Systemic milieu and age-related deterioration

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Abstract Aging is a fundamental biological process accompanied by a general decline in tissue function and an increased risk for age-related disease. The risk for cardiovascular, stroke, cancer, and neurodegenerative diseases significantly increases with aging, especially in people aged 60 years and older in the USA. Although the cellular and molecular mechanisms underlying aging and age-related disease are beginning to be unraveled, the role of the systemic milieu remains unknown. Recent studies have shown that systemic factors in young blood can revise age-related impairments and extend organismal lifespan, suggesting that the systemic milieu contains pro-aging and rejuvenating factors that play a critical role in the health and aging phenotype. In this review, we summarize the current knowledge of systemic milieu changes during the aging process and its link to age-related deterioration.

Keywords Aging · Disease · Systematic milieu · Parabiosis · Blood · Factors

Introduction

Aging is a fundamental biological process accompanied by a general decline in tissue function and an increased risk for age-related disease. The risk for cardiovascular

diseases, stroke, cancer, and neurodegenerative diseases significantly increases with aging, especially in people aged 60 years and older in the USA (Ramirez-Lassepas 1998; Arnold 1981). Systemic factors have potential intervention effects on the aging process and some age-related diseases. Therefore, new and innovative approaches to identify specific pro-aging and rejuvenating factors may be critical. According to recent studies, these anti-aging and rejuvenating factors are present in young circulation systems and can reverse age-related impairments. For example, an age-related decline in the activity of muscle progenitor cells can be restored, while damaged muscle can be successfully repaired after exposure to the young systemic milieu through heterochronic parabiosis (an approach for exchanging the systemic milieu of two organisms).

Historically, the study of the systemic milieu began with the fundamental understanding of systemic circulation and the functionality of the heart, which was introduced by William Harvey in “*Exercitatio Anatomica de Motu Cordis et Sanguinis in animalibus*” (Pasipoularides 1985). Understanding the systemic milieu was furthered with early attempts at animal to animal blood transfusions. Eventually, in 1667, John Baptiste Denys, physician to King Louis XIV of France, conducted the first animal to human blood transfusion on a 16-year-old boy with violent fever who had been bled for his illness at least 20 times in the past by removing 3 oz of blood and then injecting 9 oz of lamb’s blood (Myhre 1990). Much of Denys’s basis for using animal blood for the transfusion revolved around the idea that lamb blood was less immoral than human

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blood, which resulted in numerous other failed attempts at transfusion by Denys, eventually leading to a ban by the Pope on transfusion procedures (Learoyd 2012). In the early 1800s, a man by the name of Dr. James Blundell spent time studying the value of blood transfusions and feasibility (Baskett 2002). He then went on to perform the first successful blood transfusion in 1825, which was described in a case by C. Waller in which Dr. Blundell took fresh blood from the husband of a woman who was suffering from postpartum hemorrhage and injected it into her vein with a syringe (Waller 1825). This first successful case created many branches in medical and scientific progression with grafting, transplanting, and parabiosis. This concept of parabiosis was first introduced by Paul Bert with a protocol in 1864 (Eggel and Wyss-Coray 2014) that was further revised by Bunster and Meyer in 1933 to improve the animal's survival (Bunster and Meyer 1933). The use of parabiosis in humans is currently not performed due to the surgical complications and resulting undesirable lifestyle, similar to that of conjoined twins. However, the model has been provided insights into the role of systemic aging in age-related disease. Based on previous research, age-related cardiac hypertrophy can be reversed by exposure to a young systemic milieu, along with reduced cardiomyocyte size and molecular remodeling (Loffredo et al. 2013). In contrast, young mice exposed to an old systemic milieu exhibited reduced myogenesis (Conboy et al. 2005; Brack et al. 2007). Parabiosis helped establish the hypothesis that blood-borne factors in a healthy young organism could be beneficial for the aged, and this remarkable difference between young and old systemic milieu deserves to be studied. In this article, we reviewed the effect of young and old systemic milieu on the biological aging process.

Systemic milieu, aging, and lifespan

Evidence of the impact of the systemic milieu on lifespan is mainly from studies based on the heterochronic parabiosis model. Parabiosis is an experimental model where two animals are joined together surgically to create a shared circulation system through the connection of the skin and muscle walls, which include joining of the scapula joints, body cavities, and skin (Waskow 2010). Heterochronic parabiosis is a process that joins the vascular systems (anastomosis) of young mice with old mice, allowing for joined blood

circulation between two animals (Kamran et al. 2013). This process allows for the exchange of blood cells and soluble factors, thereby altering the systemic milieu of a paired partner. The exchanged blood circulation between organism pairs can be confirmed by *in vivo* imaging, fluorescence microscopy, and flow cytometry (Loffredo et al. 2013). Using the parabiosis model established between C57BL/6-TgN (ACTb-enhanced green fluorescent protein [EGFP]) transgenic mice and wild-type mice, GFP-positive blood cells were identified in the peripheral blood of the wild-type mice, suggesting that a shared circulation manifests between the pairs (Liu et al. 2018). The advantage of the parabiosis model is that the attached animals are free from immune reactions (Finerty 1952).

The parabiosis model is advantageous because the partnered animals share common circulating antigens, allowing cell migration and neovascularization without triggering an immunological reaction (Finerty 1952). In addition, parabiosis between male and female mice does not lead to the formation of anti H-Y antibodies (Weissman et al. 1984). Therefore, heterochronic parabiosis was not only helpful to discover and study detrimental and beneficial factors but also to assess the physiological or pathophysiological consequences of exposure to the systemic milieu in a whole organism (Eggel and Wyss-Coray 2014). Although this methodology is widely used and beneficial to explore whether circulating factors in the blood can alter tissue function, it is technically difficult to perform and is associated with high animal mortality and inflammation. The survival of parabiosis surgery is often limited due to complications, such as infection or cardiovascular diseases, which apparently occur as a consequence of parabiosis (Hall et al. 1959). In addition to technical challenges, kinetics is another consideration for the parabiosis approach (Conboy et al. 2013). Taking parabiosis and lifespan extension as an example, kinetics is a unique challenge as young parabiont animals are aging, and the “effector” parabiont age is not controlled during long-term experiments; thus, it would be challenging to design experiments in which animals undergo sequential parabiotic pairing to a series of young animals to provide more continual exposure to a youthful systemic milieu (Conboy et al. 2013).

Currently, the parabiosis model has been used to study tissue aging in the pancreas, heart, brain, muscle, and bone. Although convincing evidence regarding the effects of slowing aging or extending lifespan after

administering anti-aging remedies is limited, heterochronic parabiosis provides a novel technique to elucidate the effects of the systemic milieu on increasing the lifespan in animals possessing fatal disease (Conboy et al. 2013). Improved recovery in age-related diseases can be observed in studies focusing on myogenesis, neurogenesis, memory and cognitive abilities, cardiac hypertrophy, and gastrointestinal-related disease.

Several studies have reported the involvement of the systemic milieu in lifespan. In 1956, Pope et al. (Pope et al. 1956) used heterochronic parabiosis to study the regulation of lifespan. Both male and female hamsters were used in these parabiotic unions between animals of different ages, but no opposite sexes were used. The authors found that parabiotic pairs of young hamsters of the same age could extend the animal lifespan. In 1957, McCay et al. showed that littermates combined in youth could survive into old age, including rats and Syrian hamsters. Moreover, heterochronic parabiosis pairs survived for more than a year after parabiosis was established; these findings indicated that parabiosis seemed to be a promising technique for the study of aging by specifically determining whether the constant exchange of blood between animals of two different ages may impact chronic diseases of old age (McCay et al. 1957). In 1959, Hall et al. demonstrated lifespan extension from 15 to 24 weeks after male mice with muscular dystrophy were paired with a healthy partner using the parabiosis model (Hall et al. 1959). Notably, Ludwig and Elashoff first used the isochronic (old–old) and heterochronic parabiosis (young–old) model to systematically study the effects of heterochronic parabiosis on lifespan. These authors demonstrated that compared with old isochronic parabionts, old heterochronic parabionts had an extended lifespan (Ludwig and Elashoff 1972), which confirmed the potential anti-aging or rejuvenating effects of the youthful systemic milieu. In the twentieth century, heterochronic parabiosis has been used to study the basic questions of aging regulation and lifespan (Conboy et al. 2013). Though the molecular and cellular mechanisms underlying the aging process remain largely unexplored, these studies established the idea that the systemic milieu from healthy young animals may contribute to aging reversal in aged organisms (Conboy et al. 2013).

In summary, parabiosis is a promising technique to study lifespan, namely, the possibility of reversing the pathological changes in an old animal by bathing its tissues in the blood of a young animal. Despite its

potential value, this model is complex and not “fine-tuned”. More likely, a mix of many factors—cells and proteins—in young blood plays a “rejuvenation role”. Many efforts are required to identify some of these “enigmatic youth factors”. Future directions may include understanding why the systemic milieu can extend the lifespan in rodents. Additionally, identifying beneficial factors in young blood and detrimental molecules in old blood could provide novel and effective mechanistic and therapeutic implications for aging and age-related disease.

Systemic milieu, aging, and neurogenesis

For a long time, newly generated neurons have been considered absent from the brains of adult mammals, although Altman et al. first observed the potential capacity of proliferation in adult rodent’s brains in the 1960s (Altman and Das 1965; Altman 1962). The subventricular zone (SVZ) surrounding the lateral ventricles and the subgranular zone (SGZ) of the hippocampal dentate gyrus are considered the two main regions involved in recruiting neural stem cells (NSCs), which can continuously differentiate into neurons (Chiasson et al. 1999), astrocytes (Doetsch et al. 1999), and oligodendrocytes (Chiasson et al. 1999; Johansson et al. 1999) throughout life in mice (Yoshimura et al. 2001), rats (Jin et al. 2001), and primates (McDermott and Lantos 1991). Due to their unique location, proliferative ability, differentiation characteristics, and capability of functionally integrating existing neuronal circuitry in the central nervous system (CNS), NSCs hold great potential to repair injured brain (Jin et al. 2001; Arvidsson et al. 2002; Jin et al. 2006). Therefore, it is necessary to investigate the underlying mechanism at the molecular level of NSC behavior, particularly in the aged-damaged brain, which will provide fundamental support for the successful implementation of NSC recruitment therapy.

Regenerative capabilities significantly decline in most tissues during the aging process, largely attributable to impaired stem cell function. Similarly, growing evidence has demonstrated that neurogenesis declines with aging, as a progressive decline in the number of proliferating progenitor cells is observed in the SGZ and SVZ during aging. Although several studies have shown that intrinsic cellular changes play a role in neurogenesis decline, the systemic milieu of stem cells also apparently

represents a critical driver of age-related decline in neurogenesis. Heterochronic parabiosis studies have been used to examine the aging of somatic stem cells after attachment to animals of different ages. Evidence of the pro-aging effects of old blood on stem cell functionality comes from direct systemic injection with old blood plasma; specifically, short-term treatment with old plasma negatively affects hippocampal adult neurogenesis, which is consistent with the effects of heterochronic parabiosis (Rebo et al. 2016). Consistently, administration of old plasma over 1 month impairs the function of hippocampal-dependent learning and memory (Villeda et al. 2011). Specifically, blood from old animals, not middle-aged animals, has detrimental effects in young heterochronic parabionts (Katsimpardi et al. 2014). This finding suggests that systemic milieu changes, such as the loss of youth and accumulation of deleterious systemic factors, are the cause. For example, Villeda et al. found that young heterochronic parabionts exhibited a decline in the number of doublecortin (DCX)-, bromodeoxyuridine (BrdU)-, and sex determining region Y (SRY)-box 2 (Sox2)-positive cells in the SGZ and SVZ, as well as a decline in differentiated mature neurons (Villeda et al. 2011). Interestingly, the number of DCX-positive cells in the SGZ also declined in animals with the administration of old plasma compared with that in those receiving young plasma, suggesting that circulating factors within aged blood contribute to reduced neurogenesis with age (Villeda et al. 2011). Furthermore, mice receiving old plasma exhibited impaired behavioral assessment in terms of the contextual fear memory paradigm, which demonstrated decreased freezing.

In contrast, the exposure of the aged SVZ to young blood resulted in the activation of neural stem cell proliferation, including an increase in Ki67+ proliferating cells in the SVZ and an increase in newborn neurons in the olfactory bulb, which can be beneficial to the aging animal's reduced olfactory discrimination ability (Katsimpardi et al. 2014). Similarly, aged progenitors were restored by exposure to youthful factors through heterochronic parabiosis (Brack et al. 2007), which mainly affects proliferative Ki67+ cells, Sox2+ stem cells, and Olig2+ transit-amplifying progenitors. In the case of *Alzheimer's disease*, synaptic plasticity in the hippocampus of the aged brain has been improved by young blood, followed by improvement in cognitive function caused by age-related impairment (Villeda et al. 2014). The aged vasculature can reportedly be

affected by factors in young blood in terms of increasing blood vessel volume and cerebral blood flow (CBF) in the SVZ, along with vascular remodeling in the hippocampus and cortex in *in vivo* studies (Katsimpardi et al. 2014). Both morphology and functionality changes in the vasculature in aged animals after exposure to young factors suggest that there should be some remarkable differences between young and aged systemic milieu. Factors showing differences between the young and old were considered potential therapeutic agents for age-related disease or neurodegenerative disease. Katsimpardi et al. (Katsimpardi et al. 2014) confirmed that circulating factors, specifically, including growth differentiation factor 11 (GDF11), a transforming growth factor beta (TGF- β) family member that reverses cardiac hypertrophy in aged mice (Loffredo et al. 2013), have multiple beneficial effects in aging mice, such as stimulating vascular remodeling and increasing neurogenesis (Katsimpardi et al. 2014). Similarly, another circulating factor, CCL11 (eotaxin), is significantly increased in the old blood plasma of mice and humans. Administration of CCL11 was sufficient to induce impaired adult neurogenesis. One of these factors is chemokines (Villeda and Wyss-Coray 2013). In a recent study, daily GDF11 administration did not extend lifespan in a mouse model of premature aging (Freitas-Rodriguez et al. 2016).

In summary, these findings suggest that neurogenesis is regulated by blood-borne factors present in the systemic milieu in an age-dependent manner. Notably, the old parabiont benefits not only from young blood factors (cells and proteins) but also from young organs, including the heart, lungs, liver, kidneys, and thymus. In addition, the toxic metabolites in old parabiont can be efficiently removed by the young parabiont. All of these factors may contribute to the rejuvenated tissue stem cells (Conboy and Rando 2012; Conboy et al. 2015). Understanding how a young parabiont maintains an aged parabiont with poorly functioning organs could provide insights into the age-related decline in neurogenesis.

Systemic milieu, aging, myogenesis, and cardiac function

Muscle growth and maintenance of muscle mass are influenced by muscle stem cells referred to as myogenic stem cells, also known as satellite cells (SCs), which

progressively decline with age (Moss and Leblond 1970). Similar to neurogenesis, myogenesis is regulated by intrinsic and extrinsic signaling. In both young and old tissue, SCs are dormant and associated with postmitotic multinucleated muscle fibers but activate and give rise to proliferative myoblasts via forced activation of Notch signaling when muscle fibers are injured (Conboy et al. 2003).

A heterochronic transplantation study showed that muscles from old animals grafted into a young host improved regenerative capacity, while a young muscle grafted into an aged host impaired regenerative capabilities (Carlson and Faulkner 1989). Using the heterochronic parabiosis approach, Conboy et al. paired young and old mice as the experimental group with young–young and old–old parabionts as the controls (Ruckh et al. 2012). Five days after muscle injury, only old heteroparabionts and young isoparabionts had increased SC proliferation and myotubule formation, along with significant tissue regeneration, as demonstrated by the appearance of centrally nucleated eMHC-expressing myotubes. Conversely, damaged muscle from old isochronic parabionts regenerated poorly, along with incomplete myotube formation and prominent fibrosis at the site of injury (Ruckh et al. 2012).

GDF11 is a member of the TGF- β superfamily, a conserved family of growth factors that governs a multitude of cellular processes (Chang et al. 2001). GDF11 is decreased in the serum and spleen of aged mice (Loffredo et al. 2013) partly due to epigenetic modifications that control the aging process (Berdasco and Esteller 2012; Fraga and Esteller 2007). Sinha et al. identified GDF11 as a systemic rejuvenating factor that can exhibit positive effects in aging animals. Sinha et al. found that systematic administration of GDF11 reversed functional deterioration and renewed genomic integrity in aged SCs. Elevated GDF11 levels in aged mice also promoted muscle strength, structure, functional features, and exercise endurance capacity (Sinha et al. 2014). These findings suggest that stem cell niches are regulated systemically. GDF11 supplementation expanded the neuromuscular junction, but in the absence of injury, it did not affect gross anatomy, fat mass, or muscle mass of aged muscle. Interestingly, an important regulator of mitochondrial biogenesis, peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1 α), was elevated in the muscle derived from aged mice treated with GDF11 (Murphy and Thuret 2015), suggesting that GDF11 has an impact on mitochondrial dynamics.

GDF11 supplementation also increased basal levels of autophagy when administered to aged mice, providing evidence for another mechanism that likely causes the cellular restoration of muscle fibers (Sinha et al. 2014). The cellular and ultrastructural changes following GDF11 supplementation resulted in functional improvements, as shown by an increase in exercise endurance and augmented grip-strength (Sinha et al. 2014).

In contrast, Egerman et al. (Egerman et al. 2015) found that overexpression of GDF11 could impair SC function and reduce muscle restoration. Systemic administration of GDF11 in old mice had no effect, while in young mice, muscle regeneration was suspended due to diminished expansion and differentiation of SCs. Similarly, the injection of GDF11 (at a 3-fold-higher dose) also failed to enhance the regenerative capabilities of young muscle. These authors also found that GDF11 concentrations actually increased during aging in both young and aged rats and humans (Egerman et al. 2015). Hinken et al. confirmed that there was no evidence to show that GDF11 restored aged skeletal muscle SCs (Hinken et al. 2016). Consistently, previous studies have reported that GDF11 impairs SC expansion and differentiation, resulting in decreased myogenesis and muscle regeneration (Hinken et al. 2016; Gamer et al. 2001; Lee and Lee 2013). Therefore, the role of GDF11 in myogenesis has been questioned (Brun and Rudnicki 2015).

In addition, aging is responsible for related functional changes that lead to increased cardiovascular risk (Larson 1995). Research shows that patients with cardiac hypertrophy who have elevated diastolic pressure are at increased risk for heart failure (Aurigemma 2006). However, using heterochronic parabiosis, age-related cardiac hypertrophy was reversed by exposure to a young systemic milieu for 4 weeks, followed by reduced cardiomyocyte size and molecular remodeling (Loffredo et al. 2013). In contrast, exposing a young mouse (2 months old) to an old systemic milieu (23 months old) can inhibit myogenesis (Brack et al. 2007). Further analysis performed by proteomics identified GDF-11, a TGF- β superfamily member that declines with age, responsible for the rejuvenating effect on age-related cardiac hypertrophy in heterochronic parabiosis. Replacing circulating GDF-11 levels of old mice to young levels through daily intraperitoneal injection of recombinant GDF-11 reversed age-related cardiac hypertrophy as well, along with reducing the expression of hypertrophic markers such as atrial or B-type natriuretic peptides (ANP and BNP, respectively) and increased sarcoendoplasmic reticulum Ca²⁺ ATPase 2 (Serca2)

expression (Loffredo et al. 2013). Although the underlying mechanism of GDF-11 in human cardiac hypertrophy remains unknown, these findings regarding reversed cardiac hypertrophy with a young circulation suggest an exciting possible therapeutic approach with young blood factors, such as GDF-11, in cardiac aging. However, these findings of GDF11 as an anti-aging factor have recently been challenged (Smith et al. 2015). First, a recent study showed that endogenous levels of GDF11 were below detection limits in both young and old mice. Second, treating old mice with GDF11 had no effect on heart or myocyte size, overall cardiac structure, and cardiac pump function. Third, aged mice have greater body weights than young mice do, and 24-month-old mice have the same heart weight/bodyweight (HW/BW) ratio as 8- or 12-week-old mice, suggesting that there is no evidence of pathological hypertrophy in aged mice (Smith et al. 2015).

In summary, although there are contradictory results regarding the level of GDF11 in the aged population, most studies showed that GDF11 should be regarded as a new molecular regulator of mammalian aging with potentially broad applications (Sinha et al. 2014). In any event, unique treatment involving GDF11 and its effect on skeletal muscle, its anti-hypertrophic effect on the heart, and its beneficial effect on neurogenesis and neuronal function in aged mice should encourage further investigation of its therapeutic use for a variety of age-related diseases (Katsimpardi et al. 2014; Sinha et al. 2014). However, future preclinical and clinical studies that target GDF11 must be carefully designed to avoid unwanted side effects in skeletal or cardiac muscle or other tissues that produce or respond to GDF11, which may be particularly important in the treatment of age-related diseases (McPherron 2013).

Systemic milieu, aging, and oligodendrocyte progenitor cells

The CNS utilizes rejuvenation by remyelination to restore nerve conduction and prevent axonal degeneration (Duncan et al. 2009; Edgar and Nave 2009). Nevertheless, the efficiency of CNS remyelination decreases with age (Gilson and Blakemore 1993). One reason for this decrease is age-related impairment in the recruitment and differentiation of oligodendrocyte progenitors (Sim et al. 2002). Oligodendrocytes are specialized glial cells that myelinate CNS axons. Myelinated axons compose white matter tracts that interconnect gray matter

areas of the brain and are necessary for rapid integrated neuronal communication and cognitive function. The restoration capabilities of oligodendrocyte progenitor cells (OPCs and NG2-glia) are reduced with aging (Rivera et al. 2016). Thus, aging is one of the essential factors contributing to remyelination failure, imposing a significant restriction on therapy in the CNS (Ruckh et al. 2012). Using heterochronic parabiosis, Ruckh et al. found that despite intrinsic alterations in OPCs, exogenous signals introduced by the young systemic milieu through parabiosis were capable of providing differentiation signals and retaining efficient repair (Ruckh et al. 2012). These experiments further demonstrated that rejuvenation effects were in part due to the enhanced efficiency of young macrophages in phagocytosing myelin debris and that differences in the phagocytic activities of young versus old macrophages played a role in their differences in activating OPC function. In addition, serum-derived factors such as CCR2 and myelin protein myelin basic protein (MBP) from the young milieu contribute to rejuvenation (Ruckh et al. 2012). Following this study, Pusic and Kraig identified peripheral exosomes as potential circulating factors capable of mimicking the effects of the young milieu in increasing the number of OPCs, their differentiation, and ultimately remyelination, in the serum of young rats as well as in old rats exposed to a supplemented milieu (Pusic and Kraig 2014). In addition, the scientists used a miRNA expression array to identify miR-219, a microRNA normally found in young animals as a key component in the assembly of myelinating oligodendrocytes, which was transported by the “rejuvenating” exosomes following exposure to an enriched milieu. Furthermore, CNS myelinating in aging rats was enhanced by young exosomes *in vivo* (Pusic and Kraig 2014).

Taken together, these results show that the promyelinating effects of young systemic milieu on the aging brain involve peripheral exosome-mediated transportation of miRNAs, providing a foundation for future research to discover the predictive role of peripheral blood miRNA in human disease, which will lead to the identification of the biological processes controlled by these miRNAs.

Systemic milieu, aging, and memory

Cognitive decline is a leading cause of functional impairment worldwide, especially among a rapidly growing

aging population (Lourenco et al. 2018). However, the aging process easily results in a comprised hippocampus, evidenced by dysregulation of plasticity-related genes and declines in neurogenic activity (Murphy and Thuret 2015; Ashe 2001). Neurogenesis in the hippocampus is associated with cognitive processes such as learning and memory. Throughout life, newly generated neurons comprise a cognitive reserve that incorporates into the existing hippocampal memory circuits, contributing to the function of spatial memory (Garthe et al. 2009). Therefore, the continuous generation of new neurons in the SGZ is not only a key component of the hippocampal neurogenic reserve (Kempermann 2008) but also a structural element of the cognitive reserve (Nithianantharajah and Hannan 2009). However, based on evidence showing that aging inhibits hippocampal neurogenesis, cognitive function declines with age. As described above, the aging systemic milieu remarkably impacts neurogenesis. The role of neurogenesis in cognition is well documented (please see review (Oomen et al. 2014)). Villeda et al. demonstrated that systemically exposing young animals to old plasma results in cognitive impairments, indicating molecular changes in the aging systemic milieu (Villeda and Wyss-Coray 2013). Similarly, mice receiving old plasma showed a decline in freezing in contextual and impaired learning and memory for the platform location test during the testing phase (Villeda et al. 2011), although all mice displayed similar performance during the training phase of the radial arm water maze (RAWM) in terms of swim speeds and spatial learning capacity for the task. Furthermore, young adult mice receiving intraperitoneal injections of recombinant C-C motif chemokine 11 (CCL11) exhibited decreased freezing during contextual and impaired learning and memory deficits. Taken together, these data demonstrate that increasing pro-aging factors present in aging blood, such as CCL11, not only inhibits adult neurogenesis but also impairs the function of learning and memory (Villeda et al. 2011).

In addition to neurogenesis, synaptic plasticity declines with age and plays an important role in aging systemic milieu-mediated cognitive impairment. Long-term potentiation (LTP), which is related to cognitive function, exhibits an age-related decline in electrophysiology studies (Bliss and Collingridge 1993). Hippocampal sections obtained from young isochronic and heterochronic parabionts show a decrease in LTP in the SGZ *in vitro* (Villeda et al. 2011), suggesting that age-related systemic changes can elicit cognitive function deficits as well as reduced synaptic plasticity during aging.

The hallmark of Alzheimer's disease (AD) is progressive memory and cognitive decline with massive, progressive neuronal loss in the hippocampus and cortex. Despite progress in understanding the mechanisms involved in the death of neuronal cells with AD, effective treatment remains a mystery, largely due to inadequate knowledge of the mechanisms underlying AD pathogenesis (Eckert et al. 2018). AD transgenic mice exhibited restoration of neurogenesis, which resulted in improvement in cognitive function after exposure to neurogenic factor allopregnanolone (Wang et al. 2010). Villeda et al. reported that exposure to a young systemic milieu enhanced synaptic plasticity along with the enhanced expression of immediate early genes and the density of dendritic spines, as well as in LTP (Villeda et al. 2014). Moreover, immediate early gene expression, LTP, and cognitive function in aged mice can also be enhanced and improved by human umbilical cord blood-derived plasma (Castellano et al. 2017). At a functional level, cognitive impairments in hippocampal-dependent learning and memory in old mice were improved after long-term systemic administration of young plasma, which was proven by enhanced activation of the transcription factor cyclic adenosine monophosphate (cAMP) response element binding protein (CREB). As described above, aged mice receiving blood plasma from a young mouse improved age-related mental impairments in contextual fear conditioning, spatial learning, and memory (Villeda et al. 2014), while an old systemic milieu impaired cognitive function in a young mouse (Villeda et al. 2011). This result shows that cognitive impairments observed during aging can be partly attributed to changes in blood-borne factors. However, recent studies also indicate that factors in AD blood, such as serum amyloid P, play a role in the pathogenesis of cognitive deterioration. In addition, plasma proteins in AD patients strongly correlated with disease severity and disease progression (Hye et al. 2014). All of these studies indicate the impact of the aging systemic milieu on the pathogenesis of AD.

In summary, the best-known form of dementia is AD, but a large proportion of cognitive impairment cases in the aged population are not due to AD but rather to the normal aging process. The vast majority of older adults suffer declines in cognitive functions, interfering with their ability to participate and engage in meaningful activities (Harada et al. 2013). Thus, a better understanding of how different pathologies and age-related changes contribute to declining cognitive functions is crucial

for the rational development of therapeutics. Although the mechanisms underlying age-related cognitive decline remain poorly understood, recent studies have proven that systemic interventions that extend a healthy lifespan may prove key in our ability to restore cellular and functional decline in the aging brain. Directions for future research may include dissecting the cellular and molecular mechanisms underlying age-related cognitive decline.

Conclusion

In the twentyfirst century, the finding that heterochronic serum/plasma studies and blood transfusions can mimic the effects of heterochronic parabiosis *in vivo* provides a promising approach to research in this area (Pan et al. 2017). Furthermore, recent advances have determined that certain circulating factors have “pro-aging” or “anti-aging” effects on cells and tissues, such as neurotrophins (including GDF11 and IGF-1), neuroinflammatory cytokines (including TNF- α , IL-1, and IL-4), and miRNA, as well as the Wnt and TGF- β signaling pathways (Loffredo et al. 2013; Katsimpardi et al. 2014; Zeng et al. 2011; Lichtenwalner et al. 2001; Smith et al. 2018). The application of a heterochronic parabiosis approach using genetic alterations in these pathways would allow for direct testing of these pathways and networks in regulating cell and tissue aging (Conboy et al. 2013). In addition, increasing evidence suggests that epigenetic dysregulation is associated with stem cell aging and aging-related disease (Beerman and Rossi 2015; De Jager et al. 2014). Therefore, characterization of epigenetic profiles including genome-wide DNA methylation patterns and chromatin-wide histone modification patterns of cells exposed to heterochronic influences may allow for a truly direct molecular definition of cellular age based on epigenetic states (Conboy et al. 2013). Taken together, these findings suggest that the systemic milieu contains pro-aging and rejuvenating factors that play a vital role in the health and aging phenotype. Although the underlying mechanisms are largely unknown, multiple blood-borne factors, including cytokines, chemokines, growth factors, and/or RNAs, may be involved in the process. Identifying these genes would elucidate the biological underpinnings as well as offer clues that make certain individuals resilient to withstand inevitable age-related deterioration.

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References

- Altman J (1962) Are new neurons formed in the brains of adult mammals? *Science*. 135:1127–1128
- Altman J, Das GD (1965) Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rat. *J Comp Neurol* 124:319–336
- Arnold KG (1981) Cerebral blood flow in geriatrics—a review. *Age Ageing* 10(1):5–9
- Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O (2002) Neuronal replacement from endogenous precursors in the adult brain after stroke. *Nat Med* 8(9):963–970
- Ashe KH (2001) Learning and memory in transgenic mice modeling Alzheimer's disease. *Learn Mem* 8(6):301–308
- Aurigemma GP (2006) Diastolic heart failure—a common and lethal condition by any name. *N Engl J Med* 355(3):308–310
- Baskett TF (2002) James Blundell: the first transfusion of human blood. *Resuscitation*. 52(3):229–233
- Beerman I, Rossi DJ (2015) Epigenetic control of stem cell potential during homeostasis, aging, and disease. *Cell Stem Cell* 16(6):613–625
- Berdasco M, Esteller M (2012) Hot topics in epigenetic mechanisms of aging: 2011. *Aging Cell* 11(2):181–186
- Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. *Nature*. 361(6407):31–39
- Brack AS, Conboy MJ, Roy S, Lee M, Kuo CJ, Keller C, Rando TA (2007) Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. *Science*. 317(5839):807–810
- Brun CE, Rudnicki MA (2015) GDF11 and the mythical fountain of youth. *Cell Metab* 22(1):54–56
- Bunster E, Meyer RK (1933) An improved method of parabiosis. *The Anatomical Record* 57:339–343
- Carlson BM, Faulkner JA (1989) Muscle transplantation between young and old rats: age of host determines recovery. *Am J Phys* 256(6 Pt 1):C1262–C1266
- Castellano JM, Mosher KI, Abbey RJ, McBride AA, James ML, Berdnik D, Shen JC, Zou B, Xie XS, Tingle M, Hinkson IV, Angst MS, Wyss-Coray T (2017) Human umbilical cord plasma proteins revitalize hippocampal function in aged mice. *Nature*. 544(7651):488–492
- Chang H, Lau AL, Matzuk MM (2001) Studying TGF-beta superfamily signaling by knockouts and knockins. *Mol Cell Endocrinol* 180(1–2):39–46
- Chiasson BJ, Tropepe V, Morshead CM, van der Kooy D (1999) Adult mammalian forebrain ependymal and subependymal cells demonstrate proliferative potential, but only subependymal cells have neural stem cell characteristics. *J Neurosci* 19(11):4462–4471

- Conboy IM, Rando TA (2012) Heterochronic parabiosis for the study of the effects of aging on stem cells and their niches. *Cell Cycle* 11(12):2260–2267
- Conboy IM, Conboy MJ, Smythe GM, Rando TA (2003) Notch-mediated restoration of regenerative potential to aged muscle. *Science*. 302(5650):1575–1577
- Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA (2005) Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature*. 433(7027):760–764
- Conboy MJ, Conboy IM, Rando TA (2013) Heterochronic parabiosis: historical perspective and methodological considerations for studies of aging and longevity. *Aging Cell* 12(3): 525–530
- Conboy IM, Conboy MJ, Rebo J (2015) Systemic problems: a perspective on stem cell aging and rejuvenation. *Aging (Albany NY)* 7(10):754–765
- De Jager PL, Srivastava G, Lunnon K, Burgess J, Schalkwyk LC, Yu L, Eaton ML, Keenan BT, Ernst J, McCabe C, Tang A, Raj T, Replogle J et al (2014) Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci. *Nat Neurosci* 17(9):1156–1163
- Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A (1999) Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell*. 97(6):703–716
- Duncan ID, Brower A, Kondo Y, Curlee JF, Schultz RD (2009) Extensive remyelination of the CNS leads to functional recovery. *Proc Natl Acad Sci U S A* 106(16):6832–6836
- Eckert SH, Gaca J, Kolesova N, Friedland K, Eckert GP, Muller WE (2018) Mitochondrial pharmacology of Dimebon (latrepirdine) calls for a new look at its possible therapeutic potential in Alzheimer's disease. *Aging Dis* 9(4):729–744
- Edgar JM, Nave KA (2009) The role of CNS glia in preserving axon function. *Curr Opin Neurobiol* 19(5):498–504
- Egerman MA, Cadena SM, Gilbert JA, Meyer A, Nelson HN, Swalley SE, Mallozzi C, Jacobi C, Jennings LL, Clay I, Laurent G, Ma S, Brachat S et al (2015) GDF11 increases with age and inhibits skeletal muscle regeneration. *Cell Metab* 22(1):164–174
- Eggel A, Wyss-Coray T (2014) A revival of parabiosis in biomedical research. *Swiss Med Wkly* 144:w13914
- Finerty JC (1952) Parabiosis in physiological studies. *Physiol Rev* 32(3):277–302
- Fraga MF, Esteller M (2007) Epigenetics and aging: the targets and the marks. *Trends Genet* 23(8):413–418
- Freitas-Rodriguez S, Rodriguez F, Folgueras AR (2016) GDF11 administration does not extend lifespan in a mouse model of premature aging. *Oncotarget*. 7(35):55951–55956
- Gamer LW, Cox KA, Small C, Rosen V (2001) Gdf11 is a negative regulator of chondrogenesis and myogenesis in the developing chick limb. *Dev Biol* 229(2):407–420
- Garthe A, Behr J, Kempermann G (2009) Adult-generated hippocampal neurons allow the flexible use of spatially precise learning strategies. *PLoS One* 4(5):e5464
- Gilson J, Blakemore WF (1993) Failure of remyelination in areas of demyelination produced in the spinal cord of old rats. *Neuropathol Appl Neurobiol* 19(2):173–181
- Hall CE, Hall O, Nevis AH (1959) Prolongation of survival by parabiosis in strain 129 dystrophic mice. *Am J Phys* 196(1): 110–112
- Harada CN, Natelson Love MC, Triebel KL (2013) Normal cognitive aging. *Clin Geriatr Med* 29(4):737–752
- Hinken AC, Powers JM, Luo G, Holt JA, Billin AN, Russell AJ (2016) Lack of evidence for GDF11 as a rejuvenator of aged skeletal muscle satellite cells. *Aging Cell* 15(3):582–584
- Hye A, Riddoch-Contreras J, Baird AL, Ashton NJ, Bazenet C, Leung R, Westman E, Simmons A, Dobson R, Sattlecker M, Lupton M, Lunnon K, Keohane A et al (2014) Plasma proteins predict conversion to dementia from prodromal disease. *Alzheimers Dement* 10(6):799–807 e792
- Jin K, Minami M, Lan JQ, Mao XO, Bateur S, Simon RP, Greenberg DA (2001) Neurogenesis in dentate subgranular zone and rostral subventricular zone after focal cerebral ischemia in the rat. *Proc Natl Acad Sci U S A* 98(8):4710–4715
- Jin K, Wang X, Xie L, Mao XO, Zhu W, Wang Y, Shen J, Mao Y, Banwait S, Greenberg DA (2006) Evidence for stroke-induced neurogenesis in the human brain. *PNAS*. 103(35): 13198–13202
- Johansson CB, Momma S, Clarke DL, Risling M, Lendahl U, Frisen J (1999) Identification of a neural stem cell in the adult mammalian central nervous system. *Cell*. 96(1):25–34
- Kamran P, Sereti KI, Zhao P, Ali SR, Weissman IL, Ardehali R (2013) Parabiosis in mice: a detailed protocol. *J Vis Exp* (80): 50556
- Katsimpardi L, Litterman NK, Schein PA, Miller CM, Loffredo FS, Wojtkiewicz GR, Chen JW, Lee RT, Wagers AJ, Rubin LL (2014) Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. *Science*. 344(6184): 630–634
- Kempermann G (2008) The neurogenic reserve hypothesis: what is adult hippocampal neurogenesis good for? *Trends Neurosci* 31(4):163–169
- Larson MG (1995) Assessment of cardiovascular risk factors in the elderly: the Framingham heart study. *Stat Med* 14(16): 1745–1756
- Learoyd P (2012) The history of blood transfusion prior to the 20th century—part 1. *Transfus Med* 22(5):308–314
- Lee YS, Lee SJ (2013) Regulation of GDF-11 and myostatin activity by GASP-1 and GASP-2. *Proc Natl Acad Sci U S A* 110(39):E3713–E3722
- Lichtenwalner RJ, Forbes ME, Bennett SA, Lynch CD, Sonntag WE, Riddle DR (2001) Intracerebroventricular infusion of insulin-like growth factor-I ameliorates the age-related decline in hippocampal neurogenesis. *Neuroscience*. 107(4): 603–613
- Liu D, Lun L, Huang Q, Ning Y, Zhang Y, Wang L, Yin Z, Zhang Y, Xia L, Yin Z, Fu B, Cai G, Sun X et al (2018) Youthful systemic milieu alleviates renal ischemia-reperfusion injury in elderly mice. *Kidney Int* 94(2):268–279
- Loffredo FS, Steinhauser ML, Jay SM, Gannon J, Pancoast JR, Yalamanchi P, Sinha M, Dall'Oso C, Khong D, Shadrach JL, Miller CM, Singer BS, Stewart A et al (2013) Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy. *Cell* 153(4):828–839
- Lourenco J, Serrano A, Santos-Silva A, Gomes M, Afonso C, Freitas P, Paul C, Costa E (2018) Cardiovascular risk factors are correlated with low cognitive function among older adults across Europe based on the SHARE database. *Aging Dis* 9(1):90–101

- Ludwig FC, Elashoff RM (1972) Mortality in syngeneic rat parabionts of different chronological age. *Trans N Y Acad Sci* 34(7):582–587
- McCay CM, Pope F, Lunsford W, Sperling G, Sambhavaphol P (1957) Parabiosis between old and young rats. *Gerontologia* 1(1):7–17
- McDermott KW, Lantos PL (1991) Distribution and fine structural analysis of undifferentiated cells in the primate subependymal layer. *J Anat* 178:45–63
- McPherron AC (2013) Through thick and thin: a circulating growth factor inhibits age-related cardiac hypertrophy. *Circ Res* 113(5):487–491
- Moss FP, Leblond CP (1970) Nature of dividing nuclei in skeletal muscle of growing rats. *J Cell Biol* 44(2):459–462
- Murphy T, Thuret S (2015) The systemic milieu as a mediator of dietary influence on stem cell function during ageing. *Ageing Res Rev* 19:53–64
- Myhre BA (1990) The first recorded blood transfusions: 1656 to 1668. *Transfusion* 30(4):358–362
- Nithianantharajah J, Hannan AJ (2009) The neurobiology of brain and cognitive reserve: mental and physical activity as modulators of brain disorders. *Prog Neurobiol* 89(4):369–382
- Oomen CA, Bekinschtein P, Kent BA, Saksida LM, Bussey TJ (2014) Adult hippocampal neurogenesis and its role in cognition. *Wiley Interdiscip Rev Cogn Sci* 5(5):573–587
- Pan M, Wang P, Zheng C, Zhang H, Lin S, Shao B, Zhuge Q, Jin K (2017) Aging systemic milieu impairs outcome after ischemic stroke in rats. *Ageing Dis* 8(5):519–530
- Pasipoularides A (1985) Historical perspective: Harvey's epoch-making discovery of the circulation, its historical antecedents, and some initial consequences on medical practice. *J Appl Physiol* 114(11):1493–503
- Pope F, Lunsford W, McCay CM (1956) Experimental prolongation of the life span. *J Chronic Dis* 4(2):153–158
- Pusic AD, Kraig RP (2014) Youth and environmental enrichment generate serum exosomes containing miR-219 that promote CNS myelination. *Glia* 62(2):284–299
- Ramirez-Lassepas M (1998) Stroke and the aging of the brain and the arteries. *Geriatrics* 53(Suppl 1):S44–S48
- Rebo J, Mehdi pour M, Gathwala R, Causey K, Liu Y, Conboy MJ, Conboy IM (2016) A single heterochronic blood exchange reveals rapid inhibition of multiple tissues by old blood. *Nat Commun* 7:13363
- Rivera A, Vanzuli I, Arellano JJ, Butt A (2016) Decreased regenerative capacity of oligodendrocyte progenitor cells (NG2-glia) in the ageing brain: a vicious cycle of synaptic dysfunction, myelin loss and neuronal disruption? *Curr Alzheimer Res* 13(4):413–418
- Ruckh JM, Zhao JW, Shadrach JL, van Wijngaarden P, Rao TN, Wagers AJ, Franklin RJ (2012) Rejuvenation of regeneration in the aging central nervous system. *Cell Stem Cell* 10(1):96–103
- Sim FJ, Zhao C, Penderis J, Franklin RJ (2002) The age-related decrease in CNS remyelination efficiency is attributable to an impairment of both oligodendrocyte progenitor recruitment and differentiation. *J Neurosci* 22(7):2451–2459
- Sinha M, Jang YC, Oh J, Khong D, Wu EY, Manohar R, Miller C, Regalado SG, Loffredo FS, Pancoast JR, Hirshman MF, Lebowitz J, Shadrach JL et al (2014) Restoring systemic GDF11 levels reverses age-related dysfunction in mouse skeletal muscle. *Science* 344(6184):649–652
- Smith SC, Zhang X, Zhang X, Gross P, Starosta T, Mohsin S, Franti M, Gupta P, Hayes D, Myzithras M, Kahn J, Tanner J, Weldon SM et al (2015) GDF11 does not rescue aging-related pathological hypertrophy. *Circ Res* 117(11):926–932
- Smith LK, White CW III, Villeda SA (2018) The systemic environment: at the interface of aging and adult neurogenesis. *Cell Tissue Res* 371(1):105–113
- Villeda SA, Wyss-Coray T (2013) The circulatory systemic environment as a modulator of neurogenesis and brain aging. *Autoimmun Rev* 12(6):674–677
- Villeda SA, Luo J, Mosher KI, Zou B, Britschgi M, Bieri G, Stan TM, Fainberg N, Ding Z, Eggel A, Lucin KM, Czirr E, Park JS et al (2011) The ageing systemic milieu negatively regulates neurogenesis and cognitive function. *Nature* 477(7362):90–94
- Villeda SA, Plambeck KE, Middeldorp J, Castellano JM, Mosher KI, Luo J, Smith LK, Bieri G, Lin K, Berdnik D, Wabl R, Udeochu J, Wheatley EG et al (2014) Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. *Nat Med* 20(6):659–663
- Waller C (1825) Case of uterine hemorrhage, in which the operation of transfusion was successfully performed. *Med Phys J* 54:273–277
- Wang JM, Singh C, Liu L, Irwin RW, Chen S, Chung EJ, Thompson RF, Brinton RD (2010) Allopregnanolone reverses neurogenic and cognitive deficits in mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A* 107(14):6498–6503
- Waskow C (2010) Generation of parabiotic mice for the study of DC and DC precursor circulation. *Methods Mol Biol* 595:413–428
- Weissman IL, Jerabek L, Greenspan S (1984) Tolerance and the H-Y antigen: requirement for male T cells, but not B cells, to induce tolerance in neonatal female mice. *Transplantation* 37(1):3–6
- Yoshimura S, Takagi Y, Harada J, Teramoto T, Thomas SS, Waerber C, Bakowska JC, Breakefield XO, Moskowitz MA (2001) FGF-2 regulation of neurogenesis in adult hippocampus after brain injury. *Proc Natl Acad Sci U S A* 98(10):5874–5879
- Zeng L, Liu J, Wang Y, Wang L, Weng S, Tang Y, Zheng C, Cheng Q, Chen S, Yang GY (2011) MicroRNA-210 as a novel blood biomarker in acute cerebral ischemia. *Front Biosci (Elite Ed)* 3:1265–1272

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