ANNUAL REVIEWS

Annual Review of Biomedical Data Science

Decoding Aging Hallmarks at the Single-Cell Level

Shuai Ma,[1,2,3,*] Xu Chi,[4,*] Yusheng Cai,[1,3,*] Zhejun Ji,[3,5,*] Si Wang,[6,7] Jie Ren,[2,4,8] and Guang-Hui Liu[1,2,3,6,8]

[1]State Key Laboratory of Membrane Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China; email: mashuai@ioz.ac.cn, ghliu@ioz.ac.cn

[2]Institute for Stem Cell and Regeneration, Chinese Academy of Sciences, Beijing, China

[3]Beijing Institute for Stem Cell and Regenerative Medicine, Beijing, China

[4]CAS Key Laboratory of Genomic and Precision Medicine, Beijing Institute of Genomics, Chinese Academy of Sciences and China National Center for Bioinformation, Beijing, China; email: renjie@big.ac.cn

[5]State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China

[6]Advanced Innovation Center for Human Brain Protection and National Clinical Research Center for Geriatric Disorders, Xuanwu Hospital, Capital Medical University, Beijing, China; email: wangsi@xwh.ccmu.edu.cn

[7]Aging Translational Medicine Center, International Center for Aging and Cancer, Beijing Municipal Geriatric Medical Research Center, Xuanwu Hospital, Capital Medical University, Beijing, China

[8]University of Chinese Academy of Sciences, Beijing, China

Annu. Rev. Biomed. Data Sci. 2023. 6:129–52

First published as a Review in Advance on April 26, 2023

The *Annual Review of Biomedical Data Science* is online at biodatasci.annualreviews.org

https://doi.org/10.1146/annurev-biodatasci-020722-120642

*These authors contributed equally to this article

Keywords

aging hallmark, intervention target, mechanism, single cell, analysis tool, data resource

Abstract

Organismal aging exhibits wide-ranging hallmarks in divergent cell types across tissues, organs, and systems. The advancement of single-cell technologies and generation of rich datasets have afforded the scientific community the opportunity to decode these hallmarks of aging at an unprecedented scope and resolution. In this review, we describe the technological advancements and bioinformatic methodologies enabling data interpretation at the cellular level. Then, we outline the application of such technologies for decoding aging hallmarks and potential intervention targets and summarize common themes and context-specific molecular features in representative

organ systems across the body. Finally, we provide a brief summary of available databases relevant for aging research and present an outlook on the opportunities in this emerging field.

1. INTRODUCTION

1.1. Aging Hallmarks

Aging is associated with progressive degeneration in multiple systems across the body. At an individual level, aging occurs at a variable pace, as characterized by substantial molecular changes in cells, tissues, organs, organ systems, and the whole organism (1–5). By benchmarking aging-associated physiological changes and pathological progression, we stand to generate foundational knowledge that can help unravel mediators of the mechanisms underlying aging, and hopefully identify potential intervention targets to delay or even reverse aging. Such aging-associated molecular changes include shared features, such as genome and epigenome instability and cellular senescence (1, 5, 6), whereas others are context specific and occur at different levels, such as drivers or events specific to cell types in different tissue-specific microenvironments or systemic factors that project long-range effects across systems (2). Combined, these hallmarks reflect organismal aging and its unique features across systems.

1.2. The Benefit of Decoding Aging at Single-Cell Resolution

The complex and heterogeneous biological processes that drive the aging process across tissues and organs require investigations with a broad scope and with precision beyond that provided by conventional molecular biology approaches. For example, initiating events occurring in rare cell populations may remain undetected until downstream effects have rippled through the tissue and caused a more generic change that becomes detectable at the bulk level, obscuring the genuine triggering mechanism.

Over the past several years, substantial efforts have been dedicated to the development of single-cell omics technologies in all dimensions, including genomics, epigenomics, transcriptomics, proteomics, metabolomics, and their combinations with spatial and temporal information. Equipped with these dissection scopes at single-cell resolution, we are able to identify initial changes in a small subset of cells in a complex and heterogeneous tissue microenvironment (7, 8). Furthermore, very recently, with decreasing cost and increasing throughput, single-cell studies spanning multiple tissues and organs, or even the whole organism, have started to emerge, generating so-called atlases. In particular, recent pan-tissue studies have provided both an eagle-eye view from far above and a microscopic view for a deeper dive. Such atlases, when applied in aging, allow researchers to dissect one cell type distributed in multiple tissues, as well as to profile a broad spectrum of cell types, extending the current paradigm of aging research to a systemic level (9–13).

1.3. Current State and Opportunities

Since its first emergence in 2009 (14), single-cell omics has revolutionized many fields within the life sciences. Aging research is no exception, as summarized by a timeline of representative studies (**Figure 1**). With the tremendous expansion of single-cell omics and even multiomics, analysis tools capable of integrating multidimensional datasets are needed to uncover the underlying hallmarks of and potential targets in aging. Here, we review recent advances in technologies for analyzing the quickly expanding biomedical data, as well as generating new datasets, focusing on their application for understanding the hallmarks of and mechanisms underlying aging. We

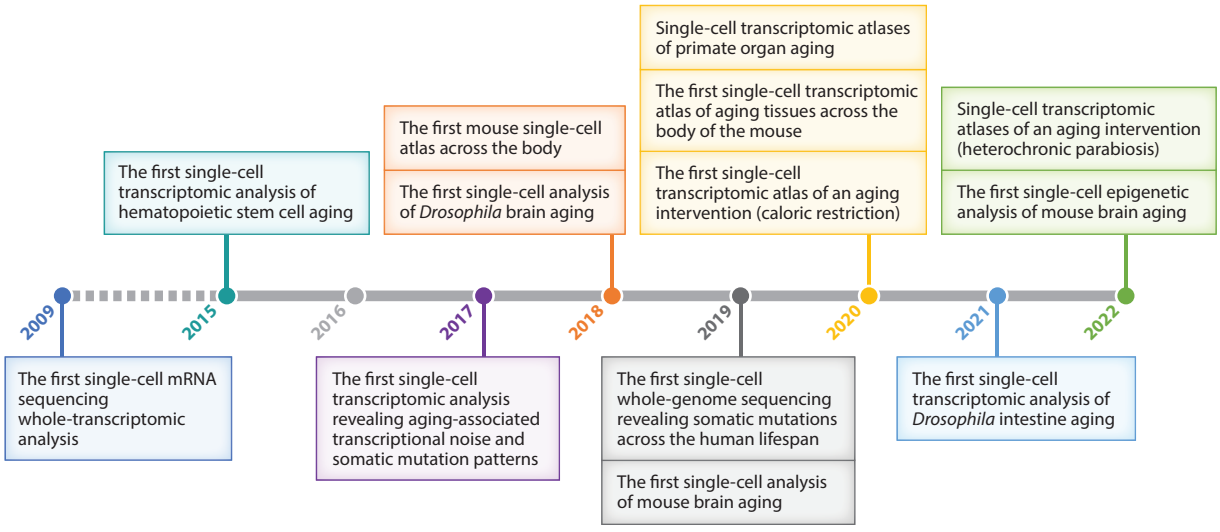

Figure 1

A timeline of the development of single-cell sequencing technologies and their applications in aging research.

further discuss outstanding questions in the field and highlight techniques that will advance our understanding of organismal aging.

2. DECODING AGING HALLMARKS THROUGH THE LENS OF SINGLE-CELL OMICS: TECHNOLOGIES AND RELATED COMPUTATIONAL METHODS

2.1. Technologies in Relation to Aging Studies

In this section, we review the breakthroughs and advancements in single-cell sequencing technologies, including single-cell transcriptomics [single-cell RNA sequencing (scRNA-seq)], genomics, epigenomics, proteomics, metabolomics, and their multimodal combinations, as well as those with spatial context. Specifically, we focus on their applications in aging studies.

2.1.1. Single-cell transcriptomics. Because aging is a gradual degeneration process composed of multiple cellular state transitions and functional changes, single-cell transcriptomics, which generates the full spectrum of gene expression levels of individual cells, has become the most widely used tool for investigating aging-associated cellular changes. The detailed nature of this technology, along with other technologies listed in Section 2.1 that create single-cell omics, has been reviewed elsewhere (15, 16). Here, we focus on its application in aging studies to identify infrequent cellular state transitions, such as adult stem cells becoming senescent, a state transition known to disrupt homeostasis and initiate degeneration in the whole tissue and across multiple systems. Among the many organ systems described in Section 3, typical examples can be found in the hematopoietic and immune system, where senescent hematopoietic stem cells (HSCs) lead to impaired adaptive immunity (17, 18). Given the power of analyzing thousands or even higher numbers of cells in parallel, scientists can now study aging via single-cell transcriptomics in almost every organ in mammals (to name a few, see 19–28), and even at the organismal level in rodents (10–13, 29), generating valuable single-cell atlases, as described in Section 4.1.

2.1.2. Single-cell genetic heterogeneity. Accumulation of DNA mutations is a hallmark of aging (1), but the heterogeneous nature of somatic mutations hinders their identification at the bulk level—they are difficult to distinguish from sequencing errors if present only in a small proportion of cells. In contrast, single-cell DNA sequencing identifies genomic changes, such as single-nucleotide variants, copy number variations, and chromosomal aneuploidy (30), enabling studies of genomic mosaicism and cell lineage tracking during physiological and pathological processes in development and aging (31). Together with other advanced methods, including whole-genome sequencing (WGS) of laser-capture microdissections (32) or single-cell-derived organoids (33), researchers can identify aging-related genomic variations in specific cells or clones in a variety of tissues. For example, a recent study reported that the accumulation of somatic mutations in human neurons is associated with aging and neurodegeneration (34). At a more general level, adult stem cells in the human small intestine, colon, and liver (33) and circulating B lymphocytes accumulate mutations steadily over time, albeit at rates that vary by tissue (35). Interestingly, and supporting the notion that the somatic mutation rate contributes to aging, single-cell WGS analysis of somatic mutation rates has shown variations across 16 mammalian species and an inverse relationship with species lifespan (36).

2.1.3. Single-cell epigenomics and chromatin organization. Single-cell epigenomics defines the epigenetic states and identities of individual cells, revealing regulators of the transcriptome and heritable changes within genotypically identical cells. With barcoding or indexing preserving information about cellular identity, various methods have transitioned from the bulk level to the single-cell level to analyze each layer that constitutes the epigenome. During aging, and unlike the relatively rare genetic mutations, the epigenetic landscape undergoes a hierarchy of large-scale organizational alterations: from nuclear deformation to compromised chromatin compartmentalization and to loss of local epigenetic identity, along with enhanced cellular heterogeneity (1, 37–39). Among aging-associated epigenetic alterations, shifts in DNA methylation and histone modifications have been most widely studied. In particular, the epigenetic clock, a method to measure chronological age with remarkable precision, was developed based on DNA methylation levels (40). Again, aided by single-cell DNA methylomes, researchers developed the epigenetic clock framework scAge to provide a more accurate chronological age of a tissue while uncovering heterogeneity in epigenetic aging among the different cell types (41). Similar to variation in DNA methylation, a recent study using EpiTOF (epigenetic time-of-flight), which can measure a broad array of chromatin marks in single immune cells, reported that variability in histone modifications also accumulates with age (42). Accordingly, heterochromatin loss and consequent activation of LINE1 elements during aging were identified specifically in excitatory neurons in the old mouse brain via single-nucleus ATAC-seq (assay for transposase-accessible chromatin using sequencing) and Paired-Tag (assay for joint profiling of histone modifications and gene expression in single nuclei) (43).

2.1.4. Spatial omics. To overcome the biases caused by digestion steps of single-cell omics, researchers developed spatial omics to extract intra- and intercellular information about DNA, RNA, the epigenome, and proteins within their native spatial context. When applied in aging research, fine-grained transcriptomes with spatial organization (i.e., spatial transcriptomes) allow for the identification of cell types with respect to tissue architecture, cell-specific molecular hallmarks, and cell–cell interactions in situ (44). In particular, and given that juxtracrine and paracrine signals operate from 0 to 200 μm (45), such spatial information is vital for understanding the interplay between aging-sensitive cell types and the aged microenvironment. Indeed, spatial transcriptomics has revealed cell state changes in specific brain regions and increased neuroinflammation and immune cell activation in old mouse brain (46, 47). However, the sequencing

depth of spatial transcriptomics is still limited compared to single-cell transcriptomics, making it less powerful in identifying the relatively confined changes in transcriptomes from young and old organisms. Therefore, the combination of spatial transcriptomics and single-cell transcriptomics is a trend in current analysis, such as in a recent preprint study of human ovarian aging (48). Nevertheless, technological advancements in spatial transcriptomics for higher resolution and more accurate incorporation of multimodal data with imaging will undoubtedly provide more insight into aging-associated molecular remodeling with the anatomical organization of cells.

2.1.5. Other single-cell omics and summary. Although nucleic acid–based analyses dominate single-cell omics, large-scale profilings of other types of molecules at the single-cell level are emerging. Instead of using RNA levels as a proxy for protein levels, single-cell proteomics can directly detect and quantify a large number [recently pushed to 1,000–3,000 (49)] of proteins from individual cells, giving a snapshot of the cellular machinery for (in principle) any given cell type and cell state. Similarly, single-cell metabolomics analyzes metabolites, products of cell metabolism that serve as systemic and microenvironmental signaling molecules in all organisms (50). Recent technical advances now even allow for the simultaneous interrogation of different modalities at the single-cell level, such as genomic mutations, gene expression, epigenetic modifications, chromatin accessibility, and higher-order organization (51). However, there are challenges and opportunities in integrating different layers of single-cell information into cellular regulatory frameworks, by either computational merging or experimental combinatorial analyses. In addition, by harnessing the long reads from third-generation sequencing (i.e., Pacific Biosciences and Oxford Nanopore sequencing platforms), researchers can now identify larger structural variations and more splicing isoforms at the single-cell level, along with epigenomic information such as that provided by scNanoATAC-seq, a long-read single-cell ATAC-seq (scATAC-seq) method based on the Nanopore platform (52). Taken together, multimodal approaches stand to generate deeper molecular insights into different cell types and dynamics; hence, we are expecting such applications to increase in aging research.

Collectively, for the majority of single-cell technologies, and even for the most developed single-cell transcriptomics, it remains challenging to scale techniques to an even larger number of cells and more molecular features per cell while maintaining or increasing accuracy for each modality and maintaining affordability. Looking to the future, in situ cell barcoding and various spatial approaches hold significant promise for further increasing throughput and multimodality. Meanwhile, approaches for the simultaneous measurement of multidimensional data or for integrated analysis after acquisition are increasingly in demand. Big data analysis is discussed below in Section 4.2.

2.2. Computational Methods and Tools for Aging Features

Advances in single-cell omics technologies have led to the generation of vast amounts of multimodal datasets, but at present, scRNA-seq analysis tools are the most developed, while tools capable of integrating multidimensional datasets are emerging quickly but still primitive. Currently, multiple protocols for single-cell sequencing analysis are available, each with its own advantages and trade-offs. While most follow similar workflows, optimization tools at each step vary, as do benchmarking studies used to make informed decisions on which tool to use. The initial workflow of scRNA-seq includes quality control, normalization, batch effect correction, data downscaling, and cell annotation. In addition to these common tools, unique features of aging require specialized implantations of downstream analysis tools to characterize systemic and multilevel hallmarks of aging, such as increased genomic instability, epigenetic heterogeneity, transcriptional noise, cell fate shift, and intercellular communication alterations. Computational tools for this purpose are described below and summarized in **Supplemental Table 1**.

Supplemental Material >

For example, increased genomic copy number variation, resulting from increased genomic instability in senescent cells, can be calculated by specific tools such as inferCNV (53), Ginkgo (54), and CopyKAT (55). Likewise, researchers have developed scT&R-seq (single-cell telomere length analysis and RNA sequencing) to simultaneously observe heterogeneity in telomere length and measure the transcriptome in a cell, which can be applied to quantify telomere shortening, a typical feature of cellular senescence (5, 56). In addition to the nucleus, mitochondrial DNA (mtDNA) also carries genomic information that impacts cell function in aging; mtDNA mutations impair mitochondrial function, which in turn leads to dysregulation of energy metabolism (57). Recently, a dedicated computational tool kit, mgatk, for mtscATAC-seq (mitochondrial single-cell assay for transposase-accessible chromatin with sequencing) was developed to call with high-confidence rare-clonal mtDNA mutations concomitant with a chromatin accessibility profile (58). These approaches make it possible to infer mtDNA heterogeneity, clonal relationships, cellular state, and accessible chromatin variation in healthy, diseased, or senescent cells.

Meanwhile, epigenetic alterations and heterogeneity serve as both a cause and consequence of the aging process. To evaluate chromatin accessibility in individual cells, researchers have widely used ArchR (59) to process and analyze scATAC-seq data with minimum memory or computational requirements. For single-cell DNA methylation, which suffers from incomplete CpG coverage, DeepCpG (60) uses a deep neural network to overcome this limit and to predict methylation states in single cells. For a comprehensive tool kit for analyzing single-cell epigenomic data, Stuart et al. developed Signac (61). In addition, through seamless compatibility with the Seurat software package for scRNA-seq (62), Signac allows for multimodal analysis, integrating DNA accessibility with gene expression, protein abundance, and mitochondrial genotype data.

Genomic/epigenomic instability contributes to transcriptional noise such that larger transcriptional variations and entropy increases were observed in single-cell transcriptomes during aging. Although noise is rarely observed in studies of early development, where single-cell omics studies have emerged and thrived, it is a common feature in the aging field and is evaluated by specialized algorithms, as listed in **Supplemental Table 1**. Specifically, within the same tissue, higher transcriptional noise is present in certain cell types, such as immune cells in aged mouse lungs, oocytes from primordial follicles, and natural killer T cells of aged ovaries, as well as in stromal cells, and even higher noise was noted in fibroblasts, endothelial cells (ECs), and pericytes of the aged skin (19, 26, 63). Underlying the elevated transcription noise, the transcriptional regulatory network becomes further impaired in aging. At present, several studies have used scRNA-seq or scATAC-seq data to analyze and compare changes in the transcriptional regulatory network and key regulators associated with aging, among which the most mainstream analysis software is SCENIC (64).

Supplemental Material >

Among pathways impacted in aging, metabolic pathways have attracted attention. Accordingly, dedicated tools (65–68) have been developed to predict changes in metabolites or metabolic pathways based on single-cell metabolomic or transcriptomic data and to evaluate metabolic disorders in different cell types, tissues, and organs intertwined with the aging process. Yet another key pathway with dedicated analysis tools is the regulation of cell cycle progression. Indeed, cell cycle arrest is a hallmark event of cellular senescence. Current methods for cell cycle prediction and gene set scoring in single-cell sequencing have important applications for this aspect of aging research. To this end, the cyclone function in the Scran software (**http://bioconductor.org/packages/release/bioc/html/scran.html**) can predict the cell cycle of each cell based on single-cell transcriptomes. Similar tools include the CellCycleScoring function of the software Seurat (62), while the software peco (69) implements machine learning methods to predict continuous cell cycle phases with scRNA-seq data.

Besides the aforementioned common pathways, cell type–specific alterations underlie key features of aging, including stem cell exhaustion and a shift or impairment of lineage differentiation capacity, which can be assessed using pseudotime analysis. Monocle (70, 71) is a well-known pseudotemporal analysis software that uses advanced machine learning (reverse graph embedding) to acquire explicit master graphs from single-cell data to rank cells, thus alleviating confounding impacts resulting from asynchrony, and accurately solving complex development processes. Another algorithm, RNA velocity (72), is based on transcriptional dynamics—on the pseudotime axis, the appearance of unspliced mRNA always precedes that of spliced mRNA, indicating the direction of differentiation of cellular gene expression. Thus, it can be used to define the start branch and endpoint of the differentiation trajectory and to predict the direction of lineages without prior knowledge.

Finally, single-cell omics aids in the analysis of the aging microenvironment. In addition to cell cycle arrest, senescent cells also secrete a large number of inflammatory factors that further promote the senescence of surrounding cells, generally referred to as the senescence-associated secretory phenotype (SASP). Various gene set scoring algorithms, such as AUCell (64), Ucell (73), and singscore (74), can calculate the expression level of gene sets in each cell, including those related to the SASP in aging. On the other hand, the SASP, along with other aging drivers, rewires intercellular communication through ligands and receptors on neighboring cells. Algorithms for predicting the probability of ligand–receptor interaction occurrence based on gene expression levels have been used in aging research, including CellphoneDB (75), SingleCellSignalR (76), CellChat (77), and NicheNet (78). Moreover, both in the tissue microenvironment and at the systemic level, the reduction of the T cell receptor (TCR) and B cell receptor (BCR) library, the imbalance of original memory cells, and the loss of effector cell plasticity are characteristic of immune aging. With the advent of single-cell TCR and BCR sequencing techniques, a series of analytical tools, such as TraCeR (79), scTCRseq (80), BASIC (81), and VDJPuzzle (82), have been developed to detect and analyze the changes in immune functions, especially key receptors of T cells and B cells during aging.

3. APPLICATIONS OF SINGLE-CELL OMICS IN AGING STUDIES: CELL TYPE–SPECIFIC HALLMARKS AND TARGETS FOR INTERVENTIONS

As summarized above, recent developments in single-cell omics technologies have paved the way toward identifying and characterizing aging hallmarks shared across organs and tissues, as well as those that are unique in organs and tissues. In this section, we summarize the current knowledge of aging hallmarks identified by applying single-cell omics technologies, ranging from aging-sensitive cell types to the intrinsic and external driving forces of aging. Specifically, aging hallmarks are described in representative organ systems, with emphasis on themes of aging-sensitive cells shared across systems and having organismal-level effects. Meanwhile, we elaborate on unique features specific to individual systems with respect to their own environments. Among these, the aberrant exit of the reversible quiescent state into senescence or exhaustion of tissue-resident stem cells is a main culprit of biased lineage differentiation, disturbed homeostasis, and impaired function, thus impacting the entire organism. Other aging-sensitive cell types include cells critical for tissue functions and microenvironmental support cells, such as ECs, fibroblasts, and resident or infiltrated immune cells. Thus, rather than a comprehensive review of all organ systems, here we aim for a summary of unique and shared hallmarks in selected systems to synthesize aging features with generality and precision. We also discuss the limitations of current studies, emerging directions, and how accumulating aging datasets open up new research opportunities.

3.1. The Hematopoietic and Immune System

The hematopoietic system is a typical example where stem cells play a pivotal role in the aging of the whole system. During aging, HSCs increase in number. In aged mice, the fraction of long-term reconstituting HSCs expands approximately sixfold (>22 months old versus 2–3 months old) (83). However, aged HSCs are less capable of maintaining a balance between quiescence, self-renewal, and differentiation into the full spectrum of lineages required to maintain homeostasis, instead demonstrating myeloid-skewed differentiation potential and megakaryocyte-platelet bias (18, 83–86). Thus, as aged HSCs fail to maintain organ homeostasis and regeneration, the aging process in the hematopoietic and immune system is promoted, altering susceptibility to autoimmune diseases (87), and even reshaping the physiological and immune landscapes of the whole organism, thus increasing vulnerability to aging-related diseases, frailty, and multimorbidity (88, 89).

Single-cell omics studies that categorize these aging-sensitive cell types, including HSCs and bone marrow niche cells, seek to dissect the underlying causes intrinsic to aged HSCs and those induced by remodeling the aging microenvironment (17, 90). Here we list three: (*a*) Intrinsically, HSCs suffer from genome instability (91) and epigenetic drift (42, 92). This may lead to clonal hematopoiesis when a substantial proportion of blood cells are derived from a single mutated HSC, which is observed in 10–20% of the elderly population (93). Recent research on clonal hematopoiesis using single-cell multiomics revealed that DNMT3A R882 mutation led to the lineage bias of myeloid cells over lymphoid cells by preferential hypomethylation of PRC2 (polycomb repressive complex 2) target genes and a specific CpG flanking motif (94). Other somatic mutations [i.e., mutations in TET2 (95), ASXL1 (96), TP53 (97), and PPM1D (98)] also frequently accompany clonal hematopoiesis. (*b*) Extrinsically, such alterations in HSCs are also induced by cross talk with aging-sensitive cell types in the bone marrow niche that send proinflammatory and unfavorable signals for HSC maintenance with aberrant metabolic programs. These age-associated hallmarks include an increased population of activated macrophages but with decreased phagocytic function (99, 100), decreased expression of surface molecules responsible for homing, egress, and differentiation of human mesenchymal stem/stromal cells in the marrow (101), and reduced levels of systemic factors received from distant systems or even diet (102). These extrinsic cues induce aging in stem cells and their differentiated derivatives, affecting not only fate decisions (quiescence, self-renewal, or differentiation) but also cell maturation. Meanwhile, single-cell imaging and transcriptome analysis have revealed that the most quiescent HSC subpopulation is protected by sinusoidal niches (marked with Nestin-dim perisinusoidal cells), which preserve their shape and number during aging (103). In a recent study on rejuvenation, researchers identified several potential targets and pathways that could reduce inflammation and restore cellular communication. Moreover, in parabiosis models, aged hematopoietic stem and progenitor cells in mice were sensitive to exposure of young blood, as reflected by a switch to a transcriptional regulatory network resembling that of a younger state (10, 29). (*c*) Since aged HSCs give rise to immune cells that circulate in the whole organism and reside in nearly all tissues (104, 105–108), single-cell studies revealed that the populations of derived immune cells present in the microenvironment are altered in various tissues across systems (11, 29). These interdependent relationships contribute to increased chronic inflammation, or inflammaging, an aging cue also discussed in the context of other systems and systemic aging.

3.2. The Nervous System

The nervous system decodes and integrates incoming sensory information and coordinates outgoing signals to and from different tissues and organs across the whole body. Given the heterogeneity among different brain regions and the rich diversity of neuronal subtypes, the dissection of

aging-associated changes at single-cell resolution is absolutely needed. Thus far, single-cell omics has helped to identify aging-sensitive cell types, including neural stem cells (NSCs) and specific types of neurons and non-neuronal cells, providing a wealth of resources for revealing aging hallmarks and potential therapeutic targets for nervous system aging and related diseases.

Although whether adult neurogenesis exists in certain species continues to be debated, particularly in humans (109–111), NSCs undoubtedly play essential roles during aging in multiple species. First, a dramatic decline in NSC number is a hallmark of the aged murine brain (112, 113). In addition, aging-associated cognitive decline has been linked with dampened NSC/neural progenitor cell (NPC) function, as manifested by increased quiescence, decreased proliferation, and differentiation bias toward astrocytes at the expense of neurons. Interestingly, changes in cell proliferation capacity are not influenced by inflammatory cytokines but likely caused by cell-intrinsic mechanisms (112, 113). Among these, the ERK/MAPK pathway appears to be critically involved in regulating age-dependent clonal expansion capacity (112). In-depth dissection of single-cell gene expression dynamics in the nonhuman primate (NHP) hippocampus also revealed age-related impairment in the division of transiently amplifying progenitor cells and compromised neuronal function along the neurogenesis trajectory (20).

In addition to mitotic NSCs/NPCs, numerous studies have demonstrated that the functional decline of postmitotic neurons also contributes to neurogenerative diseases. Conversely, individuals with cognitive resilience can retain normal cognition even when carrying pathological hallmarks of neurodegenerative diseases (114). In such individuals, single-nucleus RNA sequencing (snRNA-seq) of cortical tissues identified upregulation of *MEF2C* in a subpopulation of excitatory neurons, indicating a previously unrecognized role for MEF2 transcription factors in promoting cognitive resilience (114). In another snRNA-seq study, adaptive changes to hypocretin/orexin neuronal loss in the aged brain were found to drive age-associated sleep fragmentation (115). Intriguingly, age-accumulated DNA damage and epigenetic changes are observed in vulnerable neurons, impairing physiological functions (20, 116). For example, a single-cell WGS study investigated genome-wide somatic single-nucleotide variants (sSNVs) in single neurons from the prefrontal cortex and hippocampus of normal individuals (aged 4 months to 82 years), as well as individuals affected by early-onset neurodegeneration due to genetic disorders of DNA repair (e.g., patients with Cockayne syndrome and xeroderma pigmentosum) (34). The authors found that sSNVs increased approximately linearly with age in both brain regions (with a higher rate in the hippocampus) and were more frequent in samples with early-onset neurodegenerative diseases (34). A recent single-cell epigenome analysis also revealed age-associated decay of heterochromatin domains in excitatory neurons in the mouse brain, as evidenced by gain of chromatin accessibility at these genomic loci accompanied by the cell type–specific loss of heterochromatin and activation of LINE1 elements (43).

Single-cell omics has also helped identify age-related alterations in non-neuronal and relatively rare cell types that likely contribute to a hostile microenvironment for tissue-resident stem/progenitor cells and neurons, including glial cells, immune cells, ECs, and other niche cells. Change in the aging microenvironment is a recurrent theme in and out of the nervous system.

For instance, glial cells, such as clusters of active astrocytes and microglia exhibiting proinflammatory responses, were enriched in the aged primate hippocampus and aged mouse brain (20, 109, 117). Interestingly, an accumulation of $p16^{High}$ senescent microglia was also observed in the brains of aged humans (118). In addition, the abundance of a phagocytic/activated AD1-microglia population in patients with Alzheimer's disease was found to be closely correlated with tissue amyloid-β load, and these microglia localized to amyloid-β plaques. Meanwhile, the abundance of AD2-microglia strongly correlated with tissue phospho-tau load and was more pronounced in patients with overt tau pathology (119).

Immune cells, such as T cells, infiltrate into old neurogenic niches and thus contribute to the inflammatory microenvironment (27). In particular, T cells in aged brains are clonally expanded and are generally different from those in aged blood, suggesting that they may experience specific antigens. They also express interferon-γ, which may impair the proliferation of a subset of NSCs that have a high interferon response (27). Likewise, an accumulation of natural killer cells of the innate immune system, residing in the dentate gyrus neurogenic niche of aged brains, has been reported to impair neurogenesis in humans and mice (120).

Additionally, ECs serve as another crucial type of niche cells, whose dysfunction may lead to dysregulation of cerebral blood flow and disruption of the blood–brain barrier and promote the pathogenesis of vascular cognitive impairment (121). scRNA-seq of enriched cerebromicrovascular ECs demonstrated that there is an increased ratio of senescent ECs (~10%) in the mouse cerebral microcirculation with advanced aging (121). Another brain endothelial cell (BEC)-focused study in the hippocampus discovered that capillary BECs underwent the greatest transcriptional changes in physiological aging in comparison with arterial and venous BECs, as manifested by upregulated innate immunity and oxidative stress response pathways (122). Finally, analysis of a single-cell and spatial atlas of the choroid plexus in the aged mouse brain revealed that epithelial cells upregulate host defense programs in response to resident macrophages, which in turn upregulate interleukin-1β signaling genes (123).

In the retina, an extension of the central nervous system (CNS), scRNA-seq identified retinal pigment epithelium cells as the cell type most susceptible to aging in NHP samples, as evidenced by decreased cell density, the highest numbers of differentially expressed genes overlapping with genes underlying aging and aging-related retinal diseases, and aberrant cell–cell interactions with its two adjacent layers: the neural retina layer and the choroid layer (21). Another scRNA-seq study showed that aging of the human retina occurred earlier in the fovea than in the peripheral retina, and that MYO9A^{-} rods and a horizontal cell subtype showed greater vulnerability to aging (124). In the mouse cochlea, the entry point of the auditory system into the CNS, a recent study depicted a dynamic single-cell transcriptomic landscape of cochlear aging. In this study, aging-related transcriptomic changes pinpointed a higher age-related transcriptional fluctuation in a stria vascularis cell population, underscoring its high susceptibility to aging. Specifically, the cochlea suffers from loss of proteostasis during aging, and upregulation of the endoplasmic reticulum (ER) chaperone protein HSP90AA1 was identified as the hallmark of cochlear aging (125).

3.3. The Vascular System

The vascular system, composed of vessels that circulate blood and lymph throughout the body, differs from other organ systems in that its regeneration depends on EC behavior and assembly (126) and not on tissue stem cells. Due to the broad distribution of vessels, the aging of the vascular system could affect multiple tissues/organs and impair their functions, while aging hallmarks of vessels can be unique or shared between different tissues. For example, for blood vessels in the heart, a single-cell transcriptomic landscape of aortas and coronary arteries from young (4–6 years) and old (18–21 years) cynomolgus monkeys demonstrated decreased expression of FOXO3A in six vessel cell types, especially in ECs (127). Additionally, scRNA-seq analysis of cell–cell communications revealed that interactions between ECs and immunocytes were increased in the aged vasculature of both monkey coronary arteries and aortic arches and mouse hearts (128). BACH1 was identified as a master regulator of aging-related genes by transcriptional regulatory network analysis in monkeys and mice, suggesting an important role of BACH1 in endothelial senescence and vascular aging (128, 129). Interestingly, in mouse brains from young (3-month-old) and aged (28-month-old) C57BL/6 mice, a subpopulation of cerebromicrovascular ECs was also identified

with high expression of cellular senescence genes (121). Their senescence also induces multifaceted influences, including impairment of the blood–brain barrier and dysregulation of cerebral blood flow (121).

3.4. The Digestive System

Within the digestive system, the liver is a metabolic hub that plays an important role in the detoxification of xenobiotics, which makes this organ the prime target of genotoxicity in the body (130). Utilizing single-cell multiple displacement amplification to analyze somatic mutations in single primary hepatocytes from human donors (varying in age between 5 months and 77 years), increased frequencies of spontaneous mutation were observed at advanced age, which may causally contribute to the age-related functional decline and increased incidence of human liver diseases (130). Such increased genome instability is consistent with observations in other systems during aging.

In a study of aging in the human pancreatic tissue, scRNA-seq analysis demonstrated that levels of transcriptional noise and potential fate drift were elevated in islet endocrine cells from older donors (28). Consistent with observations in the aged liver (130), a novel mutational signature was also discovered in healthy aging endocrine cells (28). These findings suggest that genetic errors that accumulate in physiological aging can be benign, but can also lead to organ dysfunction or catastrophic transformation, as in cancer. Additionally, scRNA-seq of pancreatic islet cells from young and aged cynomolgus monkeys demonstrated an increased unfolded protein response, along with the accumulation of protein aggregates in aged beta cells (131). Further investigation found that aging-related beta cell–specific upregulation of HSP90B1, an ER-localized chaperone, impeded high-glucose-induced insulin secretion, a mechanism that may cause age-associated impairment of glucose tolerance and facilitate the development of diabetes (131).

In the intestine, age-associated changes may contribute to malnutrition in elderly individuals (132). Among the cell types in the intestine, an age-related decline in the regenerative capability of intestinal stem cells (ISCs), which regenerate the gastrointestinal epithelium, significantly perturbs intestinal homeostasis (133). Using the *Drosophila* intestine as a model to explore stem cell–intrinsic changes during aging, Tauc et al. (134) employed bulk genome-wide chromatin accessibility and transcriptome analysis combined with scRNA-seq to find that promoters of Polycomb target genes become differentially accessible in aged ISCs, resulting in increased expression of enteroendocrine cell specification genes and biased differentiation. In a related study, which reported the construction of an enteric neuronal atlas using RAISIN (ribosomes and intact single nucleus) RNA-seq for profiling intact nuclei with ribosome-bound mRNA and MIRACL-seq (mining rare cells sequencing) for label-free enrichment of rare cell types by droplet-based profiling, dysregulation of circadian genes and genes involved in age-related CNS diseases segregated with chronological age in enteric neurons, suggesting its tight correlation with intestinal and extraintestinal diseases (135). Taken together, these studies suggest that in addition to ISCs, enteric neurons play critical roles in intestinal homeostasis.

3.5. The Reproductive and Urinary System

In the reproductive organs, the biology of germ cells and somatic cells largely resembles that of stem/progenitor cells and niche-supporting cells in other systems. For germ cells (i.e., oocytes in females and spermatogenic cells in males), age-associated changes often cause attrition of germ cell pools in both sexes and dampen folliculogenesis or spermatogenesis, respectively. For the female reproductive system, scRNA-seq and single-cell whole-genome DNA methylation sequencing in both young and aged germinal vesicles of mouse oocytes uncovered mitochondrial dysfunction

and ER stress; significant changes in the expression of some metabolism-related genes, such as mitochondria-associated genes; and reduced antioxidant capability, all of which might be involved in the progression of oocyte aging (136, 137). Similar to findings in rodents, the single-cell transcriptomic landscape of ovaries from young and aged NHPs revealed aging-associated disturbance of antioxidant signaling in early-stage oocytes, indicative of oxidative damage as a crucial factor in ovarian functional decline with age (26). Interestingly, scRNA-seq in human young and aged metaphase II oocytes, obtained from patients undergoing in vitro fertilization or intracytoplasmic sperm injection, also revealed that upregulated genes were enriched for functional annotations related to oxidative stress and immune function, while downregulated genes were enriched for catalytic activity acting on DNA and glycoproteins (138, 139). For male germ cells, scRNA-seq analysis showed that age-related changes in spermatogonial stem cells (SSCs) appeared modest in aged men (>60 years old) (140, 141), whereas another study in aged NHPs found that a high susceptibility of SSCs to aging leads to their overactivated proliferation and subsequent exhaustion in the testis, a mechanism likely to contribute to age-related compromise in spermatogenesis (142). The discrepancy may result from the heterogeneity of the selected samples or the species differences between humans and NHPs, which await further investigation. Collectively, although less consistent on the male side, for female germ cells, several single-cell omics studies in mice and primates converged on mitochondrial functions as primary aging hallmarks in oocytes.

For somatic cells, the crucial niche cells that support germ cell development undoubtedly contribute to age-associated alterations (26, 143). In the ovary, an in-depth single-cell transcriptomic analysis delineated that silencing of antioxidant genes in aged monkey and human granulosa cells represented a conserved hallmark of primate ovarian aging (26). In the male reproductive system, single-cell and single-nucleus transcriptomic studies revealed cell type–specific alterations in multiple pathways, including tight junction and metabolic signaling in Sertoli cells; hedgehog signaling and testosterone production in Leydig cells; cell death and growth in testicular peritubular cells; possible developmental regression and impaired cell identity in Sertoli, Leydig, and peritubular cells; and inflammatory responses in multiple cell types (140, 142). These changes across specific somatic cell types in response to aging compromise functional support to germ cells and ultimately cause subfertility and even infertility with increased age.

Except for the male reproductive system, aging in the prostate of the urinary system is considered a major risk factor for benign prostatic hyperplasia (BPH) and prostate cancer in aged men. Mass cytometry analysis (cytometry by time-of-flight) of single-cell suspensions of total mouse prostate with a panel of metal-tagged antibodies against cell surface markers revealed an age-related increase in prostate-infiltrating lymphocytes, suggesting age-related changes in the prostate microenvironment. In addition, the study also revealed that progenitor-enriched luminal cells are expanded with aging in the mouse and human prostate, which may contribute to BPH (144).

3.6. Profiling of Systemic Aging and Intervention of the Whole Organism

In systemic aging, a progressive loss of physiological integrity and functions across the whole body increases the risk for early onset of chronic diseases and vulnerability to death. Recently, high-throughput scRNA-seq/snRNA-seq studies encompassing multiple tissues and organs across systems have facilitated a comprehensive understanding of cellular and molecular programs during systemic aging and aging intervention (10–13, 29). In detail, Tabula Muris, a single-cell transcriptome compendium of 23 tissues/organs of *Mus musculus*, revealed systemic aging hallmarks, including senescence, genomic instability, and changes in the immune system (12). A similar study of *Rattus norvegicus* also found increased immune cells and disturbed immune ecosystems in tissues throughout the body during aging (11).

Interestingly, metabolic intervention, such as caloric restriction, was found to favorably reverse aging-related inflammation by a comprehensive single-cell transcriptional landscape across various rat tissues (11). In other aging interventions, such as heterochronic parabiosis (HP), scRNA-seq analysis has helped delineate its high-resolution landscape and uncover its associated molecular hallmarks. In the HP paradigm, young and aged mice are surgically connected such that a shared circulatory system facilitates the exchange of systemic factors between parabionts (10, 29). As such, a single-cell transcriptomic atlas across aged tissues/organs and their rejuvenation in HP identified hematopoietic stem and progenitor cells as one of the most responsive cell types to HP, probably through the restoration of youthful transcriptional regulatory programs and increased lymphopoiesis (10, 145).

Altogether, these data provide a rich resource for the systemic and simultaneous exploration of age-related cellular and molecular alterations specific to individual cell types at an unprecedented resolution, with the scope of the whole organ, system, or organism (**Figure 2**). These aging hallmarks described here in selected systems also demonstrate the repeating themes of tissue and organismal aging, for example, the biology between aged stem cells (or key functional cells in certain systems) and their supportive cells, as well as cell-intrinsic drivers and extrinsic cues of aging.

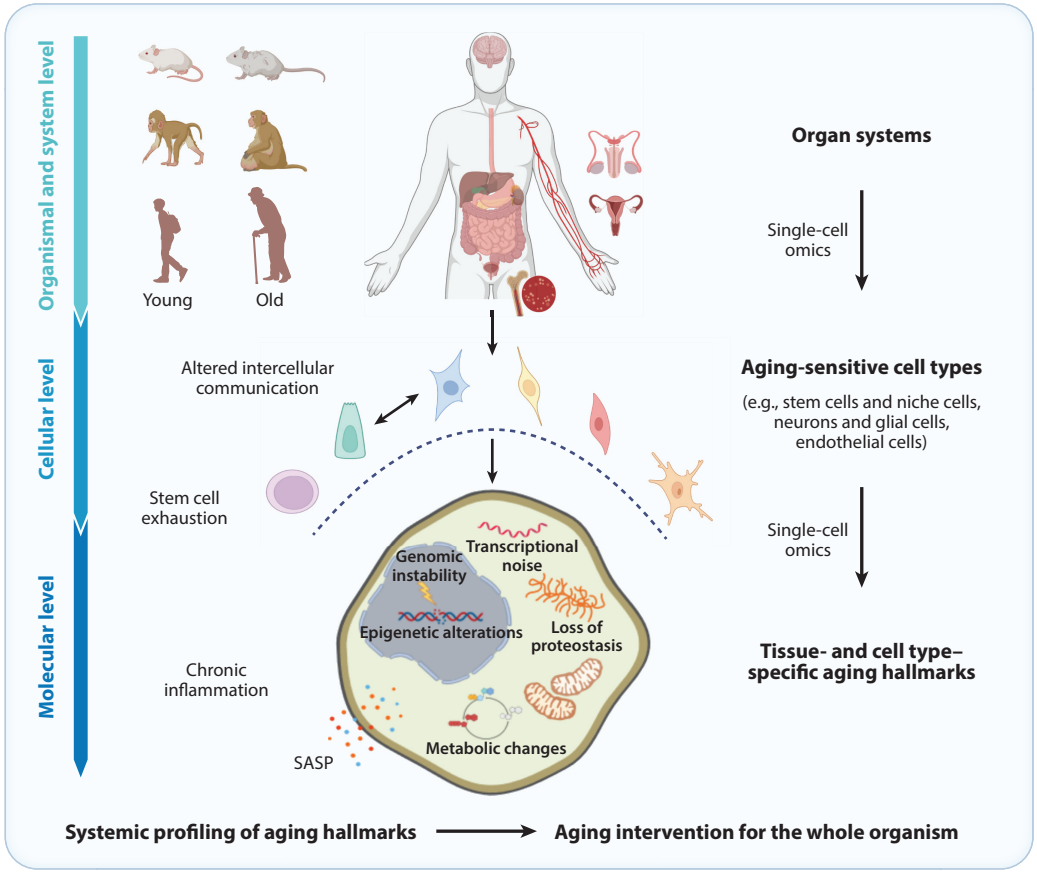

Figure 2

The applications of single-cell omics in aging studies. Single-cell omics in different tissues and organs facilitates the identification of aging-sensitive cell types, along with cell type–specific alterations, all of which constitute aging hallmarks. Abbreviation: SASP, senescence-associated secretory phenotype. Figure adapted from images created with BioRender.com.

4. DATA RESOURCES AND BIG DATA ANALYSIS

4.1. Aging-Related Databases

Cell atlases, spanning large numbers of single-cell omics datasets, help us understand how different sets of genes, encoded by the same genome of the same organism, orchestrate distinct gene programs in different cell types across tissues with distinct functions. With the development of high-throughput sequencing technology and the exponential growth of available datasets, cell atlases pertaining to aging have also emerged and expanded. However, such aging-related atlases are often scattered in large databases that focus on collecting single-cell omics data, such as the Human Cell Atlas (146), Single Cell Portal (**https://singlecell.broadinstitute.org/single_cell**), scMethBank (147), Gene Expression Nebulas (148), PanglaoDB (149), and scRNASeqDB (150). On the other hand, other databases record aging-specific gene information, such as the Human Aging Genomic Resources (containing GenAge, AnAge, GenDR, LongevityMap, and DrugAge databases) (151), AgeFactDB (152), the Digital Ageing Atlas (153), and AGEMAP (154). Most of these are knowledgebases, which compile aging phenotypes, longevity records, aging-/longevity-related genes, and factors with lifespan-extending effects, often without association with single-cell datasets. Thus, the establishment and development of more dedicated and comprehensive aging-related single-cell databases are an urgent need for advancing aging studies.

In recent years, several aging-related databases or projects have embarked in this direction, lowering the barriers to making use of single-cell datasets and thereby accelerating aging research. The Tabula Muris Senis project is a comprehensive analysis of single-cell and bulk RNA-seq data in diverse organs across the mouse lifespan. This transcriptomic atlas provides molecular information about how the most important hallmarks of aging are reflected in a broad range of cell types across various organs in mice (12). As for cross-species databases, the Aging Atlas is the first dedicated and comprehensive database encompassing cross-omics research on aging. It includes five modules: transcriptomics, epigenomics, single-cell transcriptomics, proteomics, and pharmacogenomics, among which the single-cell transcriptomics module systematically reflects tissue- and cell type–specific changes in gene expression with age in multiple species (155). The Cell Landscape is another cross-species cell profiling analysis including multiple life stages for mice, zebrafish, and *Drosophila*, providing a valuable resource for studying lineage development, maturation, and aging (156). Altogether, aging-related databases that house single-cell multimodal datasets along with knowledgebases will serve as a valuable resource for aging research, but they also call for tools for data mining and data management that meet uniform standardized rules.

4.2. Applications of Big Data Analysis in Aging Studies

With the growing body of aging-related datasets on hand, researchers are increasingly aware that aging is a systemic change involving different kinds of hallmarks in multiple tissues throughout the body; hence, systems biology and multimodal integration methods are needed to establish regulatory networks and identify key factors. Multimodal data integration methods, such as Harmony (157), Seurat v4 (62), MEFISTO (158), GLUE (graph-linked unified embedding) (159), and scJoint (160), will bring atlas-scale aging studies closer to a comprehensive characterization of the biology of aging. However, due to the high heterogeneity, there is still a need to improve the overfitting that may exist in the integration of single-cell sequencing datasets. Deep learning–based integration tools, such as best-fitting deep learning models driven by specific datasets, are being developed to solve this problem. As a consequence, big data analyses are usually facilitated by machine learning methods, such as large datasets exemplified by single-cell omics data or cohort studies. Indeed, for single-cell studies, artificial intelligence (AI) has already been applied. For example, GERAS, a neural network model with two hidden layers and rectified linear unit activation

(161), was trained on zebrafish and human pancreatic scRNA-seq data to identify age-predictive genes. Another fully connected deep learning neural network with a similar architecture has been applied in the classification of hematopoietic stem and progenitor cells of different ages and has provided explanations for the decline in the self-renewal ability of stem cells during aging (162). A recent study fitted eight machine learning models for the prediction of neuronal cell ages based on single-cell expression data plus categorical features such as neuronal cell subtypes (163). The high accuracy of these models suggested the potential for identifying age-predictive genes from single-cell transcriptomes, which may also serve as aging hallmarks and intervention targets. However, the numbers of individuals in these single-cell sequencing experiments were relatively low, raising concerns that the trained machine learning models may only suit a specific genetic background. Since the aging process is a highly heterogeneous process for different individuals, cohort studies are therefore more powerful for identifying common or specific aging hallmarks and aging-delaying targets. Indeed, although still at the bulk level, AI has been widely applied in aging cohort studies, for example, biological age prediction, biomarker discovery, and target identification (164–167).

Although scaling of single-cell analyses will no doubt provide more accurate and meaningful results, cost and data analysis remain prohibitive. Single-cell cohort studies are costly since they usually involve the sequencing of hundreds of thousands (or millions) of cells, yet several such studies have been published (168–170). For example, Zheng et al. (168) combined scRNA-seq, mass cytometry, and scATAC-seq to analyze the immune cells in peripheral blood collected from young and old subjects and patients with COVID-19. They found that the landscape of immune cells was reprogrammed with age, and that the expression of COVID-19-susceptibility-related genes was upregulated with age (168). Another study that used scRNA-seq in 20 individuals to explore sex differences in the immune system upon aging found elevated BAFF/APRIL activity in aged females and higher expression levels of inflammatory genes in aged males (169). A third report included a total of 37 women to investigate the transcriptomic changes in oocytes during aging. The research group found increased levels of genes that are associated with chromosome segregation and RNA splicing with age, while those with the mitochondrial activity were dampened in expression (170). However, these cohort- and atlas-scale studies presented additional challenges for data analysis, for which applications of AI techniques were pivotal for the identification of aging biomarkers or targets for intervention, as well as for predicting chronological and epigenetic ages (164, 166, 167).

For a reliable analysis of aging cohort studies, the heterogeneity of the genetic backgrounds and enhanced variations induced by environmental effects on different individuals need to be addressed. Indeed, Lu et al. (171) developed an analysis framework to quantify cell-to-cell expression variations (CEVs) in human immune cell populations, by which they identified the correlations between CEVs and aging. The pivotal impacts of genetic background on gene expression have recently been demonstrated by a cross-species analysis of single-cell data using deep learning models (172). It appears to be necessary for researchers to carefully interrogate the sources and effects of each individual's CEVs in cohort studies to demarcate the scope of the explanatory ability of the identified aging biomarkers. Yet another caveat of current machine learning models is the lack of interpretability, which limits their ability to explain the sources of variations and directly interpret the underlying mechanisms that apply in aging research. Accordingly, more advanced approaches to interpret and extract the biological meaning of the trained models and to explain sources for variations will help understand and expedite the applications of AI models in aforementioned aging-related aspects (**Figure 3**).

In summary, decoding the aging process with unbiased and comprehensive profiling techniques at single-cell resolution has deepened our understanding of the aging organism. Thus,

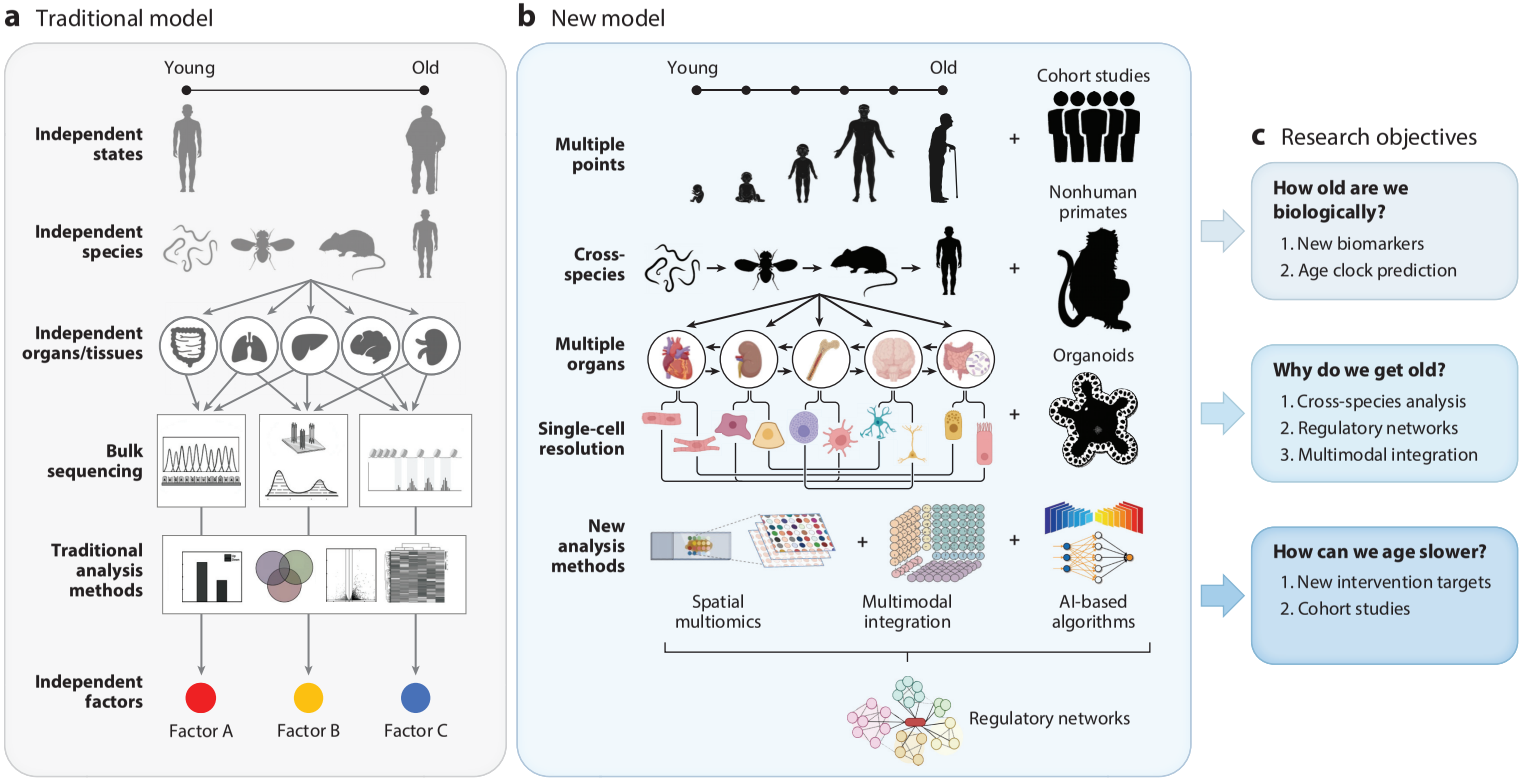

Figure 3

The development and future directions of big data analysis for aging studies. (*a*) A traditional model of aging research, for which usually only two time points (i.e., young and old ages) for a single tissue/organ of a single species are used. Data analysis on the traditional model relies on differential expression analysis, pathway enrichment analysis, etc., at the bulk level, concluding with independent factors for a specific aging process. However, with the development of single-cell technology, a new model for aging studies has emerged (*b*). The new paradigm favors continuous sampling at multiple time points on a cohort of individuals, or systematic comparisons across species and tissues/organs at single-cell resolution or in combination with nonhuman primate and organoid models. For analysis, spatial transcriptomics, multimodal integration, and artificial intelligence (AI)-based algorithms are combined to obtain system-level regulatory networks from these large datasets, rather than just independent factors. (*c*) The change in research models aids in answering the three main scientific questions of aging research: How old are we biologically? Why do we get old? And how can we age slower? Figure adapted from images created with BioRender.com.

existing knowledge gained from bulk-level targeted studies has largely been corroborated, but it has also leapfrogged beyond the boundaries of specific tissues and organs to reveal the heterogeneity of aging and its systemic paradigms. Challenges still exist at the forefront of this fast-growing area. At the same time, sequencing technology for single-cell data acquisition continues to evolve in many directions, including single-cell sequencing by third-generation sequencing (52, 173), techniques that preserve temporal and spatial contexts, and analyses using machine learning and integrating multimodal data on atlas-scale datasets. Despite the inherent difficulties in combining these cutting-edge technologies, single-cell studies in aging will eventually offer unprecedented accuracy in the identification of aging hallmarks, some of which, including the senescent cells themselves (174), can also serve as targets to facilitate the development of novel intervention strategies. As such, these efforts hold promise for answering the three most critical questions in aging research: How old are we biologically? Why do we get old? And how can we age slower?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

AUTHOR CONTRIBUTIONS

S.M., X.C., Y.C., Z.J., S.W., J.R., and G.-H.L. wrote the manuscript.

ACKNOWLEDGMENTS

The authors apologize for not being able to cite all single-cell studies and related methods in the aging field due to space limitations. This work was supported by the National Key Research and Development Program of China (grants 2020YFA0804000, 2020YFA0803401), the Strategic Priority Research Program of the Chinese Academy of Sciences (CAS) (XDA16000000), the National Key Research and Development Program of China (2020YFA0112200, 2022YFA1103700, 2018YFC2000100, 2019YFA0802202), the STI2030-Major Projects (2021ZD0202400), the National Natural Science Foundation of China (81921006, 92149301, 92168201, 82122024, 82071588, 31970597, 82201727, 82271600, 32000500, 32200610, 32121001, 81861168034), the CAS Project for Young Scientists in Basic Research (YSBR-076), the Program of the Beijing Natural Science Foundation (Z190019), the K.C. Wong Education Foundation (GJTD-2019-06, GJTD-2019-08), the Pilot Project for Public Welfare Development and Reform of Beijing-Affiliated Medical Research Institutes (11000022T000000461062), the Youth Innovation Promotion Association of the CAS (2022083), the Young Elite Scientists Sponsorship Program by CAST (YESS20200012, YESS20210002), the Informatization Plan of the CAS (CAS-WX2021SF-0301, CAS-WX2022SDC-XK14), and the Tencent Foundation (2021-1045).

LITERATURE CITED

1. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. 2013. The hallmarks of aging. *Cell* 153:1194–217
2. Cai Y, Song W, Li J, Jing Y, Liang C, et al. 2022. The landscape of aging. *Sci. China Life Sci.* 65(12):2354–454
3. He X, Memczak S, Qu J, Belmonte JCI, Liu GH. 2020. Single-cell omics in ageing: a young and growing field. *Nat. Metab.* 2:293–302
4. Zhang W, Qu J, Liu GH, Belmonte JCI. 2020. The ageing epigenome and its rejuvenation. *Nat. Rev. Mol. Cell Biol.* 21:137–50

5. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. 2023. Hallmarks of aging: an expanding universe. *Cell* 186:243–78
6. Liu X, Liu Z, Wu Z, Ren J, Fan Y, et al. 2023. Resurrection of endogenous retroviruses during aging reinforces senescence. *Cell* 186(2):287–304.e26
7. Zhou T, Kiran M, Lui KO, Ding Q. 2022. Decoding liver fibrogenesis with single-cell technologies. *Life Med*. 1:333–44
8. Fang X, Jiang M, Zhou M, Shao J, Fang X, et al. 2022. Elucidating the developmental dynamics of mouse stromal cells at single-cell level. *Life Med*. 1:45–48
9. Zou X, Dai X, Mentis A-FA, Esteban MA, Liu L, Han L. 2022. From monkey single-cell atlases into a broader biomedical perspective. *Life Med*. 1:254–57
10. Ma S, Wang S, Ye Y, Ren J, Chen R, et al. 2022. Heterochronic parabiosis induces stem cell revitalization and systemic rejuvenation across aged tissues. *Cell Stem Cell* 29:990–1005.e10
11. Ma S, Sun S, Geng L, Song M, Wang W, et al. 2020. Caloric restriction reprograms the single-cell transcriptional landscape of *Rattus norvegicus* aging. *Cell* 180:984–1001.e22
12. Tabula Muris Consort. 2020. A single-cell transcriptomic atlas characterizes ageing tissues in the mouse. *Nature* 583:590–95
13. Sun S, Ma S, Cai Y, Wang S, Ren J, et al. 2023. A single-cell transcriptomic atlas of exercise-induced anti-inflammatory and geroprotective effects across the body. *Innovation* 4:100380
14. Tang F, Barbacioru C, Wang Y, Nordman E, Lee C, et al. 2009. mRNA-Seq whole-transcriptome analysis of a single cell. *Nat. Methods* 6:377–82
15. Chappell L, Russell AJC, Voet T. 2018. Single-cell (multi)omics technologies. *Annu. Rev. Genom. Hum. Genet.* 19:15–41
16. Stuart T, Satija R. 2019. Integrative single-cell analysis. *Nat. Rev. Genet.* 20:257–72
17. Strzelecka PM, Damm F. 2021. Haematopoietic ageing through the lens of single-cell technologies. *Dis. Models Mech.* 14:dmm047340
18. Grover A, Sanjuan-Pla A, Thongjuea S, Carrelha J, Giustacchini A, et al. 2016. Single-cell RNA sequencing reveals molecular and functional platelet bias of aged haematopoietic stem cells. *Nat. Commun.* 7:11075
19. Angelidis I, Simon LM, Fernandez IE, Strunz M, Mayr CH, et al. 2019. An atlas of the aging lung mapped by single cell transcriptomics and deep tissue proteomics. *Nat. Commun.* 10:963
20. Zhang H, Li J, Ren J, Sun S, Ma S, et al. 2021. Single-nucleus transcriptomic landscape of primate hippocampal aging. *Protein Cell* 12:695–716
21. Wang S, Zheng Y, Li Q, He X, Ren R, et al. 2021. Deciphering primate retinal aging at single-cell resolution. *Protein Cell* 12:889–98
22. Zhang Y, Zheng Y, Wang S, Fan Y, Ye Y, et al. 2023. Single-nucleus transcriptomics reveals a gatekeeper role for FOXP1 in primate cardiac aging. *Protein Cell* 14:279–93
23. Jing Y, Zuo Y, Yu Y, Sun L, Yu Z, et al. 2022. Single-nucleus profiling unveils a geroprotective role of the FOXO3 in primate skeletal muscle aging. *Protein Cell*. In press. **https://doi.org/10.1093/procel/pwac061**
24. Solé-Boldo L, Raddatz G, Schütz S, Mallm J-P, Rippe K, et al. 2020. Single-cell transcriptomes of the human skin reveal age-related loss of fibroblast priming. *Commun. Biol.* 3:188
25. Mogilenko DA, Shpynov O, Andhey PS, Arthur L, Swain A, et al. 2021. Comprehensive profiling of an aging immune system reveals clonal GZMK^{+} CD8^{+} T cells as conserved hallmark of inflammaging. *Immunity* 54:99–115
26. Wang S, Zheng Y, Li J, Yu Y, Zhang W, et al. 2020. Single-cell transcriptomic atlas of primate ovarian aging. *Cell* 180:585–600.e19
27. Dulken BW, Buckley MT, Navarro Negredo P, Saligrama N, Cayrol R, et al. 2019. Single-cell analysis reveals T cell infiltration in old neurogenic niches. *Nature* 571:205–210
28. Enge M, Arda HE, Mignardi M, Beausang J, Bottino R, et al. 2017. Single-cell analysis of human pancreas reveals transcriptional signatures of aging and somatic mutation patterns. *Cell* 171:321–30.e14
29. Pálovics R, Keller A, Schaum N, Tan W, Fehlmann T, et al. 2022. Molecular hallmarks of heterochronic parabiosis at single-cell resolution. *Nature* 603:309–14

30. Gawad C, Koh W, Quake SR. 2016. Single-cell genome sequencing: current state of the science. *Nat. Rev. Genet.* 17:175–88
31. Evrony GD, Hinch AG, Luo C. 2021. Applications of single-cell DNA sequencing. *Annu. Rev. Genom. Hum. Genet.* 22:171–97
32. Li R, Di L, Li J, Fan W, Liu Y, et al. 2021. A body map of somatic mutagenesis in morphologically normal human tissues. *Nature* 597:398–403
33. Blokzijl F, De Ligt J, Jager M, Sasselli V, Roerink S, et al. 2016. Tissue-specific mutation accumulation in human adult stem cells during life. *Nature* 538:260–64
34. Lodato MA, Rodin RE, Bohrson CL, Coulter ME, Barton AR, et al. 2018. Aging and neurodegeneration are associated with increased mutations in single human neurons. *Science* 359:555–59
35. Zhang L, Dong X, Lee M, Maslov AY, Wang T, Vijg J. 2019. Single-cell whole-genome sequencing reveals the functional landscape of somatic mutations in B lymphocytes across the human lifespan. *PNAS* 116:9014–19
36. Cagan A, Baez-Ortega A, Brzozowska N, Abascal F, Coorens TH, et al. 2022. Somatic mutation rates scale with lifespan across mammals. *Nature* 604:517–24
37. Wang K, Liu H, Hu Q, Wang L, Liu J, et al. 2022. Epigenetic regulation of aging: implications for interventions of aging and diseases. *Signal Transduct. Target. Ther.* 7:374
38. Liu Z, Ji Q, Ren J, Yan P, Wu Z, et al. 2022. Large-scale chromatin reorganization reactivates placenta-specific genes that drive cellular aging. *Dev. Cell* 57(11):1347–68
39. Zhao D, Chen S. 2022. Failures at every level: breakdown of the epigenetic machinery of aging. *Life Med.* 1:81–83
40. Horvath S. 2013. DNA methylation age of human tissues and cell types. *Genome Biol.* 14:3156
41. Trapp A, Kerepesi C, Gladyshev VN. 2021. Profiling epigenetic age in single cells. *Nat. Aging* 1:1189–201
42. Cheung P, Vallania F, Warsinske HC, Donato M, Schaffert S, et al. 2018. Single-cell chromatin modification profiling reveals increased epigenetic variations with aging. *Cell* 173:1385–97.e14
43. Zhang Y, Amaral ML, Zhu C, Grieco SF, Hou X, et al. 2022. Single-cell epigenome analysis reveals age-associated decay of heterochromatin domains in excitatory neurons in the mouse brain. *Cell Res.* 32:1008–21
44. Longo SK, Guo MG, Ji AL, Khavari PA. 2021. Integrating single-cell and spatial transcriptomics to elucidate intercellular tissue dynamics. *Nat. Rev. Genet.* 22:627–44
45. Roy S, Kornberg TB. 2015. Paracrine signaling mediated at cell–cell contacts. *Bioessays* 37:25–33
46. Kiss T, Nyúl-Tóth Á, DelFavero J, Balasubramanian P, Tarantini S, et al. 2022. Spatial transcriptomic analysis reveals inflammatory foci defined by senescent cells in the white matter, hippocampi and cortical grey matter in the aged mouse brain. *GeroScience* 44:661–81
47. Allen WE, Blosser TR, Sullivan ZA, Dulac C, Zhuang XW. 2023. Molecular and spatial signatures of mouse brain aging at single-cell resolution. *Cell* 186:194–208.e18
48. Wang S, Wu M, Tang W, Chen Y, Wu C, et al. 2022. Spatiotemporal analysis of human ovarian aging at single-cell resolution. Res. Sq. rs.3.rs-1624864/v1. **https://doi.org/10.21203/rs.3.rs-1624864/v1**
49. Specht H, Emmott E, Petelski AA, Huffman RG, Perlman DH, et al. 2021. Single-cell proteomic and transcriptomic analysis of macrophage heterogeneity using ScoPE2. *Genome Biol.* 22:50
50. Wang Z, Sun F, Xiong W. 2022. Fat shapes fate: unlock the destiny of a cell with single-cell metabolomics. *Life Med.* 1:261–63
51. Wimmers F, Donato M, Kuo A, Ashuach T, Gupta S, et al. 2021. The single-cell epigenomic and transcriptional landscape of immunity to influenza vaccination. *Cell* 184:3915–35.e21
52. Hu Y, Jiang Z, Chen K, Zhou Z, Zhou X, et al. 2022. scNanoATAC-seq: a long-read single-cell ATAC sequencing method to detect chromatin accessibility and genetic variants simultaneously within an individual cell. *Cell Res.* 33:83–86
53. Patel AP, Tirosh I, Trombetta JJ, Shalek AK, Gillespie SM, et al. 2014. Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. *Science* 344:1396–401
54. Garvin T, Aboukhalil R, Kendall J, Baslan T, Atwal GS, et al. 2015. Interactive analysis and assessment of single-cell copy-number variations. *Nat. Methods* 12:1058–60
55. Gao R, Bai S, Henderson YC, Lin Y, Schalck A, et al. 2021. Delineating copy number and clonal substructure in human tumors from single-cell transcriptomes. *Nat. Biotechnol.* 39:599–608

56. Wang H, Zhang K, Liu Y, Fu Y, Gao S, et al. 2017. Telomere heterogeneity linked to metabolism and pluripotency state revealed by simultaneous analysis of telomere length and RNA-seq in the same human embryonic stem cell. *BMC Biol.* 15:114
57. Kong M, Guo L, Xu W, He C, Jia X, et al. 2022. Aging-associated accumulation of mitochondrial DNA mutations in tumor origin. *Life Med.* 1:149–67
58. Lareau CA, Ludwig LS, Muus C, Gohil SH, Zhao T, et al. 2021. Massively parallel single-cell mitochondrial DNA genotyping and chromatin profiling. *Nat. Biotechnol.* 39:451–61
59. Granja JM, Corces MR, Pierce SE, Bagdatli ST, Choudhry H, et al. 2021. ArchR is a scalable software package for integrative single-cell chromatin accessibility analysis. *Nat. Genet.* 53:403–11
60. Angermueller C, Lee HJ, Reik W, Stegle O. 2017. DeepCpG: accurate prediction of single-cell DNA methylation states using deep learning. *Genome Biol.* 18:67
61. Stuart T, Srivastava A, Madad S, Lareau CA, Satija R. 2021. Single-cell chromatin state analysis with Signac. *Nat. Methods* 18:1333–41
62. Hao Y, Hao S, Andersen-Nissen E, Mauck WM 3rd, Zheng S, et al. 2021. Integrated analysis of multimodal single-cell data. *Cell* 184:3573–87.e29
63. Zou Z, Long X, Zhao Q, Zheng Y, Song M, et al. 2021. A single-cell transcriptomic atlas of human skin aging. *Dev. Cell* 56(3):383–97
64. Aibar S, Gonzalez-Blas CB, Moerman T, Huynh-Thu VA, Imrichova H, et al. 2017. SCENIC: single-cell regulatory network inference and clustering. *Nat. Methods* 14:1083–86
65. Wagner A, Wang C, Fessler J, DeTomaso D, Avila-Pacheco J, et al. 2021. Metabolic modeling of single Th17 cells reveals regulators of autoimmunity. *Cell* 184:4168–85.e21
66. Damiani C, Maspero D, Di Filippo M, Colombo R, Pescini D, et al. 2019. Integration of single-cell RNA-seq data into population models to characterize cancer metabolism. *PLOS Comput. Biol.* 15:e1006733
67. Wu Y, Yang S, Ma J, Chen Z, Song G, et al. 2022. Spatiotemporal immune landscape of colorectal cancer liver metastasis at single-cell level. *Cancer Discov.* 12:134–53
68. Alghamdi N, Chang W, Dang P, Lu X, Wan C, et al. 2021. A graph neural network model to estimate cell-wise metabolic flux using single-cell RNA-seq data. *Genome Res.* 31:1867–84
69. Hsiao CJ, Tung P, Blischak JD, Burnett JE, Barr KA, et al. 2020. Characterizing and inferring quantitative cell cycle phase in single-cell RNA-seq data analysis. *Genome Res.* 30:611–21
70. Qiu X, Mao Q, Tang Y, Wang L, Chawla R, et al. 2017. Reversed graph embedding resolves complex single-cell trajectories. *Nat. Methods* 14:979–82
71. Cao J, Spielmann M, Qiu X, Huang X, Ibrahim DM, et al. 2019. The single-cell transcriptional landscape of mammalian organogenesis. *Nature* 566:496–502
72. La Manno G, Soldatov R, Zeisel A, Braun E, Hochgerner H, et al. 2018. RNA velocity of single cells. *Nature* 560:494–98
73. Andreatta M, Carmona SJ. 2021. UCell: robust and scalable single-cell gene signature scoring. *Comput. Struct. Biotechnol. J.* 19:3796–98
74. Foroutan M, Bhuva DD, Lyu R, Horan K, Cursons J, Davis MJ. 2018. Single sample scoring of molecular phenotypes. *BMC Bioinform.* 19:404
75. Efremova M, Vento-Tormo M, Teichmann SA, Vento-Tormo R. 2020. CellPhoneDB: inferring cell-cell communication from combined expression of multi-subunit ligand-receptor complexes. *Nat Protoc.* 15(4):1484–506
76. Cabello-Aguilar S, Alame M, Kon-Sun-Tack F, Fau C, Lacroix M, Colinge J. 2020. SingleCellSignalR: inference of intercellular networks from single-cell transcriptomics. *Nucleic Acids Res.* 48:e55
77. Jin S, Guerrero-Juarez CF, Zhang L, Chang I, Ramos R, et al. 2021. Inference and analysis of cell-cell communication using CellChat. *Nat. Commun.* 12:1088
78. Browaeys R, Saelens W, Saeys Y. 2020. NicheNet: modeling intercellular communication by linking ligands to target genes. *Nat. Methods* 17:159–62
79. Stubbington MJT, Lonnberg T, Proserpio V, Clare S, Speak AO, et al. 2016. T cell fate and clonality inference from single-cell transcriptomes. *Nat. Methods* 13:329–32
80. Redmond D, Poran A, Elemento O. 2016. Single-cell TCRseq: paired recovery of entire T-cell alpha and beta chain transcripts in T-cell receptors from single-cell RNAseq. *Genome Med.* 8:80

81. Canzar S, Neu KE, Tang Q, Wilson PC, Khan AA. 2017. BASIC: BCR assembly from single cells. *Bioinformatics* 33:425–27
82. Rizzetto S, Koppstein DNP, Samir J, Singh M, Reed JH, et al. 2018. B-cell receptor reconstruction from single-cell RNA-seq with VDJPuzzle. *Bioinformatics* 34:2846–47
83. Kowalczyk MS, Tirosh I, Heckl D, Rao TN, Dixit A, et al. 2015. Single-cell RNA-seq reveals changes in cell cycle and differentiation programs upon aging of hematopoietic stem cells. *Genome Res.* 25:1860–72
84. Kirschner K, Chandra T, Kiselev V, Flores-Santa Cruz D, Macaulay IC, et al. 2017. Proliferation drives aging-related functional decline in a subpopulation of the hematopoietic stem cell compartment. *Cell Rep*. 19:1503–11
85. Mann M, Mehta A, de Boer CG, Kowalczyk MS, Lee K, et al. 2018. Heterogeneous responses of hematopoietic stem cells to inflammatory stimuli are altered with age. *Cell Rep*. 25:2992–3005.e5
86. Florian MC, Klose M, Sacma M, Jablanovic J, Knudson L, et al. 2018. Aging alters the epigenetic asymmetry of HSC division. *PLOS Biol*. 16:e2003389
87. Li H, Zhu L, Wang R, Xie L, Ren J, et al. 2022. Aging weakens Th17 cell pathogenicity and ameliorates experimental autoimmune uveitis in mice. *Protein Cell* 13:422–45
88. Mogilenko DA, Shchukina I, Artyomov MN. 2022. Immune ageing at single-cell resolution. *Nat. Rev. Immunol.* 22:484–98
89. Leng SX, Pawelec G. 2022. Single-cell immune atlas for human aging and frailty. *Life Med*. 1:67–70
90. Verovskaya EV, Dellorusso PV, Passegué E. 2019. Losing sense of self and surroundings: hematopoietic stem cell aging and leukemic transformation. *Trends Mol. Med.* 25:494–515
91. Flach J, Bakker ST, Mohrin M, Conroy PC, Pietras EM, et al. 2014. Replication stress is a potent driver of functional decline in ageing haematopoietic stem cells. *Nature* 512:198–202
92. Adelman ER, Huang H-T, Roisman A, Olsson A, Colaprico A, et al. 2019. Aging human hematopoietic stem cells manifest profound epigenetic reprogramming of enhancers that may predispose to leukemia. *Cancer Discov*. 9:1080–101
93. Jaiswal S, Ebert BL. 2019. Clonal hematopoiesis in human aging and disease. *Science* 366:eaan4673
94. Nam AS, Dusaj N, Izzo F, Murali R, Myers RM, et al. 2022. Single-cell multi-omics of human clonal hematopoiesis reveals that *DNMT3A* R882 mutations perturb early progenitor states through selective hypomethylation. *Nat. Genet.* 54:1514–26
95. Fuster JJ, Zuriaga MA, Zorita V, MacLauchlan S, Polackal MN, et al. 2020. TET2-loss-of-function-driven clonal hematopoiesis exacerbates experimental insulin resistance in aging and obesity. *Cell Rep*. 33(4):108326
96. Fujino T, Goyama S, Sugiura Y, Inoue D, Asada S, et al. 2021. Mutant ASXL1 induces age-related expansion of phenotypic hematopoietic stem cells through activation of Akt/mTOR pathway. *Nat. Commun.* 12:1826
97. Chen S, Wang Q, Yu H, Capitano ML, Vemula S, et al. 2019. Mutant p53 drives clonal hematopoiesis through modulating epigenetic pathway. *Nat. Commun.* 10:5649
98. Hsu JI, Dayaram T, Tovy A, De Braekeleer E, Jeong M, et al. 2018. *PPM1D* mutations drive clonal hematopoiesis in response to cytotoxic chemotherapy. *Cell Stem Cell* 23:700–13.e6
99. Frisch BJ, Hoffman CM, Latchney SE, LaMere MW, Myers J, et al. 2019. Aged marrow macrophages expand platelet-biased hematopoietic stem cells via interleukin-1B. *JCI Insight* 4(10):e124213
100. Oetjen KA, Lindblad KE, Goswami M, Gui G, Dagur PK, et al. 2018. Human bone marrow assessment by single-cell RNA sequencing, mass cytometry, and flow cytometry. *JCI Insight* 3(23):e124928
101. Hennrich ML, Romanov N, Horn P, Jaeger S, Eckstein V, et al. 2018. Cell-specific proteome analyses of human bone marrow reveal molecular features of age-dependent functional decline. *Nat. Commun.* 9:4004
102. Cabezas-Wallscheid N, Buettner F, Sommerkamp P, Klimmeck D, Ladel L, et al. 2017. Vitamin A-retinoic acid signaling regulates hematopoietic stem cell dormancy. *Cell* 169:807–23.e19
103. Saçma M, Pospiech J, Bogeska R, de Back W, Mallm J-P, et al. 2019. Haematopoietic stem cells in perisinusoidal niches are protected from ageing. *Nat. Cell Biol.* 21:1309–20
104. Tabula Sapiens Consort., Jones RC, Karkanias J, Krasnow MA, Pisco AO, et al. 2022. The Tabula Sapiens: a multiple-organ, single-cell transcriptomic atlas of humans. *Science* 376:eabl4896

105. Liu Z, Zhang Z. 2022. Mapping cell types across human tissues. *Science* 376:695–96
106. Eraslan G, Drokhlyansky E, Anand S, Fiskin E, Subramanian A, et al. 2022. Single-nucleus cross-tissue molecular reference maps toward understanding disease gene function. *Science* 376:eabl4290
107. Domínguez Conde C, Xu C, Jarvis LB, Rainbow DB, Wells SB, et al. 2022. Cross-tissue immune cell analysis reveals tissue-specific features in humans. *Science* 376:eabl5197
108. Suo C, Dann E, Goh I, Jardine L, Kleshchevnikov V, et al. 2022. Mapping the developing human immune system across organs. *Science* 376:eabo0510
109. Wang W, Wang M, Yang M, Zeng B, Qiu W, et al. 2022. Transcriptome dynamics of hippocampal neurogenesis in macaques across the lifespan and aged humans. *Cell Res.* 32:729–43
110. Franjic D, Skarica M, Ma S, Arellano JI, Tebbenkamp ATN, et al. 2022. Transcriptomic taxonomy and neurogenic trajectories of adult human, macaque, and pig hippocampal and entorhinal cells. *Neuron* 110:452–69.e14
111. Ayhan F, Kulkarni A, Berto S, Sivaprakasam K, Douglas C, et al. 2021. Resolving cellular and molecular diversity along the hippocampal anterior-to-posterior axis in humans. *Neuron* 109:2091–105.e6
112. Shi Z, Geng Y, Liu J, Zhang H, Zhou L, et al. 2018. Single-cell transcriptomics reveals gene signatures and alterations associated with aging in distinct neural stem/progenitor cell subpopulations. *Protein Cell* 9:351–64
113. Kalamakis G, Brune D, Ravichandran S, Bolz J, Fan W, et al. 2019. Quiescence modulates stem cell maintenance and regenerative capacity in the aging brain. *Cell* 176:1407–19.e14
114. Barker SJ, Raju RM, Milman NEP, Wang J, Davila-Velderrain J, et al. 2021. MEF2 is a key regulator of cognitive potential and confers resilience to neurodegeneration. *Sci. Transl. Med.* 13:eabd7695
115. Li SB, Damonte VM, Chen C, Wang GX, Kebschull JM, et al. 2022. Hyperexcitable arousal circuits drive sleep instability during aging. *Science* 375:eabh3021
116. Schlachetzki JCM, Toda T, Mertens J. 2020. When function follows form: nuclear compartment structure and the epigenetic landscape of the aging neuron. *Exp. Gerontol.* 133:110876
117. Hammond TR, Dufort C, Dissing-Olesen L, Giera S, Young A, et al. 2019. Single-cell RNA sequencing of microglia throughout the mouse lifespan and in the injured brain reveals complex cell-state changes. *Immunity* 50:253–71.e6
118. Talma N, Gerrits E, Wang B, Eggen BJL, Demaria M. 2021. Identification of distinct and age-dependent $p16^{High}$ microglia subtypes. *Aging Cell* 20:e13450
119. Gerrits E, Brouwer N, Kooistra SM, Woodbury ME, Vermeiren Y, et al. 2021. Distinct amyloid-β and tau-associated microglia profiles in Alzheimer's disease. *Acta Neuropathol.* 141:681–96
120. Jin WN, Shi K, He W, Sun JH, Van Kaer L, et al. 2021. Neuroblast senescence in the aged brain augments natural killer cell cytotoxicity leading to impaired neurogenesis and cognition. *Nat. Neurosci.* 24:61–73
121. Kiss T, Nyul-Toth A, Balasubramanian P, Tarantini S, Ahire C, et al. 2020. Single-cell RNA sequencing identifies senescent cerebromicrovascular endothelial cells in the aged mouse brain. *Geroscience* 42:429–44
122. Chen MB, Yang AC, Yousef H, Lee D, Chen W, et al. 2020. Brain endothelial cells are exquisite sensors of age-related circulatory cues. *Cell Rep.* 30:4418–32.e4
123. Dani N, Herbst RH, McCabe C, Green GS, Kaiser K, et al. 2021. A cellular and spatial map of the choroid plexus across brain ventricles and ages. *Cell* 184:3056–74.e21
124. Yi W, Lu Y, Zhong S, Zhang M, Sun L, et al. 2021. A single-cell transcriptome atlas of the aging human and macaque retina. *Natl. Sci. Rev.* 8:nwaa179
125. Sun G, Zheng Y, Fu X, Zhang W, Ren J, et al. 2023. Single-cell transcriptomic atlas of mouse cochlear aging. *Protein Cell* 14:180–201
126. Wanjare M, Kusuma S, Gerecht S. 2013. Perivascular cells in blood vessel regeneration. *Biotechnol. J.* 8:434–47
127. Zhang W, Zhang S, Yan P, Ren J, Song M, et al. 2020. A single-cell transcriptomic landscape of primate arterial aging. *Nat. Commun.* 11:2202
128. Ge F, Pan Q, Qin Y, Jia M, Ruan C, et al. 2021. Single-cell analysis identify transcription factor BACH1 as a master regulator gene in vascular cells during aging. *Front. Cell Dev. Biol.* 9:786496

129. He D, Mao A, Zheng C-B, Kan H, Zhang K, et al. 2020. Aortic heterogeneity across segments and under high fat/salt/glucose conditions at the single-cell level. *Natl. Sci. Rev.* 7:881–96
130. Brazhnik K, Sun S, Alani O, Kinkhabwala M, Wolkoff AW, et al. 2020. Single-cell analysis reveals different age-related somatic mutation profiles between stem and differentiated cells in human liver. *Sci. Adv.* 6:eaax2659
131. Li J, Zheng Y, Yan P, Song M, Wang S, et al. 2021. A single-cell transcriptomic atlas of primate pancreatic islet aging. *Natl. Sci. Rev.* 8:nwaa127
132. Drozdowski L, Thomson AB. 2006. Aging and the intestine. *World J. Gastroenterol.* 12:7578–84
133. Jasper H. 2020. Intestinal stem cell aging: origins and interventions. *Annu. Rev. Physiol.* 82:203–26
134. Tauc HM, Rodriguez-Fernandez IA, Hackney JA, Pawlak M, Ronnen Oron T, et al. 2021. Age-related changes in polycomb gene regulation disrupt lineage fidelity in intestinal stem cells. *eLife* 10:e62250
135. Drokhlyansky E, Smillie CS, Van Wittenberghe N, Ericsson M, Griffin GK, et al. 2020. The human and mouse enteric nervous system at single-cell resolution. *Cell* 182:1606–22.e23
136. Zhang T, Xi Q, Wang D, Li J, Wang M, et al. 2019. Mitochondrial dysfunction and endoplasmic reticulum stress involved in oocyte aging: an analysis using single-cell RNA-sequencing of mouse oocytes. *J. Ovarian Res.* 12:53
137. Hou G, Sun QY. 2020. Maternal ageing causes changes in DNA methylation and gene expression profiles in mouse oocytes. *Zygote* 28(5):360–66
138. Zhang JJ, Liu X, Chen L, Zhang S, Zhang X, et al. 2020. Advanced maternal age alters expression of maternal effect genes that are essential for human oocyte quality. *Aging* 12:3950–61
139. Yuan L, Yin P, Yan H, Zhong X, Ren C, et al. 2021. Single-cell transcriptome analysis of human oocyte ageing. *J. Cell. Mol. Med.* 25(13):6289–303
140. Nie X, Munyoki SK, Sukhwani M, Schmid N, Missel A, et al. 2022. Single-cell analysis of human testis aging and correlation with elevated body mass index. *Dev. Cell* 57:1160–76.e5
141. Wang X, Cairns BR, Guo J. 2022. When spermatogenesis meets human aging and elevated body mass. *Life Med.* 1:267–69
142. Huang D, Zuo Y, Zhang C, Sun G, Jing Y, et al. 2022. A single-nucleus transcriptomic atlas of primate testicular aging reveals exhaustion of the spermatogonial stem cell reservoir and loss of Sertoli cell homeostasis. *Protein Cell*. In press. **https://doi.org/10.1093/procel/pwac057**
143. Makela JA, Koskenniemi JJ, Virtanen HE, Toppari J. 2019. Testis development. *Endocr. Rev.* 40:857–905
144. Crowell PD, Fox JJ, Hashimoto T, Diaz JA, Navarro HI, et al. 2019. Expansion of luminal progenitor cells in the aging mouse and human prostate. *Cell Rep.* 28:1499–510.e6
145. Wang L, Liu J, Liu H, Yazawa M, Zhu F. 2022. The secret of youth—how is systemic rejuvenation achieved at the single cell level? *Life Med.* 1:258–60
146. Regev A, Teichmann SA, Lander ES, Amit I, Benoist C, et al. 2017. The Human Cell Atlas. *eLife* 6:e27041
147. Zong W, Kang H, Xiong Z, Ma Y, Jin T, et al. 2022. scMethBank: a database for single-cell whole genome DNA methylation maps. *Nucleic Acids Res.* 50:D380–86
148. Zhang Y, Zou D, Zhu T, Xu T, Chen M, et al. 2022. Gene Expression Nebulas (GEN): a comprehensive data portal integrating transcriptomic profiles across multiple species at both bulk and single-cell levels. *Nucleic Acids Res.* 50:D1016–24
149. Franzen O, Gan LM, Bjorkegren JLM. 2019. PanglaoDB: a web server for exploration of mouse and human single-cell RNA sequencing data. *Database* 2019:baz046
150. Cao Y, Zhu J, Jia P, Zhao Z. 2017. scRNASeqDB: a database for RNA-seq based gene expression profiles in human single cells. *Genes* 8(12):368
151. Tacutu R, Thornton D, Johnson E, Budovsky A, Barardo D, et al. 2018. Human Ageing Genomic Resources: new and updated databases. *Nucleic Acids Res.* 46:D1083–90
152. Huhne R, Thalheim T, Suhnel J. 2014. AgeFactDB—the JenAge Ageing Factor Database—towards data integration in ageing research. *Nucleic Acids Res.* 42:D892–96
153. Craig T, Smelick C, Tacutu R, Wuttke D, Wood SH, et al. 2015. The Digital Ageing Atlas: integrating the diversity of age-related changes into a unified resource. *Nucleic Acids Res.* 43:D873–78
154. Zahn JM, Poosala S, Owen AB, Ingram DK, Lustig A, et al. 2007. AGEMAP: a gene expression database for aging in mice. *PLOS Genet.* 3:e201

155. Aging Atlas Consort. 2021. Aging Atlas: a multi-omics database for aging biology. *Nucleic Acids Res.* 49:D825–30
156. Wang R, Zhang P, Wang J, Ma L, E W, et al. 2022. Construction of a cross-species cell landscape at single-cell level. *Nucleic Acids Res.* 51(2):501–16
157. Korsunsky I, Millard N, Fan J, Slowikowski K, Zhang F, et al. 2019. Fast, sensitive and accurate integration of single-cell data with Harmony. *Nat. Methods* 16:1289–96
158. Velten B, Braunger JM, Argelaguet R, Arnol D, Wirbel J, et al. 2022. Identifying temporal and spatial patterns of variation from multimodal data using MEFISTO. *Nat. Methods* 19:179–86
159. Cao ZJ, Gao G. 2022. Multi-omics single-cell data integration and regulatory inference with graph-linked embedding. *Nat. Biotechnol.* 40:1458–66
160. Lin Y, Wu TY, Wan S, Yang JYH, Wong WH, Wang YXR. 2022. scJoint integrates atlas-scale single-cell RNA-seq and ATAC-seq data with transfer learning. *Nat. Biotechnol.* 40:703–10
161. Singh SP, Janjuha S, Chaudhuri S, Reinhardt S, Kränkel A, et al. 2018. Machine learning based classification of cells into chronological stages using single-cell transcriptomics. *Sci. Rep.* 8:17156
162. Arai F, Stumpf PS, Ikushima YM, Hosokawa K, Roch A, et al. 2020. Machine learning of hematopoietic stem cell divisions from paired daughter cell expression profiles reveals effects of aging on self-renewal. *Cell Syst.* 11:640–52.e5
163. Hajdarovic KH, Yu D, Hassell L-A, Evans SA, Packer S, et al. 2022. Single-cell analysis of the aging female mouse hypothalamus. *Nat. Aging* 2:662–78
164. Zhavoronkov A, Mamoshina P, Vanhaelen Q, Scheibye-Knudsen M, Moskalev A, Aliper A. 2019. Artificial intelligence for aging and longevity research: recent advances and perspectives. *Ageing Res. Rev.* 49:49–66
165. Ashiqur Rahman S, Giacobbi P, Pyles L, Mullett C, Doretto G, Adjeroh DA. 2021. Deep learning for biological age estimation. *Brief. Bioinform.* 22:1767–81
166. Rutledge J, Oh H, Wyss-Coray T. 2022. Measuring biological age using omics data. *Nat. Rev. Genet.* 23:715–27
167. Zhavoronkov A, Bischof E, Lee K-F. 2021. Artificial intelligence in longevity medicine. *Nat. Aging* 1:5–7
168. Zheng Y, Liu X, Le W, Xie L, Li H, et al. 2020. A human circulating immune cell landscape in aging and COVID-19. *Protein Cell* 11:740–70
169. Huang Z, Chen B, Liu X, Li H, Xie L, et al. 2021. Effects of sex and aging on the immune cell landscape as assessed by single-cell transcriptomic analysis. *PNAS* 118:e2023216118
170. Llonch S, Barragán M, Nieto P, Mallol A, Elosua-Bayes M, et al. 2021. Single human oocyte transcriptome analysis reveals distinct maturation stage-dependent pathways impacted by age. *Aging Cell* 20:e13360
171. Lu Y, Biancotto A, Cheung F, Remmers E, Shah N, et al. 2016. Systematic analysis of cell-to-cell expression variation of T lymphocytes in a human cohort identifies aging and genetic associations. *Immunity* 45:1162–75
172. Li J, Wang J, Zhang P, Wang R, Mei Y, et al. 2022. Deep learning of cross-species single-cell landscapes identifies conserved regulatory programs underlying cell types. *Nat. Genet.* 54:1711–20
173. Philpott M, Watson J, Thakurta A, Brown T, Oppermann U, Cribbs AP. 2021. Nanopore sequencing of single-cell transcriptomes with scCOLOR-seq. *Nat. Biotechnol.* 39:1517–20
174. Sun Y, Li Q, Kirkland JL. 2022. Targeting senescent cells for a healthier longevity: the roadmap for an era of global aging. *Life Med.* 1:103–19