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ORIGINAL ARTICLE

Heterochronic parabiosis regulates the extent of cellular senescence in multiple tissues

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Abstract An increase in the burden of senescent cells in tissues with age contributes to driving aging and the onset of age-related diseases. Genetic and pharmacologic elimination of senescent cells extends both health span and life span in mouse models. Heterochronic parabiosis in mice has been used to identify bloodborne, circulating pro- and anti-geronic factors able to drive or slow aging, respectively. However, whether factors in the circulation also regulate senescence is unknown. Here, we measured the expression of senescence and senescence-associated secretory phenotype (SASP) markers in multiple tissues from 4- to 18-month-old male mice that were either isochronically or heterochronically paired for 2 months. In heterochronic parabionts, the age-dependent increase in senescence and SASP marker expression was reduced in old mice exposed to a young environment, while senescence markers were concurrently increased in young heterochronic parabionts. These findings were supported by geropathology analysis using the Geropathology Grading Platform that showed a trend toward reduced hepatic lesions in old heterochronic parabionts. In summary, these results demonstrate that senescence is regulated in part by circulating geronic factors and suggest that one of the possible mediators of the rejuvenating effects with heterochronic parabiosis is through the reduction of the senescent cell burden.

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Introduction

A number of common aging mechanisms, termed pillars of aging, that influence life span and health span have been identified in multiple models of aging. Of these pillars, cellular senescence has received considerable attention recently as a key driver of aging as well as a potentially druggable target to prevent or treat multiple aging comorbidities (Kirkland et al. 2017; LeBrasseur et al. 2015; Tchkonia et al. 2013). Cellular senescence is an essentially irreversible growth arrest that occurs when cells experience persistent DNA damage or potential oncogenic insults (Gorgoulis et al. 2019), consistent with its role as an anticancer mechanism. Senescent cells also can secrete soluble factors, termed the senescence-associated secretory phenotype (SASP), that can induce secondary senescence both in a proximal and distal fashion. SASP factors are made up of metalloproteinases, growth factors, chemokines, and pro-inflammatory molecules that can induce cellular stress and recruit immune cell populations to sites of senescence (Coppe et al. 2010). However, with advancing age, senescent cells accumulate (Herbig et al. 2006; Waaijer et al. 2012), presumably due to inefficient removal by the immune system and their resistance to apoptosis (Campisi and d'Adda di Fagagna 2007; Zhu et al. 2015). By depleting the mitotically active progenitor pool and secreting factors that directly affect regenerative, inflammatory, and tissue remodeling processes, senescent cells contribute to age-related tissue deterioration, inflammation, fibrosis, and even hyperproliferation (Campisi and d'Adda di Fagagna 2007; Munoz-Espin and Serrano 2014). Correspondingly, genetic and/or pharmacologic clearance of senescent cells and/or suppression of the SASP is coupled with significant therapeutic benefits in murine models of natural aging (Baker et al. 2016), accelerated aging (Baker et al. 2011; Zhu et al. 2015), vascular dysfunction (Roos et al. 2016), wound healing (Demaria et al. 2014), atherosclerosis (Childs et al. 2016), lung disease (Schafer et al. 2017), diabetes (Schafer et al. 2016; Xu et al. 2015), osteoarthritis (Jeon et al. 2017), intervertebral disc degeneration (Patil et al. 2019), and osteoporosis (Farr et al. 2017).

The surgical pairing of animals, termed parabiosis, to establish a shared circulatory system has been used extensively to study the effects of circulating factors in driving disease. In addition, heterochronic parabiosis, involving the pairing of mice of young and old mice, has been used extensively in recent years to investigate whether exposure to a young circulatory system could act as a source of rejuvenation for an older animal as well as whether circulating progeronic factors from old mice can drive aging in young mice. These studies have shown the ability of circulating factors in young mice to improve myogenesis, hepatogenesis, regrowth of bone, and neurogenesis in old mice (Brack et al. 2007; Carlson et al. 2009a, b; Conboy et al. 2015; Conboy et al. 2005; Rebo et al. 2016; Villeda et al. 2011; Villeda et al. 2014; Yousef et al. 2015). Conversely, circulating factors in old mice, including β2-microglobulin (β2M), C-C Motif Chemokine Ligand 11 (CCL11), and transforming growth factor-β (TGF-β) (Carlson et al. 2009a; Smith et al. 2015; Villeda et al. 2011; Yousef et al. 2015), can drive aging in young mice.

While rejuvenating or aging effects of heterochronic parabiosis in mice have been well documented, very little is known about the impact of circulating factors on cellular senescence. To determine whether circulating factors can increase or decrease the senescent cell burden with aging in a cell non-autonomous manner, here we measured senescence (Sharpless and Sherr 2015; Yousefzadeh et al. 2019) in multiple tissues of isochronically and heterochronically paired male mice. We demonstrate that the age-related increase in markers of senescence found in old isochronic pairings was reduced in old heterochronic parabionts, whereas conversely, the markers of senescence were enhanced in young heterochronic mice. These findings were correlated with histopathologic analysis for common age-specific lesions in the mice. Taken together, these results demonstrate that senescence can be modified by circulating factors in a murine model of heterochronic parabiosis and suggest the pro- and anti-geronic effects observed in heterochronic parabiosis could be mediated, in part, via the regulation of cellular senescence.

Methods

Heterochronic parabiosis

Parabiotic mouse surgeries were performed by the Einstein Health Span Core in young (4-month-old) and old (18-month-old) male inbred C57BL/6 mice that were obtained from the National Institutes of Aging aged rodent colony. Surgical unions were performed between isochronic young, isochronic old, and heterochronic pairing of young and old mice ($n = 8$ mice per group). Following surgery, animals were kept on a partial heating pad to recover overnight. Paired mice were rigorously monitored and received subcutaneous injections of Banamine (2 mg/kg each) immediately postoperative and b.i.d. for 3 days and once daily for 4 days. Animals also received Ringers lactate subcutaneously immediately post-op and daily for 3 additional days to prevent dehydration. Animals remained conjoined for approximately 2 months before sacrifice. Samples isolated from young isochronic pairings (**Y**Y), old isochronic pairings (**O**O), young mice heterochronically paired to old mice (**Y**O), and an old mice heterochronically paired to young mice (**O**Y) are respectively denoted in Fig. 1A. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the Albert Einstein College of Medicine.

RNA isolation and qPCR

Analysis of senescence marker expression in tissues was performed as described in (Yousefzadeh et al. 2019). Tissues were harvested from euthanized animals and snap frozen in liquid nitrogen. Total RNA was isolated by Trizol extraction according to manufacturer's specifications (Thermo Fisher, Waltham, MA). Gene expression changes in $p16^{Ink4a}$, $p21^{Cip1}$, *Il1β*, *Il6*, *Mcp1*, and *Tnfα* were quantified by qRT-PCR and analyzed by ΔΔCt method. Expression was normalized to *Gapdh*. Primer sequences were as follows: *Cdkn1a* ($p21^{Cip1}$) Fwd 5'-GTCAGGCTGGTCTGCCTCCG-3′, *Cdkn1a* ($p21^{Cip1}$) Rev. 5'-CGGTCCCGTGGACAGTGAGC AG-3′; *Cdkn2a* ($p16^{Ink4a}$) Fwd 5′- CCCAACGC CCCGAACT-*3′, Cdkn2a* ($p16^{Ink4a}$) Rev. 5'-GCAGAAGAGCTGCTACGTGAA-3′; *Gapdh* Fwd 5'-AAGGTCATCCCAGAGCTGAA-3′, *Gapdh* Rev. 5'-CTGCTTCACCACCTTCTTGA-3′; *Il1β* Fwd 5′-CACAGCAGCACATCAACAAG-3′, *Il1β* Rev. 5'-GTGCTCATGTCCTCATCCTG-3′; *Il6* Fwd 5'-CTGGGAAATCGTGGAAT-3′, *Il6* Rev. 5'-CCAGTTTGGTAGCATCCATC-3′; *Mcp1* Fwd 5'-GCATCCACGTGTTGGCTCA-3′, *Mcp1* Rev. 5'-CTCCAGCCTACTCATTGGGATCA-3′; *Tnfα* Fwd 5'-ATGAGAAGTTCCCAAATGGC-3′, *Tnfα* Rev. 5'-CTCCACTTGGTGGTTTGCTA-3′.

Chemokine analysis

Tissue levels of MCP-1 were measured by ELISA (RayBiotech, Norcross, GA) according to manufacturer's specifications.

Geropathology

The GGP provides a grading system to assess biological aging in mice by measuring the pathological status of a wide range of tissues in a standardized scoring system. The grading system generates a numerical score for the total lesions in each tissue, which when averaged over the mice in the cohort provides a composite lesion score (CLS) for each tissue and mouse. Mouse tissues ($n = 8$ per tissue per group) were collected at the time of necropsy. Tissues were fixed in 10% neutral-buffered formalin for at least 24 h and stored in 70% ethanol until being processed and embedded into paraffin blocks. Tissues were sectioned and stained with hematoxylin and eosin and scored for both the presence and severity of age-related lesions by a veterinary pathologist to generate a CLS for each tissue (see Supplemental Table 1 for a list of age-related lesions scored in each tissue). This score has been confirmed to faithfully rise with chronological age in diverse mouse strains and is reflective of health span (Ladiges 2016).

Statistics

All data are expressed as the mean ± standard deviation (SD). Statistical differences among the groups were measured using one-way analysis of variance (ANOVA) with Tukey's test. A p value of ≤ 0.05 was considered statistically significant. Asterisks in the figure represent the following: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$. All statistical analyses were performed with Prism software version 8.3 (GraphPad, San Diego, CA).

Results

To investigate the impact of circulating factors on the senescent cell burden and age-related pathology of old and young mice, 4- and 18-month-old male animals were surgically paired for ~ 8 weeks before euthanasia and tissue collection (Fig. 1A). Isochronic pairings of young and old mice were included as controls. Initially, the levels of mRNA expression of senescence markers $p16^{Ink4a}$ and $p21^{Cip1}$ were measured by qPCR in order to quantitatively determine the extent of the senescent cell burden (Sharpless and Sherr 2015; Yousefzadeh et al. 2019). Significantly elevated $p16^{Ink4a}$ expression was present in multiple tissues (forebrain, liver, lung, kidney, and pancreas) of old isochronic parabionts (**O**O) compared to their younger counterparts (**Y**Y) (Fig. 1B), but not in cerebellum, heart, and muscle tissue, similar to the pattern observed in natural aging (Yousefzadeh et al. 2020). Interestingly, a significant reduction in $p16^{Ink4a}$ expression was observed in multiple tissues of old mice that were paired to young mice (**O**Y). Conversely, young heterochronic parabionts (**Y**O) had increased $p16^{Ink4a}$ expression in the liver, kidney, and pancreas, but not in the forebrain or lung.

$p21^{Cip1}$ expression was significantly elevated in all tissues of old isochronically paired mice (**O**O) except for the cerebellum and heart (Fig. 1C). In contrast, expression of $p21^{Cip1}$ was significantly reduced in multiple tissues of old mice heterochronically paired to young mice (**O**Y). A reciprocal increase in $p21^{Cip1}$ expression was observed in all tissues of the young heterochronic parabionts (**Y**O) except for cerebellum, forebrain, lung, and heart. These findings demonstrate that there is an age-related increase in cellular senescence observed in multiple tissues of old mice that is reduced by exposure to young blood through heterochronic parabiosis. Conversely, exposure of young mice to old blood increases expression of similar markers of cellular senescence.

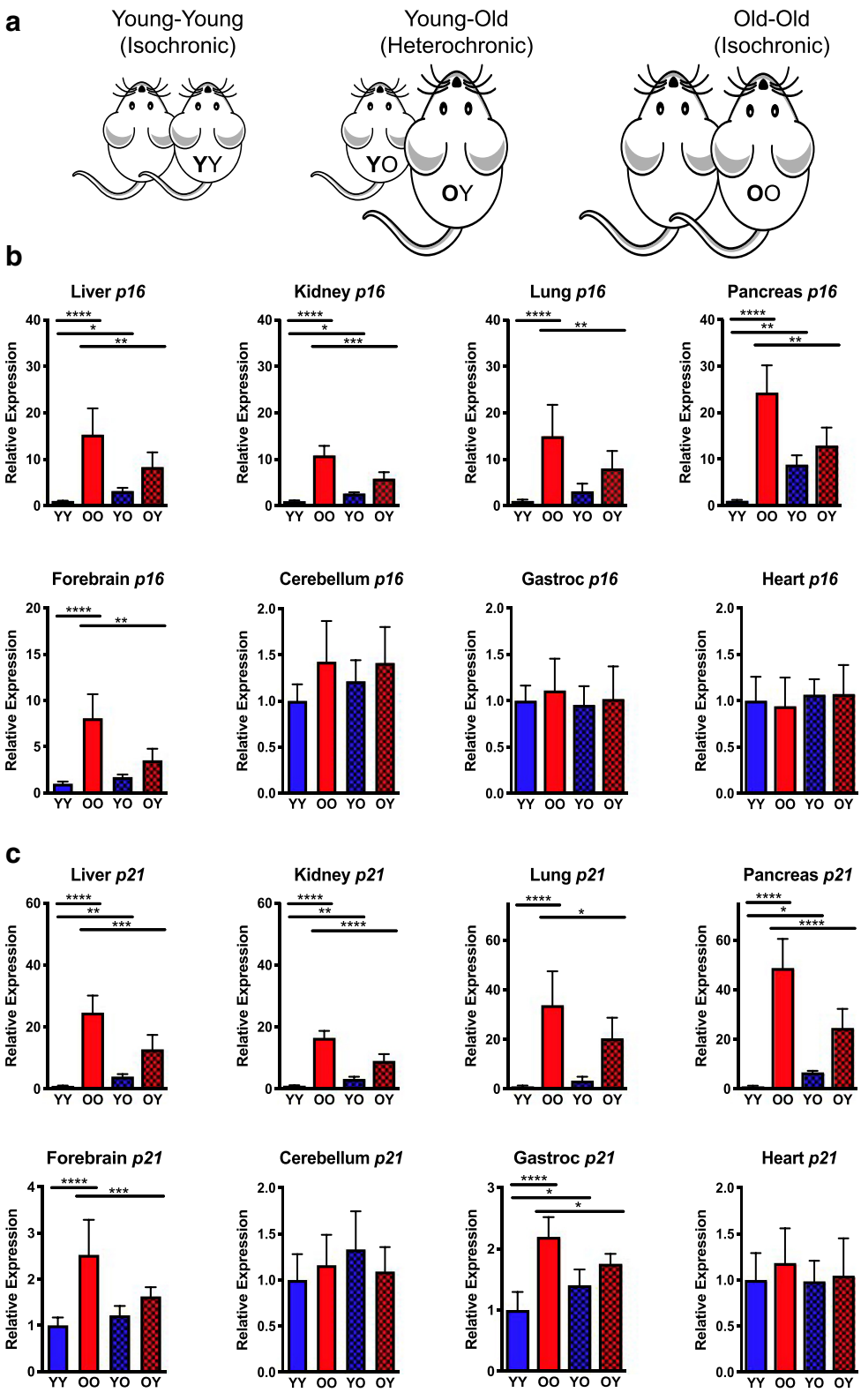
A common feature of cellular senescence is the release of soluble factors, termed the senescence-associated secretory phenotype (SASP), which can promote secondary senescence (Sharpless and Sherr 2015). These SASP factors are comprised of chemokines, cytokines, growth factors, and secreted proteases, which act on nearby cells in a paracrine manner or on distal cell populations in an endocrine manner (Coppe et al. 2010). To examine the effects of heterochronic parabiosis on SASP, mRNA expression of multiple SASP markers

Fig. 1 Expression of $p16^{Ink4a}$ and $p21^{Cip1}$ in various tissues of parabiotic mice. **A** Schematic of isochronic and heterochronic parabiotic pairings of 4- and 18-month-old mice. **B** Total RNA was isolated from snap-frozen tissues collected from parabiotic mice ($n = 5$–8 mice per group). Expression of senescence markers **B** $p16^{Ink4a}$ and **C** $p21^{Cip1}$ was measured by qPCR using the ΔΔCt method and normalized to *Gapdh* expression. Values represent the mean ± SD and one-way ANOVA with Tukey's test. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$

was quantified in tissues (Fig. 2A) with age-related increases in cellular senescence (Fig. 1). Expression of *Mcp1*, a chemokine that is responsible for monocyte recruitment and can serve as a surrogate biomarker for biological aging and frailty (Yousefzadeh et al. 2018a), was significantly increased in multiple tissues of isochronic old mice (**O**O) (Fig. 2A). Heterochronic pairing significantly reduced the *Mcp1* expression in the forebrain, kidney, liver, and lung of old mice paired with young mice (**O**Y). These gene expression findings were confirmed with analysis of tissue levels of MCP-1 protein by ELISA, which showed a similar pattern of age-related expression in the kidney and liver (Fig. 2B). Consistent with other markers, MCP-1 protein was significantly decreased in old heterochronic parabionts (**O**Y) but was increased in young heterochronic parabionts (**Y**O).

Expression of pro-inflammatory cytokines (*Il1β*, *Il6*, and *Tnfα*) was also significantly increased in old isochronically paired mice (**O**O) compared with young isochronic controls (**Y**Y) (Fig. 2A). Old heterochronic parabionts (**O**Y) experienced significant reductions in expression of these cytokines in all tissues measured. Significant increases in *Il1β* (forebrain, liver, lung, kidney), *Il6* (liver, kidney), and *Tnfα* (lung) expression were observed in young heterochronic parabionts (**Y**O) relative to young isochronic parabiont controls (**Y**Y). Concomitant with $p16^{Ink4a}$ and $p21^{Cip1}$ expression (Fig. 1), SASP marker expression elevated in old isochronic pairings (**O**O) was significantly reduced in old heterochronic parabionts (**O**Y).

The accumulation of senescent cells with aging impairs tissue homeostasis (Baker et al. 2016; Krishnamurthy et al. 2004; Wang et al. 2009; Xu et al. 2018). To determine if the transposition of senescent phenotypes was associated with histopathologic changes in the heterochronically paired mice, tissues were assessed via the Geropathology Grading Platform (GGP) as recently described to generate a composite lesion score (CSL) for each tissue (Ladiges 2016)


a
Young-Young (Isochronic)
Young-Old (Heterochronic)
Old-Old (Isochronic)
YY
YO
OY
OO
b
Liver p16
Kidney p16
Lung p16
Pancreas p16
Forebrain p16
Cerebellum p16
Gastroc p16
Heart p16
c
Liver p21
Kidney p21
Lung p21
Pancreas p21
Forebrain p21
Cerebellum p21
Gastroc p21
Heart p21
Relative Expression
YY OO YO OY

(Fig. 3). Age-related CLSs were significantly increased in the livers and kidneys of old isochronically paired mice (**O**O) relative to controls (**Y**O) (Fig. 3B). No difference was found in CLSs from the hearts of young (**Y**Y) and old (**O**O) isochronic mouse pairings, consistent with $p16^{Ink4a}$ and $p21^{Cip1}$ expression data collected in the heart (Fig. 1). Age-related lesions were modestly, but not significantly, reduced in livers from old heterochronic parabionts (**O**Y) compared with isochronic parabionts (**O**O). Similarly, hepatic lesions were slightly, albeit not significantly elevated in young mice that were paired with old mice (**Y**O) (Fig. 3B). These findings show that age-related lesions are increased in some tissues of parabiotic mice and that relatively short-term pairings of 2 months have only a modest effect on age-specific lesions.

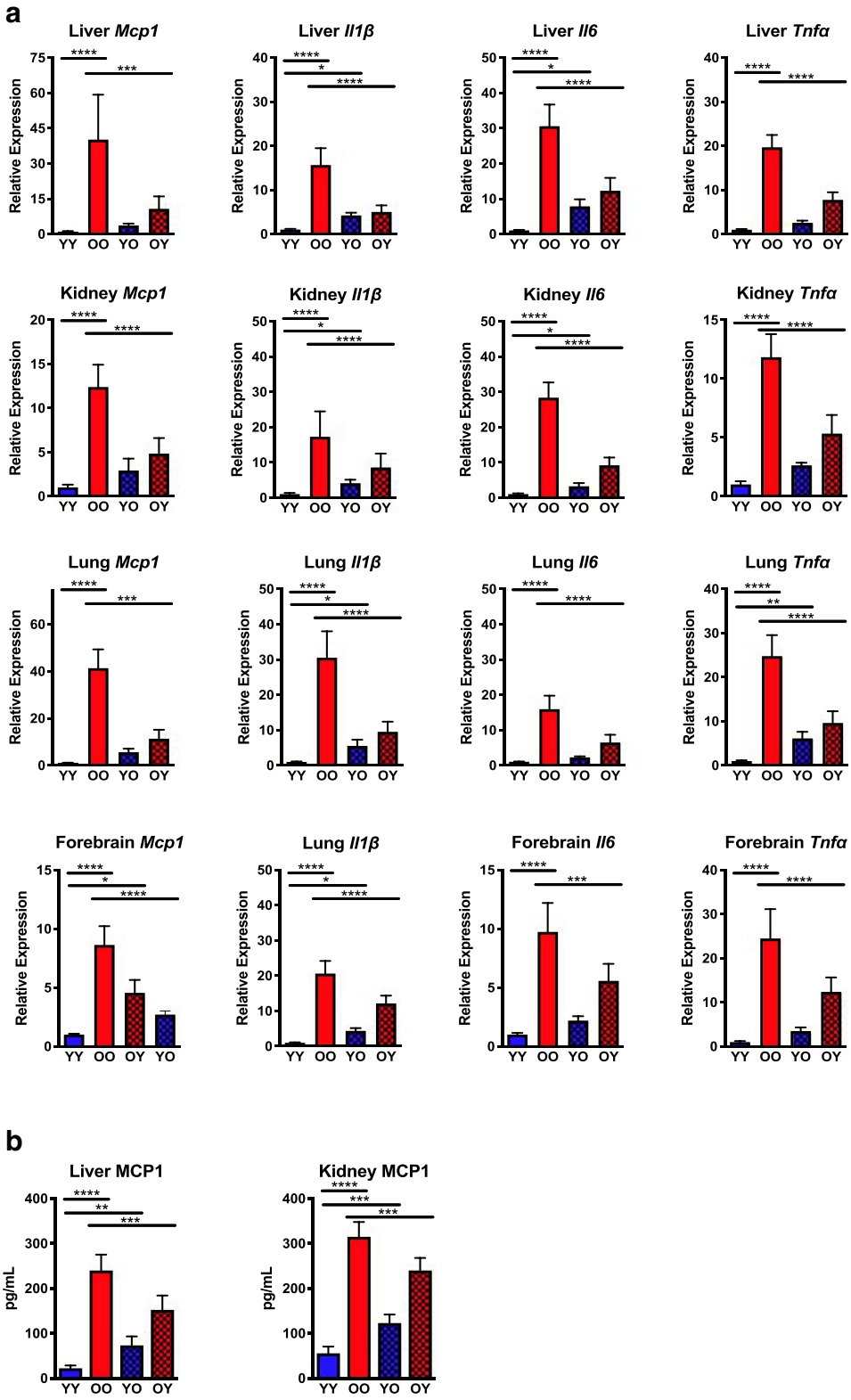
Discussion

In this study, we examined the extent of cellular senescence in multiple tissues in both isochronic and heterochronic parabiotic mice to determine whether pro- and/or anti-geronic circulating factors can modulate senescent burden that accumulates with age. Our results demonstrate that senescence was enhanced in an age-dependent manner with tissues from old isochronic pairings (**O**O) showing significantly higher levels of $p16^{Ink4a}$ and $p21^{Cip1}$ expression relative to samples from young isochronically paired mice (**Y**Y) except for the cerebellum, heart, and skeletal muscle. Similarly, SASP marker expression was elevated (Fig. 2A) in all the tissues from aged mice (**O**O) with increased senescence marker, $p16^{Ink4a}$ and $p21^{Cip1}$, expression. Tissue levels of the SASP factor, MCP-1, which can serve as a surrogate biomarker for biological age (Yousefzadeh et al. 2018a), increased in the livers and kidneys of aged mice (**O**O). The elevated senescence marker expression was reduced in tissues taken from old heterochronic parabionts (**O**Y) after exposure to a young circulation for 2 months as was SASP marker expression. In addition, the tissue levels of MCP-1 were significantly reduced in tissues from old heterochronic parabionts (**O**Y). Taken together, these findings suggest that one mechanism through which exposure to a young circulatory system can confer beneficial effects is through reduction in cellular senescence.

Conversely, exposure to an old circulatory system enhanced cellular senescence. Expression of $p16^{Ink4a}$ and $p21^{Cip1}$ was enhanced in multiple tissues in young mice paired with old mice (**Y**O). Similarly, expression of individual SASP markers was increased in tissues from young heterochronic parabionts (**Y**O). Consistent with our panel of senescence marker gene expression data, increased MCP-1 protein was present in young tissues of the heterochronic pairings (**Y**O). We also have examined the effect of heterochronic parabiosis specifically on intervertebral disc degeneration (IDD), demonstrating that exposure to old blood accelerates disc matrix degeneration in young mice (Lei et al, submitted). Conversely, old mice paired with young mice (**O**Y) exhibited a significant decrease in expression of cellular senescence and SASP markers but showed only a marginal decrease in the levels of disc MMP-13 and ADAMTS4 and aggrecan fragmentation. Taken together, these findings suggest that one mechanism in which exposure to an old circulatory system drives aging in multiple tissues is through an increase in cellular senescence.

The clearance of senescent cells has been reported to improve the histopathology of multiple tissues. Similarly, histological analysis of tissues in heterochronic parabiosis has been reported to improve the pathology of certain tissues such as heart and brain. The Geropathology Grading Platform (GGP) was developed by the Geropathology Research Network to detect the presence or absence as well as severity of age-related lesions (Supplemental Table 1) in histologic sections of murine tissues (Ladiges 2016; Ladiges et al. 2016; Ladiges et al. 2017). Lesion scores for each tissue are scored from each mouse in a cohort and averaged to generate a CLS, which appears to strongly correlate with the age of the animal. Using this platform, heart, liver, lung, and kidney sections from parabiotic mice were scored for lesions (Fig. 3A). Overall, age-associated lesions were significantly higher in old (**O**O) versus young (**Y**Y) isochronic pairings. The one exception was in heart tissue from aged isochronically paired mice (**O**O) (Fig. 3B) where there was no change in the CLS.

Fig. 2 Analysis of the senescence-associated secretory phenotype in parabiotic mice. **A** Total RNA was isolated from snap-frozen tissues collected from parabiotic mice ($n = 5$–8 mice per group). Expression of senescence-associated secretory phenotype (SASP) genes *Il1β*, *Il6*, *Mcp1*, and *Tnfα* was measured by qPCR using the ΔΔCt method and normalized to *Gapdh* expression. **B** Hepatic and renal levels of the SASP factor MCP-1 were quantified by ELISA ($n = 7$ mice per group). Values represent the mean ± SD and one-way ANOVA with Tukey's test. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$


a
Liver Mcp1
Liver Il1β
Liver Il6
Liver Tnfα
Kidney Mcp1
Kidney Il1β
Kidney Il6
Kidney Tnfα
Lung Mcp1
Lung Il1β
Lung Il6
Lung Tnfα
Forebrain Mcp1
Lung Il1β
Forebrain Il6
Forebrain Tnfα
Relative Expression
YY OO YO OY
b
Liver MCP1
Kidney MCP1
pg/mL

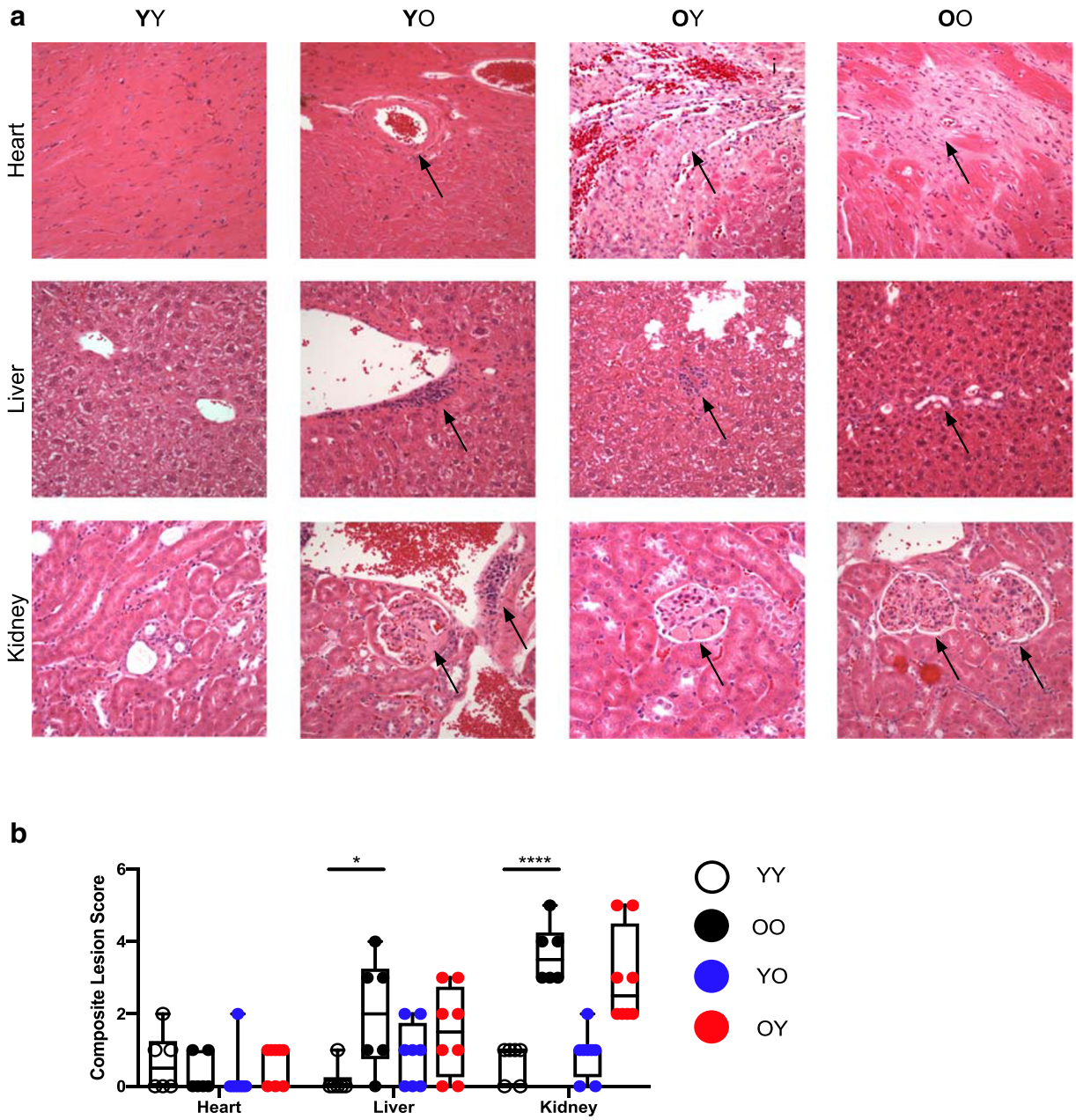


Fig. 3 Geropathological scoring of parabiotic mice**. A** Representative images of heart, liver, and kidney histologic sections from parabiotic mice. All images were taken at × 40. Heart (arteriosclerosis, **Y**O; cardiomyopathy, **O**Y, **O**O), liver (lymphoid aggregate, **Y**O, **O**Y; bile duct hyperplasia/cysts, **O**O), and kidney (nephropathy, **Y**O, **O**Y, **O**O; lymphoid aggregate, **Y**O) pathologies are indicated by arrow. **B** Histopathologic composite lesion scores (CLS) for age-related pathology in tissues determined using the Geropathology Grading Platform ($n = 6$–8 tissues per group). Values represent the mean ± SD and one-way ANOVA with Tukey's test. *$p < 0.05$, ***$p < 0.001$

A modest reduction in liver lesion scores occurred in old heterochronic parabionts (**O**Y), but was not statistically significant. Also, we did not observe a histologic improvement in disc aging in old mice (**O**Y) using a non-GGP score system (Lei et al, submitted). It is important to note that using the GGP to provide an unbiased composite lesion score did not result in an improvement in cardiac tissue pathology as previously reported

(Loffredo et al. 2013). However, longer pairings may be required to observe significant changes in age-related lesions as scored using the GGP.

Many studies have provided strong evidence that soluble factors have a potent influence on aging, and some of this data has arisen from parabiosis studies. Our results demonstrated that the age-related increase in senescence presents in old isochronic parabiosis and that senescence was modulated in old (reduced) and young (increased) mice by circulating factors. SASP marker mRNA and protein expression in tissues confirmed these findings. These results are consistent with a model where certain SASP components produced by senescent cells in the old mice contribute to driving senescence in young heterochronic parabionts. Overall, these findings from heterochronically paired mice establish that certain tissues, with an increased senescent cell burden with age, would benefit by treatment with yet to be identified factors in young serum or interventions that may promote a more youthful circulation to reduce the senescent cell burden and thus improve health span, similar to the effects observed with senotherapeutics (Chang et al. 2016; Xu et al. 2018; Yousefzadeh et al. 2018b).

It now will be important to identify the specific circulating geronic factors that play key roles in driving or suppressing the senescent cell burden. Those factors important for driving senescence could include circulating SASP factors such as TNF-α, IL-1ß, IL-1α, IL-6, or HMGB1. Future analysis of emerging markers of senescence (Haque et al. 2020; Lawrence et al. 2018; Lewis et al. 2018; Liendl et al. 2019) would provide additional insight into more comprehensively determining the senescent cell burden in parabiotic mice beyond just the classical markers ($p16^{Ink4a}$ and $p21^{Cip1}$ expression). In this regard, our preliminary analyses suggest that old murine and human serum-derived extracellular vesicles, potentially secreted by senescent cells, are able to induce senescence in cell culture. Also, CCL11 (Villeda et al. 2011) and ß2M (Smith et al. 2015), enriched in aged serum, have been implicated in driving certain age-related pathologies, but their effects on senescence are unknown. Unfortunately, even less is known about the factors in young serum important for suppressing senescence. GDF-11 (Katsimpardi et al. 2014; Loffredo et al. 2013), TIMP-2, and CSF-2 (Castellano et al. 2017) in young serum or cord blood have been reported to improve certain age-related pathologies, but whether they regulate senescence has not been reported.

In summary, these data provide evidence that markers of cellular senescence, typically associated as a manifestation of advanced age, can be rapidly induced by relatively short-term exposure to an old environment and lessened by exposure to a young environment. While we provide evidence across several distinct organs, it will be important to interrogate whether similar bidirectional effects are observed in other sites and to what extent these occur, including aorta, which was shown to have hyper-elevated levels of senescence (Khan et al. 2017; Yousefzadeh et al. 2020), adipose tissue, which is known to harbor a substantial number of senescent cells (Palmer et al. 2019; Xu et al. 2015) as well the brain, where senescence appears to occur particularly in endothelial cells and other cells to drive neurodegenerative changes (Bussian et al. 2018; Musi et al. 2018; Zhang et al. 2019). Comprehensive analysis into what particular cell types are affected by senescence in the brains of parabiotic mice, using spatial genomics or mass imaging, could provide a better understanding of the beneficial effects of parabiosis on cognition. For example, the senescent cell burden in astrocytes, a cell type implicated in causing cognitive decline (Csipo et al. 2020), is likely to be altered by the rejuvenating effects of parabiosis. Ultimately, a better understanding of the cell autonomous and non-autonomous drivers of senescence in diverse tissues, their interplay, and potential to be regulated should help to facilitate progress in the development of interventions to effectively target senescence as a means to delay aging.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical declaration Animal studies were performed in accordance with the Institutional Animal Care and Use Committee at the Albert Einstein College of Medicine.

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