MULTIOMICS

A multiomic atlas for the exploration of healthy aging in human monocytes

An extensive multiomic resource using human monocytes reveals large-scale remodeling of DNA methylation landscapes in healthy aging and accelerated or pathological aging contexts. This work provides an invaluable resource with important implications for the study of age-related changes in immune function.

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Older animals and humans show a decreased ability to mount appropriate vaccine responses to fight pathogens and to recover from infections[1]. Such progressive immune dysfunction is linked to the common molecular and physiological aberrations that come with old, dubbed 'hallmarks' or 'pillars' of aging[2,3] and is characterized by a decline in the effectiveness of the adaptive immune system and an abnormal increased baseline activation of the innate immune system. Consequently, a state of chronic sterile inflammation, referred to as 'inflamm-aging'[3], develops in older individuals, which may itself contribute to driving frailty and other age-related conditions. Although age-related inflammation has been proposed to stem from a number of sources (for example, senescent cell cytokine secretion, response to microbial leakage, and so on), the dysfunction of immune cells is believed to be central to inflamm-aging. A better understanding of the breakdown of molecular networks leading to such dysfunction could help design interventions to improve immune responses in older individuals.

In a new study, Shchukina and colleagues[4] leveraged the power of a parallel multiomics approach to compile an unprecedented atlas of immune aging in a cohort of healthy humans, encompassing both the characterization of a key circulating innate immune cell type (that is, monocytes) through epigenomics with DNA methylation and five histone modifications, transcriptomics and proteomics (Fig. 1), as well as a characterization of plasma, the standard milieu they are exposed to, at the metabolomic and proteomic levels. Monocytes are a key cell type in the vertebrate innate immune system derived from bone marrow progenitors, which are abundant in circulation and are thus very tractable for such large-scale studies. Classical monocytes comprise approximately 90% of circulating monocytes in humans

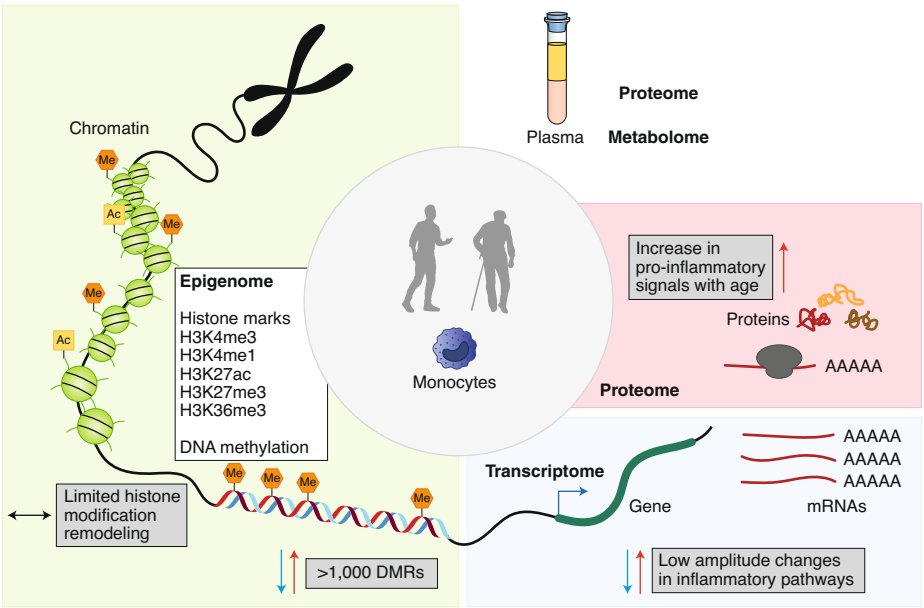

Fig. 1 | An atlas of immune aging in healthy men. Immune dysfunction is a common aberration observed in aged individuals. Shchukina and colleagues[4] leveraged a parallel multiomics approach, spanning epigenome, transcriptome and proteome analyses, to provide a comprehensive understanding of 'omic' differences between young (~27 years old) and older (~64 years old) individuals in monocytes, a key innate immune cell type, as well as in plasma, the milieu they are in constant contact with. Through enhanced reduced representation bisulfite sequencing-based DNA methylation profiling, >1,000 DMRs between young and older age groups were identified. Transcriptome and proteome analyses detected small but consistent trends for increased expression for molecules linked to pro-inflammatory signaling. Five histone modifications assessed, including H3K4me3, H3K4me1, H3K27ac, H3K27me3 and H3K36me3, did not undergo significant rearrangement with age.

and are known for their high phagocytic potential. Upon activation, monocytes can infiltrate tissues and differentiate into dendritic cells and macrophages, two key populations of antigen-presenting cells, which serve as important bridges between the innate and adaptive immune systems.

To eliminate potential confounding factors that may introduce age-irrelevant bias, the authors defined strict inclusion criteria to focus primarily on the impact of aging in the absence of pathology (that is, 'healthy' aging), only enrolling donors with a normal body mass index, non-smokers and without ongoing infections or cancer diagnoses, or any inflammatory conditions. Together, the study used samples from 20 healthy young (~27 years old) and 20 healthy older (~64 years old) males of white ethnicity.

Using a combination of RNA sequencing (RNA-seq) and proteomic profiling, the authors interrogated how gene and protein expression are impacted in monocytes from

their healthy aging cohort. The authors noted that transcriptional changes in aging monocytes were rather limited in amplitude. Based on high variability and comparison with a larger microarray cohort data, the authors conclude that the RNA-seq data may be partially underpowered to detect the rather small transcriptional changes undergone by monocytes. However, pathway enrichment analysis results were consistent with an age-related skew in a more pro-inflammatory state consistent with the notion of inflamm-aging, albeit of a smaller scale, likely due to the strict inclusion health inclusion criteria for this cohort. In contrast, the authors observed more robust, if still rare, changes in protein expression levels. Surprisingly, changes at the transcriptomic and proteomic levels were poorly correlated, first reinforcing that transcription and translation are not equivalent, and second suggesting that dysfunction in post-transcriptional regulation may be driving a number of aging phenotypes.

An important conserved characteristic of aging is the widespread alteration of epigenomic landscapes, which encompass DNA methylation and histone modifications[5]. DNA methylation predominantly occurs at cytosine residues that are part of CpG dinucleotides. Although its impact on gene expression can be context-dependent, DNA methylation is primarily thought to be an evolutionarily conserved form of transcription repression. Relevant to aging, accumulating studies have shown that DNA methylation is widely regulated with aging across species. Profiling of DNA methylation through arrays is the most widely used and affordable method to assess methylation states, especially in aging research, and was the key technology that led to the concept of aging 'epigenetic clocks'[6]. Array-based techniques can provide a somewhat biased view of the methylome, since they can only detect methylation levels in a set of limited and pre-defined regions of the genome. The authors opted to use enhanced reduced representation bisulfite sequencing-based DNA methylation profiling, which allows assessment of genome-wide DNA methylation states at an exquisite resolution. Through their analysis, authors identified >1,000 differentially methylated regions (DMRs) between the young and older age groups. Approximately half of the age-related DMRs were hypermethylated, and were enriched at promoter regions. In contrast, DMRs with age-related DNA hypomethylation occurred at more distal elements and were generally linked to the upregulation of associated genes. Interestingly, the authors observed greater individual-to-individual variability in methylome landscapes in the older age group, consistent with the notion of age-related epigenetic drift[7]. In contrast, few changes were detected in the histone modification landscapes in a reproducible manner across individuals, suggesting that histone modifications may change in a less stereotypical manner than DNA methylation profiles over the course of healthy aging in human monocytes. The differential impact of age on DNA methylation compared to post-translational histone modifications may be due to the more dynamic nature of histone modifications.

Finally, the authors investigated whether identified DMRs had clinical and physiological significance in individuals older than in their cohort, in pathological contexts with accelerated aging phenotypes and in relation to genetic variants of clinical significance. In HIV patients for instance, they found that the methylation status of age-related DMRs in leukocytes appeared 'older' than the chronological age of the donors, consistent with the notion that HIV accelerates aging of the immune system. Interestingly, hypomethylated DMRs were enriched to overlap significant variants within the human leukocyte antigen locus, which may have implications for age-related changes in antigen presentation potential in myeloid cells.

The most surprising conclusion from this study is that the extent and amplitude of age-related changes in primary classical monocytes at the transcriptomic, epigenomic and proteomic levels were much more limited than anticipated. This observation may result from the impact of several factors: (i) the strict inclusion criteria restricted the study to 'healthy' individuals only, who may exhibit fewer changes compared to individuals who experience 'normal' or 'average' aging and are thus more prone to inflammatory disease; (ii) monocytes themselves, being freshly generated throughout life, may accumulate fewer changes with aging compared to longer-lived cells; and (iii) cellular remodeling patterns are more variable among older individuals (that is, due to 'biological noise'), and thus identification of coordinated and reproducible changes in molecular networks may be difficult, if not impossible. Instead, an accumulative and combinatorial effect of stochastic changes in cellular landscapes may partially drive age-associated dysfunctions. Moreover, since the authors observed many more epigenetic changes than RNA or protein changes, it is possible that age-related molecular changes or phenotypic dysfunction may be exacerbated upon infectious challenges.

An important point that will deserve further investigation is whether similar observations would be made when including donors from both sexes, as well as encompassing additional ethnic groups. Accumulating evidence supports the notion that both the overall aging process[8] and human immune cell aging[9] are extremely sex dimorphic. Indeed, estrogen signaling is a known modulator of immune cell phenotypes[10], including monocytes, and estrogen deprivation in post-menopausal women may lead to larger changes in molecular and cellular phenotypes with aging than in their male counterparts. Additionally, individuals from different ethnic groups have been shown to present with varying mortality rates and susceptibility to age-associated chronic diseases. Concurrently, traditional epigenetic clock analyses on multiple tissues showed significant differences in the DNA methylation landscape of individuals from different ethnic backgrounds[11]. Thus, future studies should investigate the nature, extent and amplitude of age-related remodeling in cells from aging women and donors from non-white ethnic groups.

By providing a comprehensive epigenomic, transcriptomic and proteomic characterization of monocyte aging in a well-controlled and homogeneous human cohort, this study provides a comprehensive and valuable resource for research into immune aging. The discovery of specific DNA methylome signatures of healthy human aging in a purified cell type (monocytes) will also undoubtedly help shed light on the functional meaning of DNA methylation clocks. Thus, this resource will undoubtedly help generate new hypotheses and research directions to understand the underlying mechanisms driving age-related immune dysfunction. ❐

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Competing interests

The authors declare no competing interests.