF-PROGRAMMED-EMBRYO-SENESCENCE-IS.
11 Muñoz-Espín D, Cañamero M, Maraver A, et al. Programmed cell senescence during mammalian embryonic development. *Cell*. 2013;155:1104. https://doi.org/10.1016/j.cell.2013.10.019.
12 Storer M, Mas A, Robert-Moreno A, et al. Senescence is a developmental mechanism that contributes to embryonic growth and patterning. *Cell*. 2013;155:1119–1130. https://doi.org/10.1016/j.cell.2013.10.041.
13 Hernandez-Segura A, Nehme J, Demaria M. Hallmarks of cellular senescence. *Trends Cell Biol*. 2018;28:436–453. https://doi.org/10.1016/j.tcb.2018.02.001.
14 Cohn RL, Gasek NS, Kuchel GA, Xu M. The heterogeneity of cellular senescence: insights at the single-cell level. *Trends Cell Biol*. 2023;33:9–17. https://doi.org/10.1016/j.tcb.2022.04.011.
15 Gorgoulis V, Adams PD, Alimonti A, et al. Cellular senescence: defining a path forward. *Cell*. 2019;179:813–827. https://doi.org/10.1016/j.cell.2019.10.005.
16 González-Gualda E, Baker AG, Fruk L, Muñoz-Espín D. A guide to assessing cellular senescence in vitro and in vivo. *FEBS J*. 2021;288:56–80. https://doi.org/10.1111/febs.15570.
17 Debacq-Chainiaux F, Erusalimsky JD, Campisi J, Toussaint O. Protocols to detect senescence-associated beta-galactosidase ({SA}-betagal) activity, a biomarker of senescent cells in culture and in vivo. *Nat Protoc*. 2009;4:1798–1806. https://doi.org/10.1038/nprot.2009.191.
18 Kurz DJ, Decary S, Hong Y, Erusalimsky JD. Senescence-associated (beta)-galactosidase reflects an increase in lysosomal mass during replicative ageing of human endothelial cells. *J Cell Sci*. 2000;113(Pt 2):3613–3622.
19 Adams PD, Ivanov A, Pawlikowski J, et al. Lysosome-mediated processing of chromatin in senescence. *J Cell Biol*. 2013;202:129–143. https://doi.org/10.1083/jcb.201212110.

senescent cells by the immune system). Senescent cells generated through these mechanisms display a Senescence-Associated Secretory Phenotype (SASP) which will interact with other senescent cells and the rest of the microenvironment. Within tumours, the SASP may facilitate immune evasion, tumour dormancy and recurrence, promote cancer cell growth and tumour invasiveness and the acquisition of resistance to further treatment. In off-target tissues, it may lead to chronic inflammation, organ/tissue dysfunction and systemic side-effects. Created with BioRender.com.

20 Rattan SIS, Hayflick L, eds. *Cellular ageing and replicative senescence*. Cham: Springer International Publishing; 2016. https://doi.org/10.1007/978-3-319-26239-0.
21 Vitale I, Jemaà M, Galluzzi L, Metivier D, Castedo M, Kroemer G. Cytofluorometric assessment of cell cycle progression. *Methods Mol Biol*. 2013;965:93–120. https://doi.org/10.1007/978-1-62703-239-1_6.
22 Kumari R, Jat P. Mechanisms of cellular senescence: cell cycle arrest and senescence associated secretory phenotype. *Front Cell Dev Biol*. 2021;9:645593. https://doi.org/10.3389/fcell.2021.645593.
23 Wagner K-D, Wagner N, Wagner K-D, Wagner N. The senescence markers p16INK4A, p14ARF/p19ARF, and p21 in organ development and homeostasis. *Cells*. 2022;11:1966. https://doi.org/10.3390/CELLS11121966.
24 Nelson G, von Zglinicki T. Monitoring DNA damage during cell senescence. *Methods Mol Biol*. 2013;965:197–213. https://doi.org/10.1007/978-1-62703-239-1_13.
25 Bárcena C, Osorio FG, Freije JMP. Detection of nuclear envelope alterations in senescence. *Methods Mol Biol*. 2013;965:243–251. https://doi.org/10.1007/978-1-62703-239-1_16.
26 Ryu SJ, Oh YS, Park SC. Failure of stress-induced downregulation of Bcl-2 contributes to apoptosis resistance in senescent human diploid fibroblasts. *Cell Death Differ*. 2007;14:1020–1028. https://doi.org/10.1038/sj.cdd.4402091.
27 Hampel B, Wagner M, Teis D, Zwerschke W, Huber LA, Jansen-Dürr P. Apoptosis resistance of senescent human fibroblasts is correlated with the absence of nuclear IGFBP-3. *Aging Cell*. 2005;4:325–330. https://doi.org/10.1111/j.1474-9726.2005.00180.x.
28 de Mera-Rodríguez JA, Álvarez-Hernán G, Gañán Y, Martín-Partido G, Rodríguez-León J, Francisco-Morcillo J. Is senescence-associated β-galactosidase a reliable in vivo marker of cellular senescence during embryonic development? *Front Cell Dev Biol*. 2021;9:623175. https://doi.org/10.3389/fcell.2021.623175.
29 Ito Y, Hoare M, Narita M. Spatial and temporal control of senescence. *Trends Cell Biol*. 2017;27:820–832. https://doi.org/10.1016/j.tcb.2017.07.004.
30 Da Silva-Álvarez S, Guerra-Varela J, Sobrido-Cameán D, et al. Cell senescence contributes to tissue regeneration in zebrafish. *Aging Cell*. 2020;19:e13052. https://doi.org/10.1111/acel.13052.
31 Feng T, Meng J, Kou S, et al. CCN1-induced cellular senescence promotes heart regeneration. *Circulation*. 2019;139:2495–2498. https://doi.org/10.1161/CIRCULATIONAHA.119.039530.
32 Yun MH, Davaapil H, Brockes JP. Recurrent turnover of senescent cells during regeneration of a complex structure. *Elife*. 2015;4:e05505. https://doi.org/10.7554/eLife.05505.
33 Demaria M, Ohtani N, Youssef SA, et al. An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. *Dev Cell*. 2014;31:722–733. https://doi.org/10.1016/j.devcel.2014.11.012.
34 Chiche A, Le Roux I, von Joest M, et al. Injury-induced senescence enables in vivo reprogramming in skeletal muscle. *Cell Stem Cell*. 2017;20:407–414.e4. https://doi.org/10.1016/j.stem.2016.11.020.
35 Ritschka B, Storer M, Mas A, et al. The senescence-associated secretory phenotype induces cellular plasticity and tissue regeneration. *Genes Dev*. 2017;31:172–183. https://doi.org/10.1101/gad.290635.116.
36 Jurk D, Wilson C, Passos JF, et al. Chronic inflammation induces telomere dysfunction and accelerates ageing in mice. *Nat Commun*. 2014;2:4172. https://doi.org/10.1038/ncomms5172.
37 Baker DJ, Wijshake T, Tchkonia T, et al. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. *Nature*. 2011;479:232–236. https://doi.org/10.1038/nature10600.
38 Baker DJ, Childs BG, Durik M, et al. Naturally occurring p16 Ink4a-positive cells shorten healthy lifespan. *Nature*. 2016;530:184–189. https://doi.org/10.1038/nature16932.
39 Childs BG, Li H, van Deursen JM, et al. Senescent cells: a therapeutic target for cardiovascular disease. *J Clin Invest*. 2018;128:1217–1228. https://doi.org/10.1172/{JCI95146}.
40 Di Micco R, Krizhanovsky V, Baker D, d'Adda di Fagagna F. Cellular senescence in ageing: from mechanisms to therapeutic opportunities. *Nat Rev Mol Cell Biol*. 2021;22:75–95. https://doi.org/10.1038/s41580-020-00314-w.
41 Raffaele M, Vinciguerra M. The costs and benefits of senotherapeutics for human health. *Lancet Healthy Longev*. 2022;3:e67–e77. https://doi.org/10.1016/S2666-7568(21)00300-7.
42 Zhang L, Pitcher LE, Prahalad V, Niedernhofer LJ, Robbins PD. Targeting cellular senescence with senotherapeutics: senolytics and senomorphics. *FEBS J*. 2023;290:1362–1383. https://doi.org/10.1111/febs.16350.
43 Milanovic M, Fan DNY, Belenki D, et al. Senescence-associated reprogramming promotes cancer stemness. *Nature*. 2018;553:96–100. https://doi.org/10.1038/nature25167.
44 Gonzalez-Meljem JM, Apps JR, Fraser HC, Martinez-Barbera JP. Paracrine roles of cellular senescence in promoting tumourigenesis. *Br J Cancer*. 2018;118:1283–1288. https://doi.org/10.1038/s41416-018-0066-1.
45 Gonzalez-Meljem JM, Martinez-Barbera JP. Adamantinomatous craniopharyngioma as a model to understand paracrine and senescence-induced tumourigenesis. *Cell Mol Life Sci*. 2021;78:4521–4544. https://doi.org/10.1007/s00018-021-03798-7.
46 Arwert EN, Hoste E, Watt FM. Epithelial stem cells, wound healing and cancer. *Nat Rev Cancer*. 2012;12:170–180. https://doi.org/10.1038/nrc3217.
47 Yamauchi M, Barker TH, Gibbons DL, Kurie JM. The fibrotic tumor stroma. *J Clin Invest*. 2018;128:16–25. https://doi.org/10.1172/JCI93554.
48 Boulter L, Bullock E, Mabruk Z, Brunton VG. The fibrotic and immune microenvironments as targetable drivers of metastasis. *Br J Cancer*. 2021;124:27–36. https://doi.org/10.1038/s41416-020-01172-1.
49 Muñoz-Espín D, Rovira M, Galiana I, et al. A versatile drug delivery system targeting senescent cells. *EMBO Mol Med*. 2018;10:e9355. https://doi.org/10.15252/emmm.201809355.
50 Schafer MJ, White TA, Iijima K, et al. Cellular senescence mediates fibrotic pulmonary disease. *Nat Commun*. 2017;8:14532. https://doi.org/10.1038/ncomms14532.
51 Chen X, Xu H, Hou J, et al. Epithelial cell senescence induces pulmonary fibrosis through Nanog-mediated fibroblast activation. *Aging*. 2020;12:242–259. https://doi.org/10.18632/aging.102613.
52 Justice JN, Nambiar AM, Tchkonia T, et al. Senolytics in idiopathic pulmonary fibrosis: results from a first-in-human, open-label, pilot study. *eBioMedicine*. 2019;40:554–563. https://doi.org/10.1016/J.EBIOM.2018.12.052.
53 Wang L, Lankhorst L, Bernards R. Exploiting senescence for the treatment of cancer. *Nat Rev Cancer*. 2022;22:340–355. https://doi.org/10.1038/s41568-022-00450-9.
54 Schmitt CA, Wang B, Demaria M. Senescence and cancer — role and therapeutic opportunities. *Nat Rev Clin Oncol*. 2022;19:619–636. https://doi.org/10.1038/s41571-022-00668-4.
55 Prasanna PG, Citrin DE, Hildesheim J, et al. Therapy-induced senescence: opportunities to improve anticancer therapy. *J Natl Cancer Inst*. 2021;113:1285–1298. https://doi.org/10.1093/jnci/djab064.
56 Melmed S, Kaiser UB, Lopes MB, et al. Clinical biology of the pituitary adenoma. *Endocr Rev*. 2022;43:1003–1037. https://doi.org/10.1210/ENDREV/BNAC010.
57 Müller HL, Merchant TE, Warmuth-Metz M, Martinez-Barbera J-PP, Puget S. Craniopharyngioma. *Nat Rev Dis Primers*. 2019;5:75. https://doi.org/10.1038/s41572-019-0125-9.
58 Müller HL. Childhood craniopharyngioma: treatment strategies and outcomes. *Expert Rev Neurother*. 2014;14:187–197. https://doi.org/10.1586/14737175.2014.875470.
59 Haston S, Pozzi S, Carreno G, et al. MAPK pathway control of stem cell proliferation and differentiation in the embryonic pituitary provides insights into the pathogenesis of papillary craniopharyngioma. *Development*. 2017;144:2141–2152. https://doi.org/10.1242/dev.150490.
60 Larkin S, Karavitaki N. Recent advances in molecular pathology of craniopharyngioma. *F1000Res*. 2017;6:1202. https://doi.org/10.12688/f1000research.11549.1.
61 Apps JR, Martinez-Barbera JP. *Pathophysiology and genetics in craniopharyngioma. Pituitary tumors: a comprehensive and interdisciplinary approach*. Elsevier; 2021:53–66. https://doi.org/10.1016/B978-0-12-819949-7.00020-2.
62 Martinez-Barbera JP, Andoniadou CL. Biological behaviour of craniopharyngiomas. *Neuroendocrinology*. 2020;110:797–804. https://doi.org/10.1159/000506904.
63 Gaston-Massuet C, Andoniadou CL, Signore M, et al. Increased Wingless (Wnt) signaling in pituitary progenitor/stem cells gives rise to pituitary tumors in mice and humans. *Proc Natl Acad Sci U S A*. 2011;108:11482–11487. https://doi.org/10.1073/pnas.1101553108.
64 Andoniadou CL, Gaston-Massuet C, Reddy R, et al. Identification of novel pathways involved in the pathogenesis of human

adamantinomatous craniopharyngioma. *Acta Neuropathol*. 2012;124:259–271. https://doi.org/10.1007/s00401-012-0957-9.
65 Apps JR, Hutchinson JC, Arthurs OJ, et al. Imaging invasion: micro-CT imaging of adamantinomatous craniopharyngioma highlights cell type specific spatial relationships of tissue invasion. *Acta Neuropathol Commun*. 2016;4:57. https://doi.org/10.1186/s40478-016-0321-8.
66 Andoniadou CL, Matsushima D, Mousavy-gharavy SN, et al. The Sox2 + population of the adult murine pituitary includes progenitor/stem cells with tumour-inducing potential. *Cell Stem Cell*. 2013;13:433–445.
67 Gonzalez-Meljem JM, Haston S, Carreno G, et al. Stem cell senescence drives age-attenuated induction of pituitary tumours in mouse models of paediatric craniopharyngioma. *Nat Commun*. 2017;8:1819. https://doi.org/10.1038/s41467-017-01992-5.
68 Jiang Y, Yang J, Liang R, et al. *Single-cell RNA sequencing highlights intratumor heterogeneity and intercellular network featured in adamantinomatous craniopharyngioma*. 2023.
69 Haston S, Gonzalez-Gualda E, Morsli S, et al. Clearance of senescent macrophages ameliorates tumorigenesis in KRAS-driven lung cancer. *Cancer Cell*. 2023;41:1242–1260.e6. https://doi.org/10.1016/j.ccell.2023.05.004.
70 Apps JR, Carreno G, Gonzalez-Meljem JM, et al. Tumour compartment transcriptomics demonstrates the activation of inflammatory and odontogenic programmes in human adamantinomatous craniopharyngioma and identifies the MAPK/ERK pathway as a novel therapeutic target. *Acta Neuropathol*. 2018;135:757–777. https://doi.org/10.1007/s00401-018-1830-2.
71 Donson AM, Apps J, Griesinger AM, et al. Molecular analyses reveal inflammatory mediators in the solid component and cyst fluid of human adamantinomatous craniopharyngioma. *J Neuropathol Exp Neurol*. 2017;76:779–788. https://doi.org/10.1093/jnen/nlx061.
72 Villa C, Baussart B, Assié G, Raverot G, Roncaroli F. The World Health Organization classifications of pituitary neuroendocrine tumours: a clinico-pathological appraisal. *Endocr Relat Cancer*. 2023;30:e230021. https://doi.org/10.1530/ERC-23-0021.
73 Chen J, Schmidt RE, Dahiya S. Pituitary adenoma in pediatric and adolescent populations. *J Neuropathol Exp Neurol*. 2019;78:626–632. https://doi.org/10.1093/jnen/nlz040.
74 Walz PC, Drapeau A, Shaikhouni A, et al. Pediatric pituitary adenomas. *Childs Nerv Syst*. 2019;35:2107–2118. https://doi.org/10.1007/s00381-019-04293-y.
75 Chesnokova V, Zonis S, Rubinek T, et al. Senescence mediates pituitary hypoplasia and restrains pituitary tumor growth. *Cancer Res*. 2007;67:10564–10572. https://doi.org/10.1158/0008-5472.CAN-07-0974.
76 Mongi-Bragato B, Grondona E, Sosa LDV, et al. Pivotal role of NF-κB in cellular senescence of experimental pituitary tumours. *J Endocrinol*. 2020;245:179–191. https://doi.org/10.1530/JOE-19-0506.
77 Sabatino ME, Grondona E, Sosa LDV, et al. Oxidative stress and mitochondrial adaptive shift during pituitary tumoral growth. *Free Radic Biol Med*. 2018;120:41–55. https://doi.org/10.1016/j.freeradbiomed.2018.03.019.
78 Ben-Shlomo A, Deng N, Ding E, et al. DNA damage and growth hormone hypersecretion in pituitary somatotroph adenomas. *J Clin Invest*. 2020;130:5738–5755. https://doi.org/10.1172/JCI138540.
79 Chesnokova V, Zonis S, Barrett R, et al. Excess growth hormone suppresses DNA damage repair in epithelial cells. *JCI Insight*. 2019;4:e125762. https://doi.org/10.1172/jci.insight.125762.
80 Chesnokova V, Zonis S, Apostolou A, et al. Local non-pituitary growth hormone is induced with aging and facilitates epithelial damage. *Cell Rep*. 2021;37:110068. https://doi.org/10.1016/j.celrep.2021.110068.
81 Chesnokova V, Zhou C, Ben-Shlomo A, et al. Growth hormone is a cellular senescence target in pituitary and nonpituitary cells. *Proc Natl Acad Sci U S A*. 2013;110:E3331–E3339. https://doi.org/10.1073/pnas.1310589110.
82 Apaydin T, Zonis S, Zhou C, et al. WIP1 is a novel specific target for growth hormone action. *iScience*. 2023;26:108117. https://doi.org/10.1016/j.isci.2023.108117.
83 Alexandraki KI, Munayem Khan M, Chahal HS, et al. Oncogene-induced senescence in pituitary adenomas and carcinomas. *Hormones (Basel)*. 2012;11:297–307.
84 Manojlovic-Gacic E, Skender-Gazibara M, Popovic V, et al. Oncogene-induced senescence in pituitary adenomas—an immunohistochemical study. *Endocr Pathol*. 2016;27:1–11. https://doi.org/10.1007/s12022-015-9405-4.
85 Sabatino ME, Petiti JP, Del Valle Sosa L, et al. Evidence of cellular senescence during the development of estrogen-induced pituitary tumors. *Endocr Relat Cancer*. 2015;22:299–317. https://doi.org/10.1530/ERC-14-0333.
86 Sapochnik M, Haedo MR, Fuertes M, et al. Autocrine IL-6 mediates pituitary tumor senescence. *Oncotarget*. 2017;8:4690–4702. https://doi.org/10.18632/oncotarget.13577.
87 Taniguchi-Ponciano K, Andonegui-Elguera S, Peña-Martínez E, et al. Transcriptome and methylome analysis reveals three cellular origins of pituitary tumors. *Sci Rep*. 2020;10:19373. https://doi.org/10.1038/s41598-020-76555-8.
88 Lv L, Zhang S, Hu Y, et al. Invasive pituitary adenoma-derived tumor-associated fibroblasts promote tumor progression both in vitro and in vivo. *Exp Clin Endocrinol Diabetes*. 2018;126:213–221. https://doi.org/10.1055/S-0043-119636/ID/R08-2017-0305-ENDO-0017/BIB.
89 Marques P, Barry S, Carlsen E, et al. Pituitary tumour fibroblast-derived cytokines influence tumour aggressiveness. *Endocr Relat Cancer*. 2019;26:853–865. https://doi.org/10.1530/ERC-19-0327.
90 Shay JW, Roninson IB. Hallmarks of senescence in carcinogenesis and cancer therapy. *Oncogene*. 2004;23:2919–2933. https://doi.org/10.1038/sj.onc.1207518.
91 Le ONL, Rodier F, Fontaine F, et al. Ionizing radiation-induced long-term expression of senescence markers in mice is independent of p53 and immune status. *Aging Cell*. 2010;9:398–409. https://doi.org/10.1111/j.1474-9726.2010.00567.x.
92 Wang B, Kohli J, Demaria M. Senescent cells in cancer therapy: friends or foes? *Trends Cancer*. 2020;6:838–857. https://doi.org/10.1016/j.trecan.2020.05.004.
93 Zhu W, Zhang X, Yu M, Lin B, Yu C. Radiation-induced liver injury and hepatocyte senescence. *Cell Death Discov*. 2021;7. https://doi.org/10.1038/s41420-021-00634-6.
94 Liao E-C, Hsu Y-T, Chuah Q-Y, et al. Radiation induces senescence and a bystander effect through metabolic alterations. *Cell Death Dis*. 2014;5:e1255. https://doi.org/10.1038/cddis.2014.220.
95 Flor AC, Wolfgeher D, Wu D, Kron SJ. A signature of enhanced lipid metabolism, lipid peroxidation and aldehyde stress in therapy-induced senescence. *Cell Death Discov*. 2017;3:17075. https://doi.org/10.1038/cddiscovery.2017.75.
96 Ewald JA, Desotelle JA, Wilding G, Jarrard DF. Therapy-induced senescence in cancer. *J Natl Cancer Inst*. 2010;102:1536–1546. https://doi.org/10.1093/jnci/djq364.
97 He S, Sharpless NE. Senescence in health and disease. *Cell*. 2017;169:1000–1011. https://doi.org/10.1016/j.cell.2017.05.015.
98 Collado M, Gil J, Efeyan A, et al. Tumour biology: senescence in premalignant tumours. *Nature*. 2005;436:642. https://doi.org/10.1038/436642a.
99 Braig M, Lee S, Loddenkemper C, et al. Oncogene-induced senescence as an initial barrier in lymphoma development. *Nature*. 2005;436:660–665. https://doi.org/10.1038/nature03841.
100 Schoetz U, Klein D, Hess J, et al. Early senescence and production of senescence-associated cytokines are major determinants of radioresistance in head-and-neck squamous cell carcinoma. *Cell Death Dis*. 2021;12:1162. https://doi.org/10.1038/s41419-021-04454-5.
101 Fitsiou E, Soto-Gamez A, Demaria M. Biological functions of therapy-induced senescence in cancer. *Semin Cancer Biol*. 2022;81:5–13. https://doi.org/10.1016/j.semcancer.2021.03.021.
102 Czarnecka-Herok J, Sliwinska MA, Herok M, et al. Therapy-induced senescent/polyploid cancer cells undergo atypical divisions associated with altered expression of meiosis, spermatogenesis and EMT genes. *Int J Mol Sci*. 2022;23:8288. https://doi.org/10.3390/ijms23158288.
103 Saleh T, Tyutyunyk-Massey L, Gewirtz DA. Tumor cell escape from therapy-induced senescence as a model of disease recurrence after dormancy. *Cancer Res*. 2019;79:1044–1046. https://doi.org/10.1158/0008-5472.CAN-18-3437.
104 Demaria M, O'Leary MN, Chang J, et al. Cellular senescence promotes adverse effects of chemotherapy and cancer relapse. *Cancer Discov*. 2017;7:165–176. https://doi.org/10.1158/2159-8290.CD-16-0241.
105 Peng X, Wu Y, Brouwer U, et al. Cellular senescence contributes to radiation-induced hyposalivation by affecting the stem/progenitor cell niche. *Cell Death Dis*. 2020;11:854. https://doi.org/10.1038/s41419-020-03074-9.

106 Kutyna MM, Kok CH, Lim Y, et al. A senescence stress secretome is a hallmark of therapy-related myeloid neoplasm stromal tissue occurring soon after cytotoxic exposure. *Leukemia*. 2022;36:2678–2689. https://doi.org/10.1038/s41375-022-01686-y.

107 Stoddart A, Wang J, Fernald AA, et al. Cytotoxic therapy–induced effects on both hematopoietic and marrow stromal cells promotes therapy-related myeloid neoplasms. *Blood Cancer Discov*. 2020;1:32–47. https://doi.org/10.1158/2643-3230.bcd-19-0028.

108 Murali B, Ren Q, Luo X, et al. Inhibition of the stromal p38MAPK/MK2 pathway limits breast cancer metastases and chemotherapy-induced bone loss. *Cancer Res*. 2018;78:5618–5630. https://doi.org/10.1158/0008-5472.CAN-18-0234.

109 Prieto LI, Sturmlechner I, Graves SI, et al. Senescent alveolar macrophages promote early-stage lung tumorigenesis. *Cancer Cell*. 2023;41:1261–1275.e6. https://doi.org/10.1016/j.ccell.2023.05.006.

110 Salam R, Saliou A, Bielle F, et al. Cellular senescence in malignant cells promotes tumor progression in mouse and patient glioblastoma. *Nat Commun*. 2023;14:441. https://doi.org/10.1038/s41467-023-36124-9.

111 Nacarelli T, Fukumoto T, Zundell JA, et al. NAMPT inhibition suppresses cancer stem-like cells associated with therapy-induced senescence in ovarian cancer. *Cancer Res*. 2020;80:890–900. https://doi.org/10.1158/0008-5472.CAN-19-2830.

112 Jaber S, Warnier M, Leers C, et al. Targeting chemoresistant senescent pancreatic cancer cells improves conventional treatment efficacy. *Mol Biomed*. 2023;4:4. https://doi.org/10.1186/s43556-023-00116-4.

113 Dörr JR, Yu Y, Milanovic M, et al. Synthetic lethal metabolic targeting of cellular senescence in cancer therapy. *Nature*. 2013;501:421–425. https://doi.org/10.1038/nature12437.

114 Meng J, Li Y, Wan C, et al. Targeting senescence-like fibroblasts radiosensitizes non-small cell lung cancer and reduces radiation-induced pulmonary fibrosis. *JCI Insight*. 2021;6:e146334. https://doi.org/10.1172/jci.insight.146334.

115 Kim JH, Brown SL, Gordon MN. Radiation-induced senescence: therapeutic opportunities. *Radiat Oncol*. 2023;18:10. https://doi.org/10.1186/s13014-022-02184-2.

116 Selt F, Sigaud R, Valinciute G, et al. BH3 mimetics targeting BCL-XL impact the senescent compartment of pilocytic astrocytoma. *Neuro Oncol*. 2023;25:735–747. https://doi.org/10.1093/neuonc/noac199.

117 Buhl JL, Selt F, Hielscher T, et al. The senescence-associated secretory phenotype mediates oncogene-induced senescence in pediatric pilocytic astrocytoma. *Clin Cancer Res*. 2019;25:1851–1866. https://doi.org/10.1158/1078-0432.CCR-18-1965.

118 Balakrishnan I, Danis E, Pierce A, et al. Senescence induced by BMI1 inhibition is a therapeutic vulnerability in H3K27M-mutant DIPG. *Cell Rep*. 2020;33. https://doi.org/10.1016/j.celrep.2020.108286.

119 Carpenter VJ, Saleh T, Gewirtz DA. Senolytics for cancer therapy: is all that glitters really gold? *Cancers (Basel)*. 2021;13:1–25. https://doi.org/10.3390/cancers13040723.

120 Hanahan D. Hallmarks of cancer: new dimensions. *Cancer Discov*. 2022;12:31–46. https://doi.org/10.1158/2159-8290.CD-21-1059.

121 Bavik C, Coleman I, Dean JP, Knudsen B, Plymate S, Nelson PS. The gene expression program of prostate fibroblast senescence modulates neoplastic epithelial cell proliferation through paracrine mechanisms. *Cancer Res*. 2006;66:794–802. https://doi.org/10.1158/0008-5472.CAN-05-1716.

122 Bancaro N, Calì B, Troiani M, et al. Apolipoprotein E induces pathogenic senescent-like myeloid cells in prostate cancer. *Cancer Cell*. 2023;41:602–619.e11. https://doi.org/10.1016/J.CCELL.2023.02.004.

123 Kolodkin-Gal D, Roitman L, Ovadya Y, et al. Senolytic elimination of Cox2-expressing senescent cells inhibits the growth of premalignant pancreatic lesions. *Gut*. 2022;71:345–355. https://doi.org/10.1136/GUTJNL-2020-321112.

124 Plakhova N, Panagopoulos V, Vandyke K, Zannettino ACW, Mrozik KM. Mesenchymal stromal cell senescence in haematological malignancies. *Cancer Metastasis Rev*. 2023;42:277–296. https://doi.org/10.1007/S10555-022-10069-9.

125 Ellison-Hughes GM. First evidence that senolytics are effective at decreasing senescent cells in humans. *eBioMedicine*. 2020;56:102473. https://doi.org/10.1016/J.EBIOM.2019.09.053.

126 Hickson LTJ, Langhi Prata LGP, Bobart SA, et al. Senolytics decrease senescent cells in humans: preliminary report from a clinical trial of Dasatinib plus Quercetin in individuals with diabetic kidney disease. *eBioMedicine*. 2019;47:446–456. https://doi.org/10.1016/J.EBIOM.2019.08.069.

127 Apps JR, Muller HL, Hankinson TC, Yock TI, Martinez-Barbera JP. Contemporary biological insights and clinical management of craniopharyngioma. *Endocr Rev*. 2023;44:518–538. https://doi.org/10.1210/endrev/bnac035.