Single cell analyses of aging, inflammation and senescence

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Summary

1. Single-cell RNA-seq in Aging Research

Cellular aging remains a poorly understood process, and the advent of single-cell RNA-seq (scRNA-seq) provides an exciting opportunity to study the aging phenotypes of cellular subpopulations, and explore how these may relate to aging-related decline and disease. To this end we conducted a review of the literature to identify aging studies using scRNA-seq and evaluate their impact and similarities.

2. Identification of Studies (see slide 3)

We identified 20 studies in total, including 2 multi-tissue studies in mice (Tabula Muris Senis and Calico), 14 single-tissue *in vivo*, and 3 *in vitro* studies. We evaluated each study by assessing which tissue(s) were studied, the number of animals and cells studies, whether cellular heterogeneity (thought to increase with age) was studied and how, and what platforms were used.

3. Aging Biomarkers in Single-cell RNAseq (see slide 4)

To further evaluate scRNA-seq as a technology to study aging, we performed some simple analyses comparing the fractions of cells within liver cell types (Slide4: Figure 1) and tissues (Slide 4: Figure 2) that expressed various marker genes of cellular senescence, an important aging process. We found that inflammation markers (e.g. IL1b) in particular were expressed at a higher ratio in aged cells, and that markers tended to be expressed at consistent ratios between studies. Further we found that senescence markers such as p16/CDKN2A are often highly expressed (“strong up”) in a few cells of specific cell-types, reflecting the frequently suspected role of senescent cells as a “minority of bad guys”.

Prominent scRNA-seq Studies in Aging Research

Multi-tissue Studies

Tabula Muris Senis (Almanzar et al., 2020)

Tissues:	bladder, bone marrow, brain (cerebellum, cortex, hippocampus and striatum), fat (brown, gonadal, mesenteric and subcutaneous), heart and aorta, kidney, large intestine, limb muscle and diaphragm, liver, lung, mammary gland, pancreas, skin, spleen, thymus, tongue and trachea
Animals/Cells:	~530 000 cells from 30 male & female C57BL/6 J N mice: 6 age groups: 1 mo, 3 mo, 18 mo, 21 mo, 24 mo, 30 mo
Heterogeneity:	Not investigated
Exp. setup:	10x Genomics, also FACS-based for the 3 mo & 24 mo age groups; Nova-seq 6000
Remarks:	The number of p16-expressing cells doubled in old mice, and in the 10x Genomics data, p16 expression doubled as well.

Calico Study (Kimmel et al., 2019)

Tissues:	kidney, lung, spleen
Animals/Cells:	~55 000 cells from 7 male C57Bl/6 mice: 7 mo, and 22/23 mo
Heterogeneity:	Not investigated
Exp. setup:	10X Genomics; HiSeq 4000
Remarks:	No change in p16 expression, nor of any specific senescence-related gene signature.

Single-tissue Studies (2 of 14 *in vivo*)

Aging T-cell Atlas (Elyahu et al., 2019)

Tissues:	blood ($CD4^+$ T-cells)
Animals/Cells:	~24 000 cells from 8 C57BL/6 mice (of unspecified gender), 2–3 mo and 22–24 mo
Heterogeneity:	Not investigated
Exp. setup:	10x Genomics GemCode Chromium, Illumina NextSeq 500
Remarks:	Investigation of subsets of cells associated with chronic inflammation and immunity decline, but no specific investigation of marker genes of aging or senescence.

Aging Lung Atlas (Angelidis et al., 2019)

Tissues:	lung
Animals/Cells:	~15 000 cells from 15 C57BL/6 mice, 3 mo and 24 mo
Heterogeneity:	increasing transcriptional noise, similar to Enge et al. (2017)
Exp. setup:	DropSeq; Illumina HiSeq4000
Remarks:	Increased cholesterol biosynthesis in type-2 pneumocytes and lipofibroblasts and altered relative frequency of airway epithelial cells were observed.

Senescence and Inflammation Markers: Insights and Consistencies

Figure 1. Fraction of cells expressing 10 aging-related marker genes in liver cells of the Tabula Muris Senis.

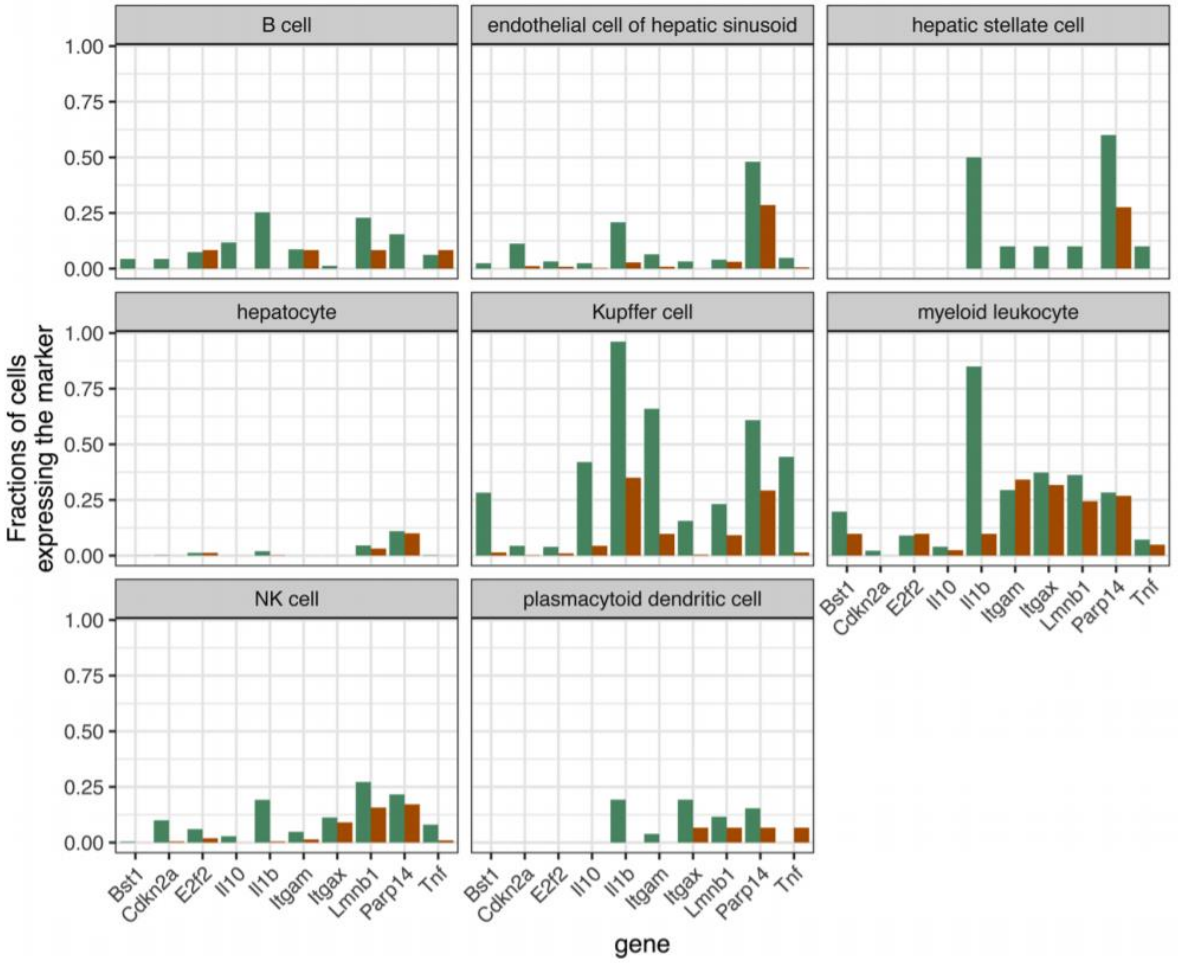
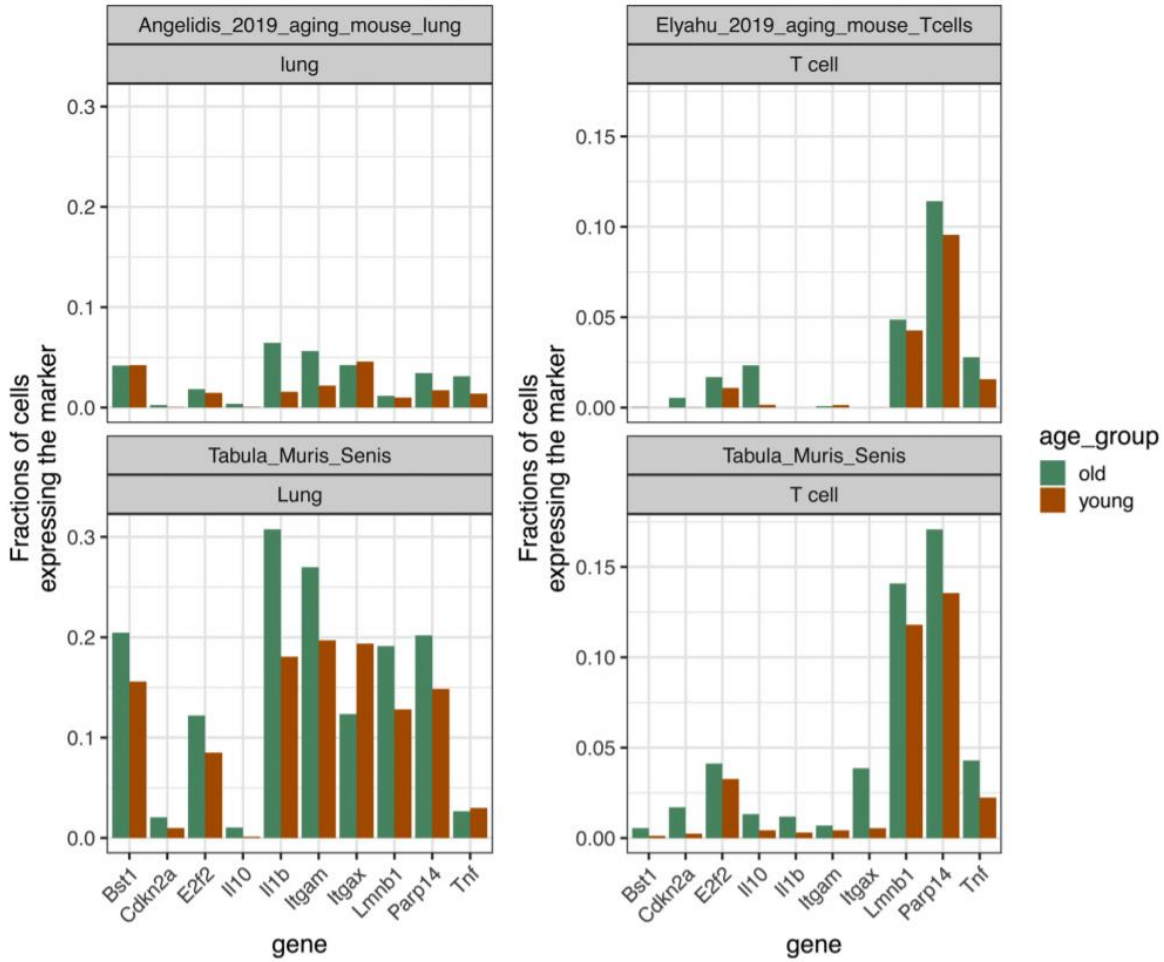


Figure 2. Fraction of cells expressing 10 markers in lung cells and T cells of the Tabula Muris Senis, compared to the Aging Lung Atlas and the Aging T-cell Atlas.



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