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Commentary

Challenges and opportunities for modeling aging and cancer

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Age is among the main risk factors for cancer, and any cancer study in adults is faced with an aging tissue and organism. Yet, pre-clinical studies are carried out using young mice and are not able to address the impact of aging and associated comorbidities on disease biology and treatment outcomes. Here, we discuss the limitations of current mouse cancer models and suggest strategies for developing novel models to address these major gaps in knowledge and experimental approaches.

Thanks to continuous advances in medicine, the average human life expectancy has increased significantly. In the US, the proportion of the population over 65 years of age is projected to surpass the number of people 18 years or younger within the next 30 years. Thus, the management of aging-related diseases, including cancer, becomes a major challenge. Cancer can affect individuals across the lifespan; however, most cases are diagnosed in individuals over 65 years of age, with 60% of new cancer diagnoses and 70% of cancer deaths occurring in this population. Yet, cancer research has largely overlooked the role of aging. Indeed, cancer studies are mostly conducted using young animals. Therefore, they cannot directly address the impact of aging on various aspects of cancer initiation, progression, heterogeneity, and response to therapy. The disconnect between aging and cancer research is also illustrated by the underrepresentation of patients older than 65 in clinical trials. This is a problem because older individuals display comorbidities likely to influence drug efficacy and toxicity that will not be fully captured in younger individuals. Thus, key questions related to tumorigenesis, therapeutic efficacy, and resistance are not fully resolved in a large fraction of patients with cancer.

Recently, the aging biology and cancer biology fields have appeared to move toward greater convergence. Advances in the geroscience field, which seeks to connect the biology of aging to the biology of aging-related diseases, can also be leveraged for a better understanding of cancer among older adults. This includes testing geroscience-guided therapies as a means of improving clinical outcomes for older patients with cancer. Importantly, successful translation of any findings from such research into actionable interventions will require pre-clinical investigation and validation using robust experimental systems capable of comodeling aging and cancer together. Whereas progress has been made in each field using a range of vertebrate and invertebrate model systems, there is a shortage of models enabling studies on aging and cancer simultaneously. Here, we summarize the cancer and aging fields and the limitations of current cancer models. We propose a strategy to construct and validate novel models that are much needed to accelerate progress in pre-clinical discovery and translation to therapy (Figure 1).

Features of systemic aging to consider in cancer model development

Aging is a complex biologic and physiologic process accompanied by significant changes to cellular and molecular components of tissues and organ systems.[1] Therefore, the choice of a model system should be determined by the aspects of aging that one wishes to model, e.g.,

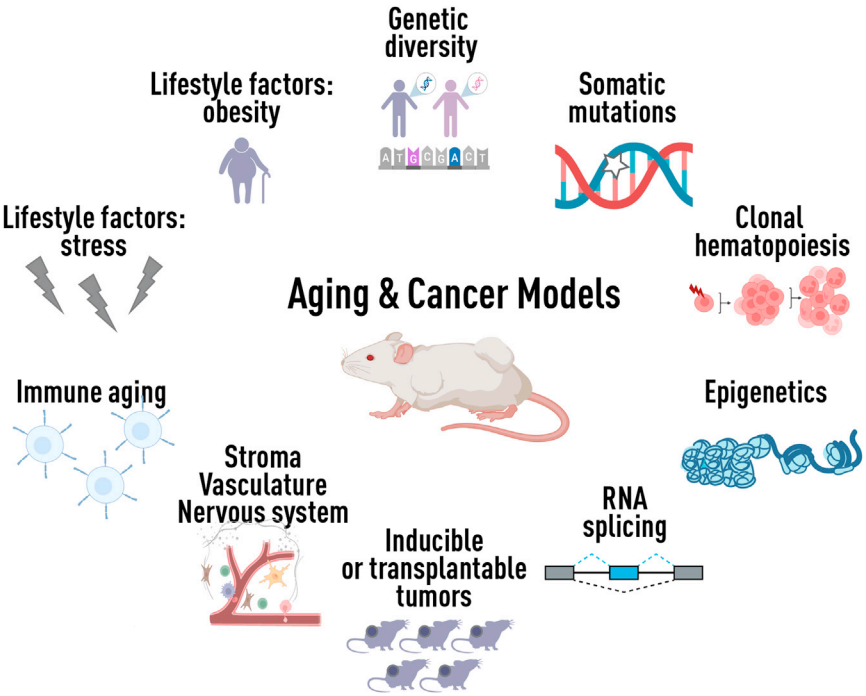

Figure 1. Developing new models for age-related cancers

Key features to be modeled include molecular processes such as the impact of genetic diversity; the accumulation of mutations with age including mutations associated with clonal hematopoiesis, epigenetics, and post-transcriptional alterations; aging of the immune system; and age-related remodeling of the microenvironment, as well as environmental factors such as stress and obesity. Accounting for these features will improve models for testing therapeutic and prevention strategies. Inducible transgenic animals or spontaneous transplantable models would enable the study of tumors in older animals.

biological aging, chronological aging, immune aging (locally vs. systemic), inflammaging, or senescence and aging of the microenvironment (considering tissue-specificity differences).

At the cellular level, aging is characterized, among other changes, by senescence, mitochondrial dysfunction, and altered metabolic programs. At the molecular level, aged tissues exhibit genomic changes including the accumulation of somatic mutations, as well as epigenetic,[1] transcriptional, and post-transcriptional remodeling.[2] On a systemic level, aging is often accompanied by cognitive dysfunction, muscle and bone loss, changes in hormonal and endocrine levels and function, immune dysfunction, and chronic inflammation known as inflammaging.[1] The complex role for senescence in aging and cancer requires careful dissection in the context of model systems.[1] It is also known that aging-induced changes in distal metastatic microenvironments promote the efficient reactivation of dormant cancer cells.[3] A fundamental open question is how these biological hallmarks of aging influence cancer initiation, progression, and therapeutic responses. Furthermore, these hallmarks—and the diseases of aging they give rise to—exist not in isolation but in combination, can influence each other's impact, and can display heterogeneity both within an individual and between individuals.

The development of relevant cancer and aging models requires a better understanding of how to measure aging at the molecular and cellular level; how to quantify the rates and mechanisms by which tissues and cell types age; how to determine which mechanisms are shared or tissue-/cell-type specific; and ultimately, which aging-related changes lead to disease. For example, inflammaging is widely observed in many aging-related diseases, but whether its role is causal, and if so how, is not fully understood.[1,4] Thus, a critical need exists to identify genetic, genomic, and cellular diversity underlying the variation in cancer penetrance as a function of inflammation and aging. It is also increasingly appreciated that not all tissues and organ systems age at the same rate in an individual. Moreover, the extent to which cellular and tissue aging differs between individuals is not clear, although increasing evidence points to the fact that "biological age" does not directly correlate with chronological age and that biological age differs between individuals, reflecting lifestyle and medical history.

In vivo models need to be designed to understand not only the influence of age but also biological sex, hormone signaling, heritable genetic background, immune status, diet, physical activity, exposure to infectious agents, environmental insults, and inflammation over a lifetime. In addition, models that better incorporate the contribution and possible additive or synergistic effects of other aging-related comorbidities detected in patients with cancer are needed. Furthermore, while some older adults with comorbidities go on to develop cancer, many do not, and the underlying mechanisms of such resilience are unknown. Understanding which combination(s) of aging-related hallmarks and comorbidities are truly causal and predictive of specific types of cancer offers opportunities for early diagnosis, intervention, and possibly cure among older adults. Such knowledge would facilitate development of diagnostic tests to identify risk of cancer development among otherwise healthy older individuals, enabling an opportunity for early intervention and cancer prevention.

Modeling mutations and genetic alterations

Aging models of cancer should enable us to dissect which cellular and molecular components have the greatest impact on the cell of origin of cancer, on the fitness of initiated pre-neoplastic cells, and on mechanisms of tumor progression and metastasis. A major limitation of most murine tumor models, including some of the classical models that uncovered key oncogenes or tumor suppressors (e.g., *Trp53*, *Brca1*, *Her2*), is that they use young, inbred animals, often between 4 and 8 weeks of age, which are equivalent to ~15- to 20-year-old humans. These models enable studies of cancer development in a short time frame but do not accurately reflect or recapitulate the cellular or molecular milieu of older

individuals or of genetically diverse populations within whom tumors arise. Creating inducible models to activate cancer-driving oncogenes or inactivate tumor suppressors in older animals (over 18 months of age) in a tissue-specific manner would enable the monitoring of how these changes alter epithelial cells vs. the immune system vs. stromal components with age. Similarly, approaches to model the stepwise accumulation of mutations in individual cells over time—in a manner similar to what occurs in humans—would be valuable. These cellular changes could be further linked with underlying epigenetic and transcriptomic alterations to nominate molecular drivers of aging-related cancers in a cell context-considered manner.

Aging and cancer models should also incorporate somatic mutations, which accumulate over an organism's lifespan and can impact the function of the host. This phenomenon is best illustrated by clonal hematopoiesis, where a mutated clone has a fitness advantage and contributes to the production of a substantial proportion of mature blood cells.[5] Mutations in genes involved in epigenetic regulation (*DNMT3A*, *TET2*, *ASXL1*) account for the majority of mutation-driven clonal hematopoiesis in humans.[5] These mutations are rare in the young but are highly prevalent in older adults, with 10%–20% of those older than age 70 harboring a clone of appreciable size. Clonal hematopoiesis has been shown to transform into acute myeloid leukemia *in vivo* by induction of additional cooperating leukemogenic driver mutation(s).[6] While less than 1% of clonal hematopoiesis cases convert to blood cancer per year,[5] the impact of clonal hematopoiesis on the myeloid compartment even in the absence of leukemic transformation might lead to myeloid cell dysfunction in the tissue.

Finally, multiple studies have described the accumulation with age of cells with mutations often classified as oncogenic in phenotypically normal human tissues, some of which are even present since birth. Yet, these are often not sufficient alone to drive tumorigenesis. Therefore, there is a need for models that enable one to measure the aging-related changes in fitness of cells bearing tumor-associated mutations, including in model systems that would be agnostic to tumor cell of origin.

Modeling immune aging

The decline of the immune system with age is reflected in the increased susceptibility to infectious diseases, poorer responses to vaccination, and increased prevalence of cancer and autoimmune and other chronic diseases. Aging is associated with significant changes to both innate and adaptive immunity.[1,4] For example, hematopoiesis is skewed toward myelopoiesis, while lymphopoiesis retracts with age.[7] In the periphery, T cells go through major age-related changes, including a reduction in naive T cells due to thymic involution, as well as increases in the numbers of terminally differentiated memory T cells and exhausted T cells.[8,9] Phenotypic and functional alterations are also observed in myeloid cells including dendritic cells and macrophages.[10] These age-related alterations are further impacted by sex. The clinical implications of age-related changes in immune function for cancer development and response to therapy are not well understood. Early studies highlight differences in age-stratified responses to immunotherapy (e.g., immune checkpoint blockade) that depend on cancer type. Understanding those cancer type-specific responses will be critical as we think about age- and sex-stratified therapies for patients with cancer.

Similar age-related changes in immune populations have also been observed in some strains of mice commonly used for cancer research.[11,12] Therefore, cancer models relying on young, inbred animals do not properly reflect the immune microenvironment in which tumors arise in older patients, but it may be possible that some mouse strains would be appropriate for studying "immunological age." Indeed, neither humans nor mice need to be geriatric to show immune aging phenotypes. Several studies highlight the fact that a mouse naturally aged to 10–14 months, while not geriatric, is immunologically old. For example, changes in thymic output of 40-week-old mice are similar to the age-related changes of the human thymus, and in mice, thymic output is the same at 100 weeks as it is at 40 weeks.[11] In fact, using geriatric mice, which is associated with morbidity of mice reaching the end of their lifespan, could potentially confound results of cancer research studies, thereby necessitating development of novel models driven by specific questions.

Another key limitation of many current aging and/or cancer mouse models is that they are kept in aseptic conditions and are not exposed to external triggers such as diet, carcinogens, viruses, bacteria, or vaccines that stimulate the immune system over a human lifetime. Chronic exposure to pro-inflammatory environmental factors is a plausible mechanism by which aging and cancer development are accelerated. This concept could be rigorously tested in mouse models, for example, by chronic activation of the immune system via vaccination or exposure to inflammation.

Modeling treatment response

Aging cancer models will be highly valuable to dissect how aging affects responses to different cancer therapies and to identify therapeutic agents or their combinations that are more effective in older animals and are associated with decreased side effects. For example, while most patients with cancer are still treated with chemotherapies, the dosing, pharmacokinetics, and pharmacodynamics are remain poorly understood in older patients because most compounds are tested in clinical trials in younger patients. Similarly, efficacy of immunotherapies in older patients with aged immune systems remains unclear, as discussed above. In addition, defining disease across the age spectrum is necessary. For example, recent molecular profiling of human cancers reveals that some adult cancers look quite different in younger vs. older adults (e.g., triple-negative breast cancer).[13] Another example is provided by the observed differences in responses to VEGF-targeting therapies in younger vs. older patients with melanoma, the latter of which have little benefit.[14] As highlighted earlier, age-stratified responses to therapies seem to differ across cancer types; therefore, we should take caution when making generalized assumptions about how older patients will respond to a given therapy. Cancer models to test age-stratified therapies would thus be very useful and clinically relevant. Finally, cancer and associated drug treatments can impact aging hallmarks in patients and induce frailty and other side effects over the lifespan. Senolytics have been proposed to offset the aging-promoting effects of cancer treatment, but their efficacy has yet to be

evaluated in pre-clinical models. Senescent cells can also accumulate in tumors and may, paradoxically, promote tumor relapse, metastasis, and resistance to therapy, observations that form the basis for ''one-two punch'' therapeutic strategies that begin with traditional cancer therapies followed by senolytics.[1]

Modeling genetic diversity of host

A major challenge is how to robustly model the biological complexity and heterogeneity of age-related cancers in genetically identical inbred strains of mice. These strains, the most common being *C57BL/6*, offer important *in vivo* context and the ability to experimentally manipulate specific genes of interest and graft human patient tumors to study both biological mechanisms as well as potential chemotherapies. However, the lack of genetic diversity in standard inbred strains is not representative of human genetic diversity. These factors could partly explain the poor translatability of some biological discoveries and, more importantly, of many chemotherapeutic agents in human trials. Layering in the additional biological complexity of aging in the study of cancer, a phenomenon that is heavily dependent on genetics, will further necessitate addressing genetic diversity in model systems.

Mouse diversity panels are a powerful platform to model human genetic diversity and determine how individual genetic variation contributes to the complexity of aging and cancer. By using aged, genetically diverse collaborative cross (CC)/diversity outbred (DO) mice displaying a wide range of phenotypic variation in tumor susceptibility, we might uncover new insights into how genetic background influences aging-associated cellular processes and potentially identify shared biochemical pathways between mouse and human. For example, the DO population, a heterogeneous outbred stock derived from eight founder inbred strains,[15] is an ideal resource for genetic mapping to determine the extent to which genetic modifiers of biological aging phenotypes intersect with genetic modifiers of cancer. DO mice could be used for phenotypic, epigenetic, and transcriptomic profiling across a variety of tissues in a cross-sectional study examining different ages (e.g.,12, 18, and 24 months of age) alongside comprehensive histopathological assessments to identify pre-neoplastic or neoplastic lesions.

Genetic background is also expected to influence the dynamics of initiating and resolving inflammation at epithelial barriers; polymorphism-regulating host responses combined with somatic mutation rate and type in different tissues likely represent a critical factor in determining tissue-specific response. Indeed, disease risk variants affect gene expression in context- and cell-type-dependent manners in humans. The human immune response to microbes, sterile tissue damage, or environmental perturbagens activating inflammation is highly divergent and is differentially regulated by age, genetic disposition, epigenomic state of responding immune cell(s), and environmental/lifestyle factors. Genetically diverse mice could further be used to incorporate and investigate acute or chronic inflammatory or immune stimulation. Analysis of aging-associated host and cancer behavior changes alongside comparable multi-omics data in humans will help us to select the diversity strains and models most representative of humans to study specific cancer behaviors. A key strength of this integrative cross-species approach is that it would enable the identification of actionable thresholds of transition over the course of chronological or biological aging, paving the way for interventions at such transition points to prevent or delay cancer progression. These models would also enable us to identify genetic and genomic markers of healthy aging vs. frailty, some of which might be detectable in early development. These signatures could inform how we use geroscience-guided therapies to improve clinical outcomes for older cancer patients.

Conclusions

The dearth of research at the intersection of aging and cancer is a vexing issue given that cancer is mostly a disease of older adults. Better understanding of the interplay between aging and cancer will be instrumental to improving cancer outcomes in older patients. Studies incorporating integrative mouse models of cancer and aging are therefore very much needed and should be coordinated via consortium-type efforts. The inevitable challenge is that all pre-clinical *in vivo* models are not human. Thus, care must be taken in extrapolating data from mice to human, and new generation models need to account for environmental factors as discussed above. Expanding the toolkit for cross-species integrative analyses in the future will help us identify and ideally account for those differences. We acknowledge that there are other *in vivo* models besides mice, such as rats, that might be better models for estrogen-sensitive tumors, as well as *in vitro* models like primary cells or organoids. However, *in vitro* models are limited in their ability to model cell-cell interactions, microenvironments, and time-dependent systemic effects, all of which are critical during aging. Addressing these and other questions in cancer and aging biology will require greater cross-divisional alignment in the case of federal agencies like NIH and increased strategic interest and support in this research area by additional funders. Such work must be reviewed by panels of experts that span both aging and cancer biology who can both appreciate the need for this work while acknowledging the even greater complexity of addressing research questions at this intersection. Successful development of clinically relevant models of age-related cancers will enable us to test therapeutic interventions and ultimately prevention strategies.

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DECLARATION OF INTERESTS

K. Polyak serves on the scientific advisory boards of Novartis, Vividion Therapeutics, Ideya Biosciences, and Scorpion Therapeutics; holds equity options in Scorpion Therapeutics; has received

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honorarium from Astra-Zeneca, New Equilibrium Biosciences, and Roche in the past 12 months; and receives sponsored research funding from Novartis. K. Politi is coinventor on a patent for EGFRT790M mutation testing issued, licensed, and with royalties paid from Molecular Diagnostics/Memorial Sloan Kettering Cancer Center. She reports research funding to her institution from AstraZeneca, Roche/Genentech, Boehringer Ingelheim, and D2G Oncology, and consulting for AstraZeneca and Jannssen. K. Palucka is a cofounder of Guardian Bio and holds equity and receives research support from Guardian Bio. She is a member of the scientific advisory board and holds equity from Cue Biopharma. She received research support from Merck in the past. O.A. has received research support from Sanofi and Pacbio in the past. J.J.T. receives patent royalties from Fate Therapeutics. She has also received research support from H3 Biomedicine, Inc. L.M.C. reports consulting services for Cell Signaling Technologies, AbbVie, the Susan G. Komen Foundation, and Shasqi; has received reagent and/or research support from Cell Signaling Technologies, Syndax Pharmaceuticals, ZelBio, Inc., Hibercell, Inc., Acerta Pharma, Prospect Creek Foundation, the Susan G. Komen Foundation, and National Foundation for Cancer Research; and has participated in advisory boards for Syndax Pharmaceuticals, Carisma Therapeutics, Inc., CytomX Therapeutics, Inc., Kineta, Inc., Hibercell, Inc., Cell Signaling Technologies, Alkermes, Inc., Genenta Sciences, Pio Therapeutics, Pty., Ltd., PDX Pharmaceuticals, Inc., NextCure, Guardian Bio, the AstraZeneca Partner of Choice Network, the Lustgarten Foundation, and the NIH/NCI-Frederick National Laboratory Advisory Committee.

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