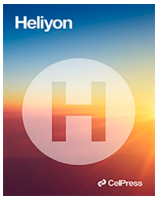
Heliyon 10 (2024) e32652

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Review article

Rejuvenation of young blood on aging organs: Effects, circulating factors, and mechanisms

Meng-Nan Liu [a,1,**], Qi Lan [a,1], Hao Wu [a,1], Cai-Wei Qiu [b,1,*]

[a] National Traditional Chinese Medicine Clinical Research Base, The Affiliated Traditional Chinese Medicine Hospital, Southwest Medical University, Luzhou, 646000, PR China

[b] Research Center of Combine Traditional Chinese and Western Medicine, Prophylaxis and Treatment of Organ Fibrosis by Integrated Medicine of Luzhou Key Laboratory, The Affiliated Traditional Chinese Medicine Hospital, Southwest Medical University, Luzhou, 646000, Sichuan, PR China

A R T I C L E I N F O

Keywords:
Aging
Heterochronic parabiosis
Young blood
Circulating factors
Rejuvenation

A B S T R A C T

Aging causes degenerative changes in organs, leading to a decline in physical function. Over the past two decades, researchers have made significant progress in understanding the rejuvenating effects of young blood on aging organs, benefiting from heterochronic parabiosis models that connect the blood circulation of aged and young rodents. It has been discovered that young blood can partially rejuvenate organs in old animals by regulating important aging-related signaling pathways. Clinical trials have also shown the effectiveness of young blood in treating aging-related diseases. However, the limited availability of young blood poses a challenge to implementing anti-aging therapies on a large scale for older individuals. As a promising alternative, scientists have identified some specific anti-aging circulating factors in young blood that have been shown to promote organ regeneration, reduce inflammation, and alleviate fibrosis associated with aging in animal experiments. While previous reviews have focused primarily on the effects and mechanisms of circulating factors on aging, it is important to acknowledge that studying the rejuvenating effects and mechanisms of young blood has been a significant source of inspiration in this field, and it will continue to be in the future. In recent years, new findings have emerged, further expanding our knowledge in this area. This review aims to summarize the rejuvenating effects and mechanisms of young blood and circulating factors, discussing their similarities and connections, addressing discrepancies in previous studies, outlining future research directions, and highlighting the potential for clinical translation in anti-aging interventions.

* Corresponding author. Research Center of Traditional Chinese and Western Medicine, Prophylaxis and Treatment of Organ Fibrosis by Integrated Medicine of Luzhou Key Laboratory, The Affiliated Traditional Chinese Medicine Hospital, Southwest Medical University, 182 Chunhui Road, Longmatan District, Luzhou, 646000, Sichuan, PR China.

** Corresponding author. National Traditional Chinese Medicine Clinical Research Base, Affiliated Traditional Chinese Medicine Hospital, Southwest Medical University, Luzhou, 646000, PR China.

E-mail addresses: liumengnan@swmu.edu.cn (M.-N. Liu), Lanky_3111@163.com (Q. Lan), 993175631@qq.com (H. Wu), qiucaiwei@swmu.edu.cn (C.-W. Qiu).

[1] All authors contributed equally to this article and considered as the first author.

https://doi.org/10.1016/j.heliyon.2024.e32652
Received 29 May 2024; Accepted 6 June 2024
Available online 15 June 2024

1. Introduction

Senescence is characterized as a relatively inactive, non-dividing, and irreversible cellular state [1]. The aging process entails a significant decrease in body functions and an increased vulnerability to diseases. Scientists have dedicated extensive efforts to researching various interventions aimed at mitigating the effects of aging. Some methods that have shown promising results in animal models include stem cell transplantation [2], exercise [3], and calorie restriction [4]. However, the translation of these approaches from animals to humans poses practical and unresolved challenges, demanding perseverance in the long run. For instance, in the case of stem cell transplantation, the issue of immune rejection needs to be addressed. Prolonged use of immunosuppressants can compromise the body's ability to resist infectious diseases, thus limiting the widespread application of stem cell transplantation. Slowing down the aging process often requires a considerable amount of exercise, which may inadvertently cause harm to elderly individuals who commonly experience skeletal and/or muscle decline. Moreover, many older adults struggle with digestive disorders or debilitating diseases that contribute to concerns regarding malnutrition. Calorie restriction is clearly unsuitable for individuals facing such challenges. Furthermore, certain anti-aging drugs that have shown success in basic research, such as nicotinamide riboside, have been found to have no proven anti-aging effects in clinical trials [5].

Considering the limitations of the aforementioned methods [2–4], an important solution is to investigate the disparities between the elderly and the young, and determine whether addressing these deficiencies could delay the aging process. This approach potentially offers wider applicability and safety. Over the past two decades, studies have demonstrated significant anti-aging effects of young blood in aged animals. In 1980, Butenko and Gubriĭ developed the heterochronic parabiosis model (HPB) by connecting the circulatory systems of young (Het-Y) and old (Het-O) mice by sewing paired mirror-image incisions on the skin, extending from the elbow joint to the knee joint [6]. The same-age pairs of young or old mice served as controls. In 2005, Conboy et al. used the HPB model and observed the rejuvenating effect of young blood on muscle and liver of aged mice [7]. Since then, researchers have found that young blood rejuvenates multiple aging organs and revealed their effects on a few key anti-aging signaling pathways. Furthermore, a recent clinical study confirmed the beneficial effects of human umbilical cord blood plasma (hUCBP) therapy on various aging indicators in elderly individuals [8]. These findings offer promise for the treatment of age-related conditions.

However, as a result of ethical concerns and the limited availability of blood, there has been increasing attention on the use of anti-aging circulating factors found in young blood. Studies conducted on naturally aging rodents have already demonstrated that injecting multiple circulating factors can have a rejuvenating effect on various aging organs. These interventions of young blood and circulating factors have been shown to exert their effects through known anti-aging signaling pathways, such as promoting proliferation, combating oxidative stress, and reducing inflammation. This review aims to examine the similarities and connections between these mechanisms and effects, address discrepancies in previous studies, propose future research directions, and emphasize the potential for clinical implementation of anti-aging interventions.

2. The biological characteristics of aging influenced by young blood

A recent report has compiled a summary of 12 aging markers [9]. Among these markers, there are some that have shown potential for reversal in older animals through the infusion of young blood.

2.1. Stem cell aging and cellular senescence

Telomere shortening is a crucial internal factor contributing to cellular aging. Telomeres, which consist of repeated DNA sequences TTAGGG, are situated at the ends of chromosomes. Their primary function is to prevent the loss or fusion of chromosomes during cell division. However, telomeres gradually shorten with each round of cell division. At a certain point known as the Hayflick limit, telomeres become too short to bind an adequate amount of telomere cap proteins. As a result, the exposed DNA ends trigger the DNA damage response (DDR) that activates the protein p53, leading to the expression of p21 and p16. Ultimately, this causes the cell cycle to be blocked, inhibiting proliferation [10].

When aging occurs, the number and function of tissue stem cells, such as hematopoietic stem cells (HSC), muscle stem cells (MuSC), and neural stem cells (NSC), are diminished [11], resulting in the decreased proliferation ability and the age-related tissue repair dysfunction [12]. In addition, the aging of somatic cells also occurs with increasing age [11]. Increased activity of aging-related β-galactosidase (SA-β-Gal) and the enhanced expression of p21 and p16 are common markers of senescent cells.

2.2. DNA methylation

DNA methylation is an important form of epigenetic modification, referring to the process in which a methyl group binds to the cytosine base to form 5-methylcytosine (5 mC) by DNA methyltransferase. The 5 mC is demethylated through ten-eleven translocation proteins (Tet) that converse the methyl group of 5 mC to a hydroxymethyl group (CH_2OH). DNA methylation typically occurs at CpG sites (cytosine followed by guanine), forming methylated CpG islands, which are often located in the promoter regions of genes. This modification can influence gene expression, thereby regulating cellular function and developmental processes. To detect DNA methylation sites, the reduced-representation bisulfite sequencing (RRBS) method can be employed. By measuring changes at several hundred specific CpG sites, the RRBS-based epigenetic clock can accurately estimate the chronological age of various species, including humans [13].

2.3. Chronic inflammation

Markers of aging, such as epigenetic dysregulation, protein instability, impaired autophagy function, and the accumulation of senescent cells, can all contribute to chronic inflammation [9]. The hallmark of age-related inflammation is the ability to sustain a low-grade, chronic inflammatory background even in the absence of infection or clinical disease [14]. Inflammation poses a risk factor for the decline in tissue repair and regenerative abilities, which is linked to numerous age-related diseases [14].

2.4. Mitochondrion aging and oxidative stress

Mitochondrial aging is strongly associated with the aging process of cells. It is characterized by a decrease in cellular respiratory capacity and a decline in the stable state of mitochondrial membrane potential. These changes are often attributed to an increased production of reactive oxygen species (ROS) [15]. The presence of ROS worsens telomere damage, leading to DNA damage reactions and the onset of cellular senescence [16]. There is currently a belief that restoring the impaired function of aging mitochondria can help alleviate cell senescence [17].

3. Rejuvenating effects of young blood on aging organs

Young blood has demonstrated its potential in mitigating aging-related symptoms, promoting cell growth and repair, and restraining age-associated chronic inflammation and fibrosis in older individuals (Fig. 1). Additionally, single-cell sequencing analysis

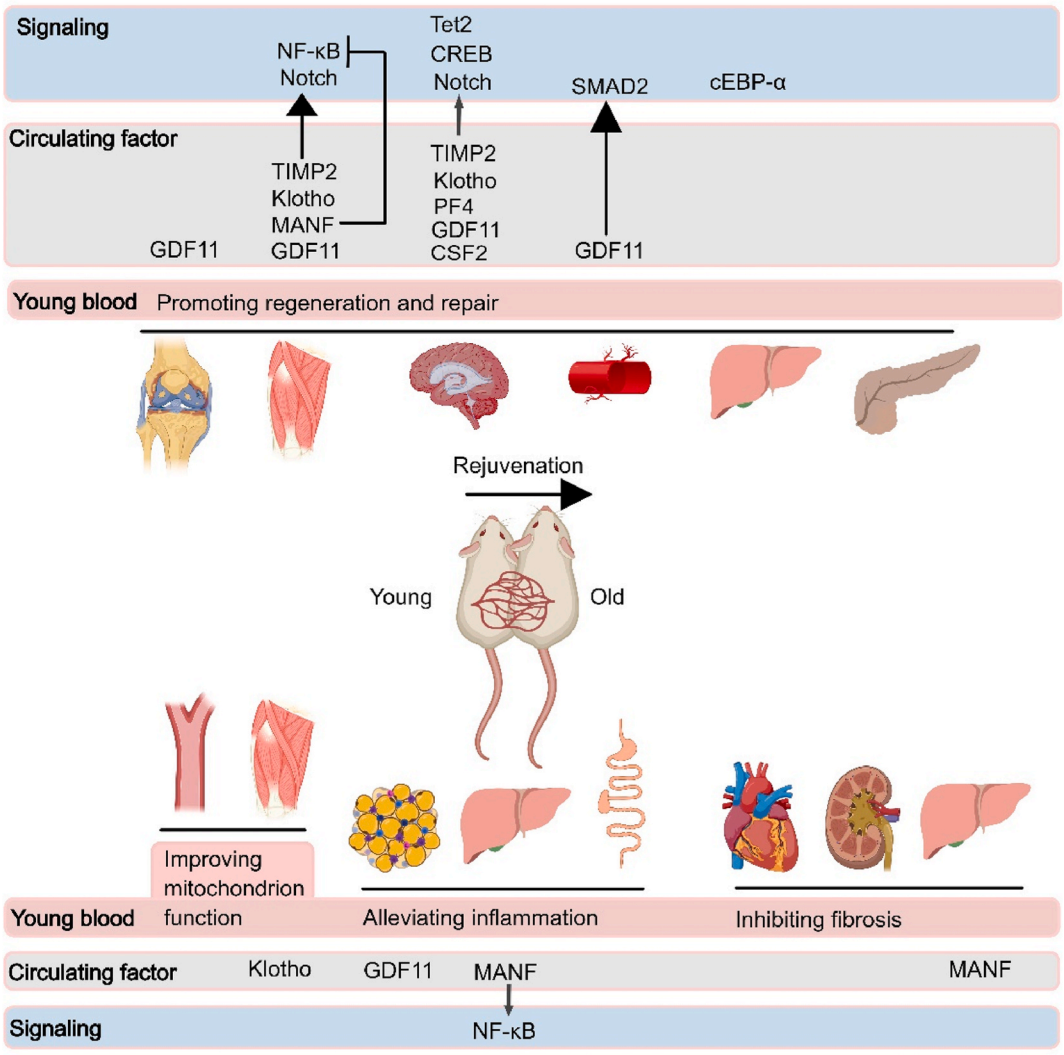


Fig. 1. The rejuvenation effects and molecular mechanism of young blood and circulating factors.

reveals that young blood effectively reverses age-related gene expressions in a diverse range of cell types (51 in total) derived from the 20 main organs and tissues of elderly animals [18].

3.1. Improving neurogenesis in aging

Cognitive abilities, including learning and memory, gradually decline with age. In adult humans, the subgranular zone (SGZ) located in the dentate gyrus of the hippocampus retains the capacity for neurogenesis. However, as individuals age, there is a significant decrease in the number of neural stem cells (NSCs) in the SGZ, leading to a subsequent decline in neurogenesis [19] and cognitive impairment [20]. It is worth noting that learning and memory are closely tied to synaptic plasticity, which refers to the ability to regulate the strength of synaptic connections between neurons. As synaptic plasticity decreases with age, memory decline becomes evident in aging animals [21]. Additionally, older adults often experience myelin degeneration, which is crucial for protecting nerve fibers and facilitating nerve conduction. Studies on young rodents have demonstrated the critical role of myelin in consolidating newly formed memories [22]. HPB improves remyelination in experimentally induced demyelination within the aged brain [23], induces structural changes in the brain vessels, improves blood flow, and activates NSC [24]. Furthermore, the injection of young blood improves synaptic plasticity in the hippocampus and ameliorates age-related cognitive decline in older animals [25].

The overexpression of Tet2 in the DG through lentivirus administration led to a significant increase in proliferation and differentiation in NSCs. In young adult mice lacking the NSC Tet2 gene ($Tet2^{-/-}$), their ability to learn and memorize was compromised. This was evaluated by measuring their performance in both the radial arm water maze and contextual fear conditioning paradigms [26]. In the hippocampus of mice, Tet2 and 5hmC levels decrease with age by qPCR or Slot blot measurement [26]. HPB significantly increases Tet2 expression in the hippocampus and improves cognitive function in aged mice [26].

CREB, the cyclic AMP response element binding protein, is a transcription factor responsible for regulating the expression of various proteins, including brain-derived neurotrophic factor (BDNF) [27]. BDNF, in turn, is responsible for modulating synaptic plasticity [28]. Villeda et al. conducted a study where they observed a decrease in phosphorylated CREB levels with aging. However, they found that injecting young blood increased phosphorylated CREB, dendritic spine density, and enhanced long-term potentiation (LTP) in older mice [25].

3.2. Enhancing regeneration of aging MuSCs

As individuals age, skeletal muscle undergoes a gradual decline in both mass and function. Muscle satellite cells (MuSC), which are normally in a resting state, can re-enter the cell cycle and differentiate into skeletal muscle cells when prompted by physiological signals, such as injury [29]. The number and functionality of MuSCs decrease with age, resulting in impaired repair [30]. HPB has been shown to restore MuSC regeneration in muscle injuries caused by dry ice [7] and cardiotoxin [31]. Aged MuSC progeny exhibit mitochondrial dysfunction; however, when cultured with young serum, they demonstrate a significantly higher basal oxygen consumption rate and a 1.5-fold increase in the reduced form of phospholipids in the mitochondrial inner membrane [32]. Moreover, the expression of the Notch ligand Delta is impaired in aging MuSCs and it is reversed by young mouse serum [7].

3.3. Enhancing the regenerative capacity of aging HSCs

Hematopoietic stem cells (HSCs) play a crucial role in the regeneration of blood cells. However, in aged HSCs, the absence of autophagy leads to the accumulation of aged mitochondria and an increase in production of reactive oxygen species (ROS). These changes have negative effects on the self-renewal and regeneration abilities of HSCs [33].

A study conducted by Ho et al. demonstrated that young blood is unable to rejuvenate aging HSCs. When HSCs from Het-O were transplanted into recipients exposed to lethal doses of radiation, no differences were observed in blood engraftment and lineage distribution when compared with the transplanted HSCs from Iso-O parabionts [34].

However, single-cell RNA sequencing reveals that genes associated with cytokine production, hematopoiesis, chromatin organization, circadian rhythm, and cell cycle are negatively regulated in HSCs in old mice, The negative changes were reversed with the use of HPB [35]. Because single-cell sequencing can detect changes in gene expression at the single-cell level, it is plausible that young blood rejuvenates a subset of HSCs.

3.4. Enhancing bone regeneration in the aging

Bone marrow stromal cells (BMSCs) are a type of immature cells in the bone marrow and have the ability to differentiate into osteoclasts or osteoblasts. Osteoclasts are responsible for absorbing old and worn bone tissue, while osteoblasts aid in the synthesis and mineralization of the bone matrix. Due to the fact that bone resorption tends to increase and bone synthesis declines with age, there is considerable interest in finding interventions that can simultaneously reduce bone resorption and promote bone synthesis [36,37]. In old mice, it has been observed that HPB improves bone repair [37], enhances chondrocyte proliferation and cartilage matrix production [38], and increases extracellular matrix (ECM) in the discs [39].

3.5. Reducing cardiac hypertrophy in aging

In the aging heart, there is a gradual decrease in the number of cardiomyocytes accompanied by an increase in cell volume. Heart

failure in elderly individuals is primarily linked to diastolic dysfunction caused by myocardial hypertrophy [40]. HPB has been found to reverse age-related cardiac hypertrophy [41].

3.6. Promoting liver regeneration in the context of aging

The aging rate of the liver is relatively slower compared to other organs. However, in elderly individuals, the telomeres in the liver significantly shorten [42]. The DNA methylation in the human liver undergoes significant remodeling with age, but it generally stabilizes after the age of 60 [43]. The duration and success rate of liver transplantations from elderly donors are comparable to those of younger donors [43]. In comparison to young animals, the liver in the elderly shows increased chronic inflammation and fibrosis. HPB and young blood injection promote liver cell proliferation in the aging liver [7] or in the aging liver subjected to 70 % hepatectomy [44,45].

CCAAT/enhancer binding protein alpha (C/EBP-alpha) is a highly expressed transcription repressor in the liver. In the aging liver, the accumulation of the complex C/EBP-alpha-Brahma-HDAC1 leads to the repression of E2F-dependent promoters and subsequently inhibits liver cell proliferation [46]. The HPB model demonstrates that young blood has the capability to enhance the proliferation of aging liver cells by suppressing the formation of the C/EBP-alpha complex [7].

Zhang and colleagues conducted a 3-month HPB, followed by a 2-month detachment period, in pairs of mice. The use of RRBS-based epigenetic clocks allowed for the accurate prediction of the liver's actual age in old control mice. Furthermore, it was found that the biological age of the liver in mice with Het-O was significantly reduced compared to mice with Iso-O during HPB, with a reduction of 22.3 $\pm$ 4.2 %. Even after detachment, there was still a 16.1 $\pm$ 3.4 % reduction in biological age [47]. Studies using single-cell genomics have revealed notable alterations in gene expression in liver cells during the aging process. These changes can be effectively reversed when exposed to young blood [18]. Another study using single-cell genomics found that young blood has the ability to counteract inflammation and impaired fatty acid metabolism in the liver of older individuals [48].

3.7. Alleviating renal fibrosis: a potential therapeutic approach for renal disease

Although aging does not decrease the number of kidney units or overall renal function, it is observed that aging does lead to the development of SA-β-Gal $^{+}$ tubules and renal interstitial fibrosis. However, it has been discovered that these changes can be effectively reversed through the use of HPB [49].

3.8. Improvement in other aging organs

HPB has demonstrated its potential in preventing the age-related decline in pancreatic β-cell replication [50]. Furthermore, elderly rats that received young blood plasma exhibited a decrease in lymphocyte infiltration in the intestine [51]. In the case of adipose tissue, HPB has shown to alleviate senescence and inflammation in aging mice [52]. The systemic delivery of hUCBP to mice has the ability to alleviate neuroinflammation associated with senescence and improve olfactory function by modulating the peripheral Treg cells [53]. IIn a single-cell sequencing study, it was observed that young blood effectively restores the reduced gene expression of various electron

Table 1
Rejuvenation effects and mechanism of - circulating factors.

Trial	Factors	Treatment	Organ affected	mechanism	Effects
(Castellano et al., 2017)	rmTIMP2	50 μg/kg, i.p., every other day, for 8 days	brain, dentate gyrus	activates Notch-p53	improves learning and memory
(Castellano et al., 2017)	rGM-CSF	50 μg/kg, i.p., every other day, for seven times	brain, dentate gyrus	activates MAPK-CREB-BDNF	improves learning and memory
(Hou et al., 2023)	rhMANF	0, 15, 30, 60 μg/mouse, i.v., twice a week, for 2 weeks	CCl_4-injured liver	inhibits TLR4–NF-κB	alleviates liver inflammation and fibrosis
(Sousa et al., 2023)	rhMANF	2, 4 μg/mouse, i.m., daily, for 4 days	$BaCl_2$-injured muscle	inhibits TLR4–NF-κB	promotes muscle regeneration
(Sahu et al., 2021)	EVs	5×10^8–7.5×10^8 EVs, i.m.	CTX-injured muscle	Klotho mRNA	promotes muscle regeneration
(Chu et al., 2022)	EVs	50 μl; 2 mg/Kg body weight, i.v., once	liver after HS	?	enhanced antioxidation
(Park et al., 2023)	rmPF4	20 μg/kg, i.p., daily	brain, hippocampus	activates platelet to release PF4	enhances cognition
(Loffredo et al., 2013)	rGDF11	0.1 mg/kg, i.p., Daily, for 30 days	cardiac hypertrophy	?	reverses cardiac hypertrophy in female mice
(Sinha et al., 2014)	rGDF11	0.1 mg/kg, i.p., Daily, for 30 days	dry ice-injured muscle	?	promotes muscle regeneration
(Katsimpardi et al., 2014)	rGDF11	0.1 mg/kg, i.p., Daily, for 4 weeks	brain	?	improves memory
(Li et al., 2020)	rGDF11	1.1 mg/kg, i.p., daily, for 16 weeks	knee joint	?	promotes chondrocyte growth

transport chain subunits (e.g., Cox6c, Cox7c, Ndufa1, Ndufa3, Atp5k, and Uqcr11) in different cell types [18]. Additionally, in aged mice, there is a significant decrease in mitochondrial number and increase in oxidative stress in blood vessels, but these effects can be reversed through the use of HPB [54].

4. Rejuvenating effects and mechanism of circulating factors

The anti-aging effects of young blood have been shown in animal experiments, encompassing various aging organs and senescence-related signaling pathways (Fig. 1). Due to the limited availability and ethical concerns surrounding young blood, scientists are now investigating the anti-aging effects of circulating factors. This review focuses on the circulating factors (Table 1) that decline with age, omitting those that increase with age.

4.1. The enhancement of synaptic plasticity and muscle regeneration through the activation of the Notch/p53 pathway by TIMP-2

TIMP-2, a tissue inhibitor of MMPs, is highly abundant in UCBP and diminishes with age. In aged mice, the administration of TIMP-

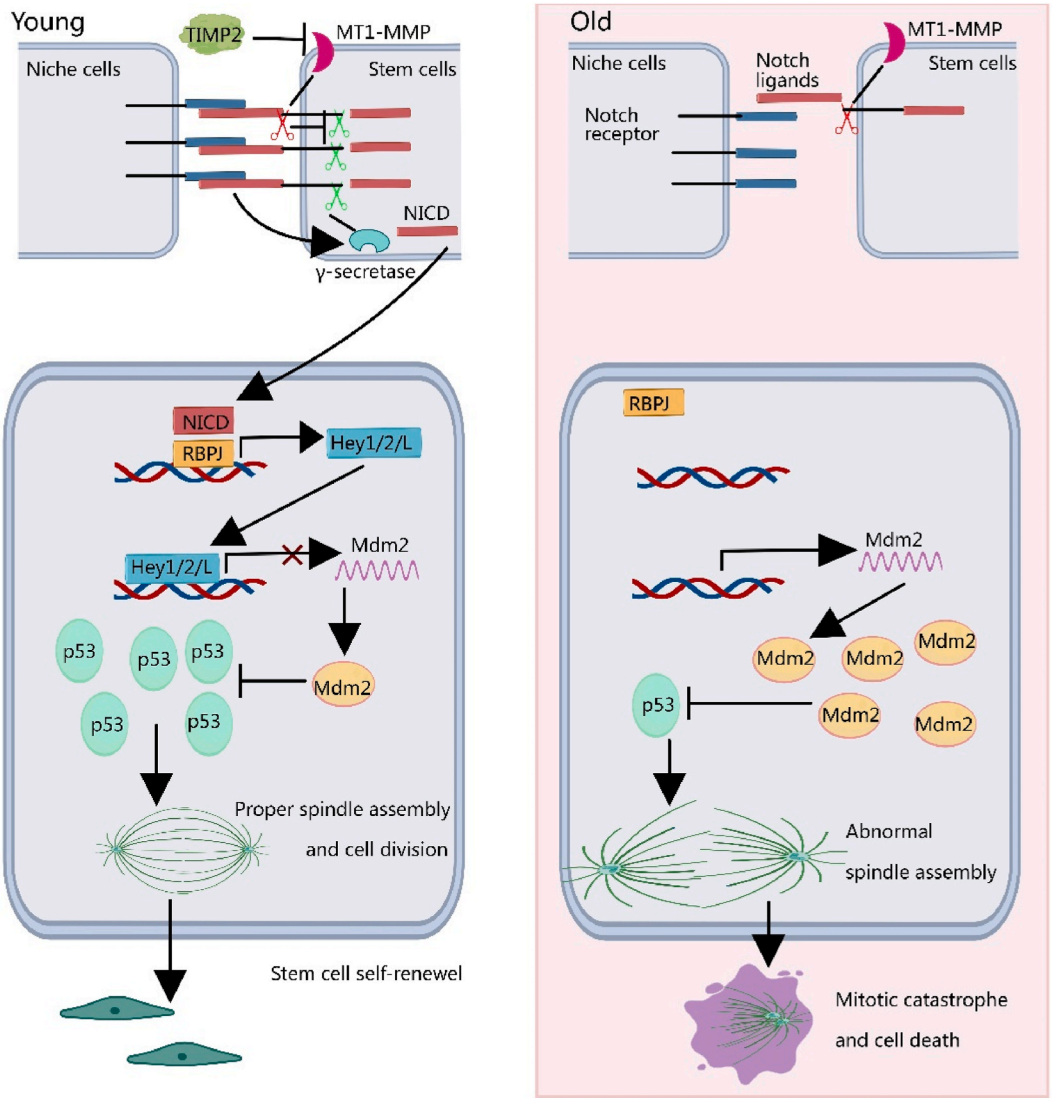


Fig. 2. TIMP2 protects Notch/p53 signaling and protects cell proliferation. TIMP2 inhibits MT1-MMP which cuts the extracellular part of Dll1 and inhibits Notch ligand-receptor binding. Notch ligand-receptor binding activates γ-secretase to cut the intracellular part of Dll1 to form DICD. DICD enters the nucleus and up-regulates Hey1 expression. Hey1 binds to the Mdm2 promoter and inhibits Mdm2 expression. Mdm2 binds to p53 and promotes p53 degradation. Thus, TIMP2 protects Notch signaling and p53 ensuring proper spindle formation and cell division (Referring to Liu et al., 2018, PMID: 30244867).

2 promotes muscle regeneration [55]. Delta-like protein 1 (Dll1) functions as a Notch ligand. When Dll1 binds to Notch, the intracellular portion of the ligand is cleaved by γ-secretase, resulting in the formation of the Delta intracellular domain (DICD). DICD translocates to the nucleus and up-regulates the expression of Hey1, which inhibits Mdm2 expression and prevents degradation of p53 [55]. MT1-MMP is a membrane type 1 matrix metalloproteinase that breaks down extracellular matrix (ECM) components. MT1-MMP binds to and cleaves the extracellular domain of Notch ligand Dll1, thereby blocking Notch ligand/receptor binding and Notch/p53 signaling [56]. In aged mice, the decrease in TIMP-2 fails to inhibit MT1-MMP, subsequently suppressing Notch signaling and enhancing p53 degradation (Fig. 2). As p53 is crucial for proper spindle formation during cell division, the administration of TIMP-2 restores muscle regeneration in old mice [52].

Administration of hUCBP and TIMP-2 leads to a significant improvement in synaptic plasticity, as evidenced by enhanced long-term potentiation (LTP) in the aging hippocampus. Additionally, these treatments promote learning, as demonstrated by improved fear-conditioning freezing response in elderly mice [57]. These findings suggested that TIMP2 activates Notch/p53 signaling to enhance synaptic plasticity in the elderly, as p53 has been shown to enhance hippocampal synaptic plasticity and cognition [58] (Supplemental Fig. 1).

Unlike the notable upregulation of Notch ligand expression resulting from young blood infusion [7], TIMP2 does not induce an increase in Notch ligand expression. Instead, it functions by inhibiting the cleavage of Notch ligands mediated by MT1-MMP in the extracellular domain.

4.2. GM-CSF enhances cognitive recognition in aging mice via activation of MAPK/ERK/CREB/BDNF signaling

CSF2, also referred to as granulocyte-macrophage colony-stimulating factors (GM-CSF), is synthesized by various cell types, such as macrophages, T cells, and endothelial cells. GM-CSF is employed in clinical settings for the recovery of diminished white blood cells [59]. Moreover, the expression of CSF2 declines with age in human plasma. Castellano et al. conducted a study demonstrating that the administration of GM-CSF through intraperitoneal injection enhances learning and memory in aging mice [57]; however, they did not investigate the underlying mechanism behind this improvement.

CREB, a transcription factor, regulates the expression of Egr-1 and BDNF. The activation of MAPK triggers CREB kinase pp90RSK, resulting in the phosphorylation and activation of CREB [60]. In the study, it was observed that GM-CSF stimulates the MAPK/ERK signaling pathway (as illustrated in Fig. 3) [61]. After 20 min of GM-CSF stimulation in TF-1 cells, increased levels of phosphorylation were detected in cell extracts for both ERK and CREB. However, when the MAPK inhibitor PD98059 was used, these increased levels of

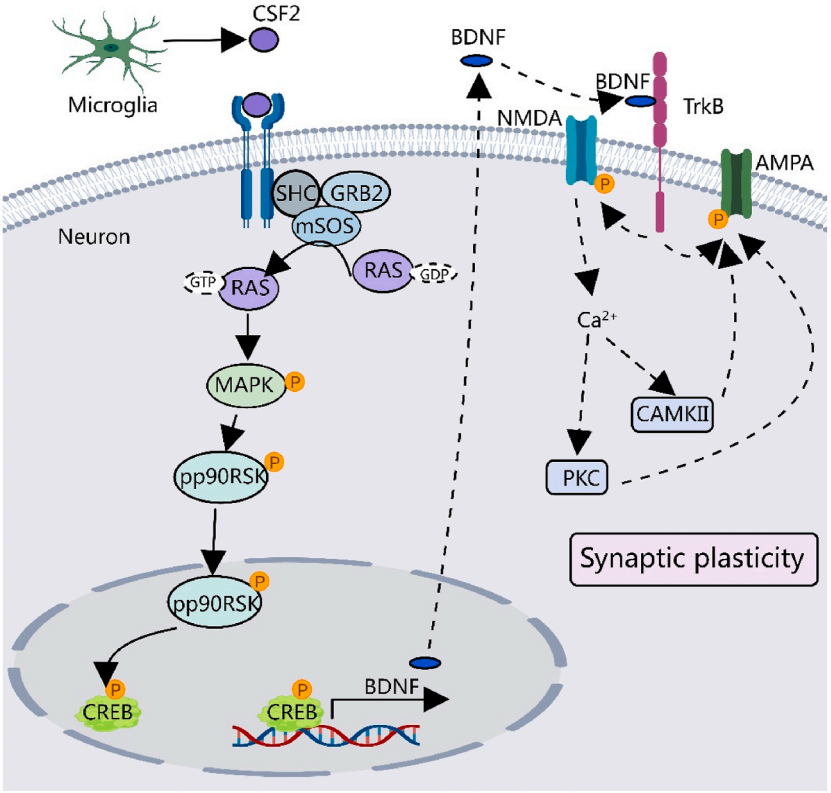


Fig. 3. GM-CSF activates MAPK/pp90RSK/CREB/BDNF signaling to enhance synaptic plasticity (Referring to Laar et al., 2012, PMID: 22323450; and Amidfar et al., 2020, PMID: 32603820).

phosphorylation were eliminated [60]. Based on this review, it is speculated that in old mice, GM-CSF may regulate the MAPK/ERK/CREB/BDNF signaling pathway (as indicated in Fig. 3). This speculation is supported by previous studies demonstrating that CREB improves synaptic plasticity and cognition in old mice [25], and that BDNF can enhance synaptic plasticity and cognition [62].

4.3. MANF mitigates inflammatory response in the senescent hepatic tissue while facilitating skeletal muscle repair through suppression of S100a8/A9-TLR4-NF-κB

Mesencephalic astrocyte-derived neurotrophic factor (MANF) is a cytokine secreted by both neurons and non-neuronal cells, playing a significant role in promoting the survival and proper functioning of neurons [63]. It has been observed that the levels of MANF decrease in the blood of both aged humans and mice [64]. Moreover, in old mice, the administration of HBP has been found to reduce the number of $CD68^{+}$ macrophages and the levels of the inflammatory factor IL6. Importantly, these beneficial effects are dependent on the presence of MANF. Furthermore, intraperitoneal injection of recombinant human MANF (rMANF) in aged mice has been shown to exert similar effects to HBP in the liver [64].

Using the Co-Immunoprecipitation (Co-IP) method, Hou et al. discovered that MANF has the ability to bind to S100A8, consequently preventing the formation of the S100A8/A9 heterodimer. Additionally, MANF hinders the interaction between S100A8/A9 and Toll-like receptor 4 (TLR4), thereby inhibiting the TLR4/nuclear factor-κB signaling pathway in monocytes. Consequently, MANF promotes the differentiation of monocytes into $F4/80^{+}Ly6C^{low}$ pro-repair macrophages, while simultaneously preventing the formation of $Ly6C^{high}$ inflammatory macrophages. This mechanism plays a critical role in reducing CCl_4-induced liver inflammation and fibrosis (Fig. 4) [65].

By utilizing the same mechanism, MANF improves muscle regeneration and repair after $BaCl_2$ injury by increasing the presence of $F4/80^{+}Ly6C^{low}$ pro-repair macrophages, which efficiently remove localized debris (Fig. 4) [66].

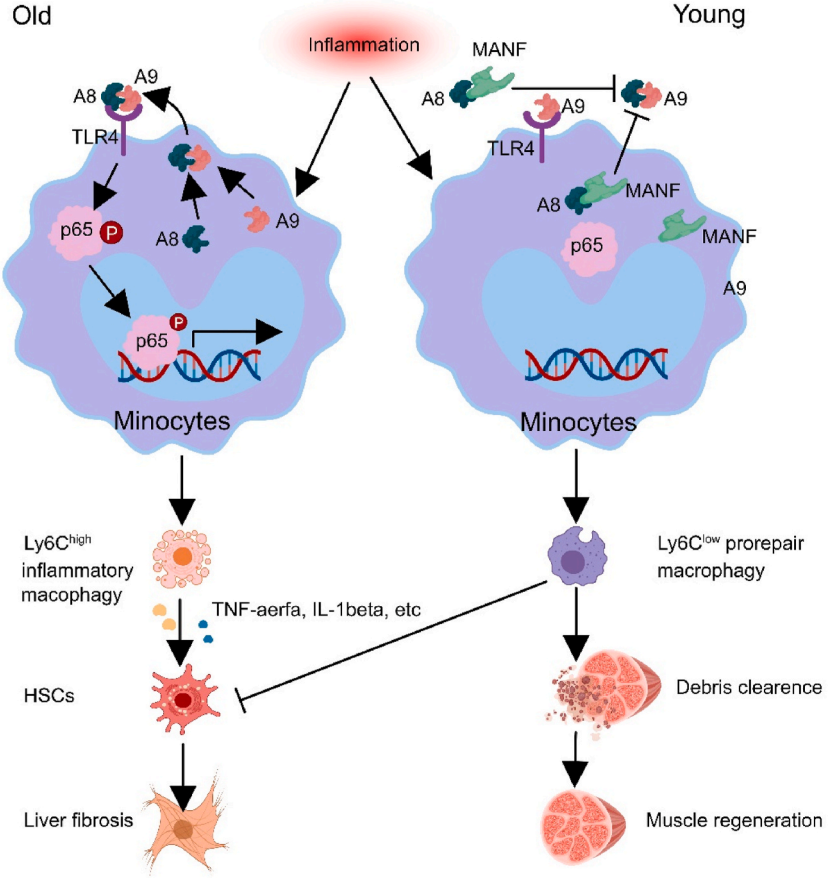


Fig. 4. MANF inhibits TLR4/NF-κB signaling to promote muscle regeneration and alleviate liver fibrosis. MANF binds to S100A8, and prevents the formation of the S100A8/A9 heterodimer, which inhibits TLR4/NF-κB signaling. Thus, MANF induces monocytes to differentiate into $F4/80^{+}Ly6C^{low}$ prorepair macrophages but not $Ly6C^{high}$ inflammatory macrophages. MANF alleviates liver fibrosis and promotes muscle regeneration in old mice (Referring to Hou et al., 2023, PMID: 37799387).

4.4. Klotho enhances neurogenesis and muscle regeneration in the aging process

Klotho is a transmembrane protein implicated in the aging process. It is found in two forms: membrane-bound and soluble. Studies have shown that mice overexpressing Klotho exhibit a 30 % increase in lifespan [67].

4.4.1. Improving aging synaptic regeneration through platelet-stimulated release of PF4

Klotho has been shown to enhance cognitive function in elderly mice [68,69] and aged non-human primates, specifically Rhesus macaques [70]. However, due to its inability to cross the blood-brain barrier (BBB) [71], it is suggested that klotho may impact the brain through an intermediary mechanism.

PF4, a protein released by activated platelets, plays a crucial role in promoting the formation of blood clots [72]. Notably, there is a significant decline in PF4 levels in plasma among older mice compared to their younger counterparts [73]. Recent research by Park et al. has shown that Klotho induces platelets to release platelet factor 4 (PF4) in old mice. Additionally, it has been observed that the administration of His-tagged PF4 via peripheral means can effectively cross the blood-brain barrier and enter the brain [71]. Importantly, inhibiting platelet activity through the administration of aspirin and clopidogrel impedes the cognitive improvement mediated by Klotho [71].

Further research was conducted to administer PF4 at a concentration equivalent to post-exercise levels in the blood. The findings revealed that this treatment significantly increased the number of $Ki67^{+}$/$doublecortin^{+}$ neurons in the subgranular zone (SGZ) of the dentate gyrus in old mice, leading to an improvement in cognition. This improvement was demonstrated through performance in new object location and contextual fear conditioning tasks [73]. Furthermore, the systemic administration of PF4 effectively reduced neuroinflammation in aged mice. This reduction was observed through a decrease in the expression of pro-inflammatory genes such as Tnf, Nfkb1, and Il1b in the hippocampus, as well as a decrease in the activation of CD68-positive microglia [21].

4.4.2. The beneficial effects of klotho in extracellular vesicles (EVs) on muscle regeneration in aging

Extracellular vesicles (EVs) play a vital role in intercellular communication [74] and decrease in abundance as individuals age [32]. The administration of EVs derived from young blood has shown to enhance aged muscle regeneration by improving mitochondrial respiration in aged MsSCs. However, it should be noted that if Klotho mRNA is depleted from the EVs of young individuals, these beneficial effects will be nullified [32].

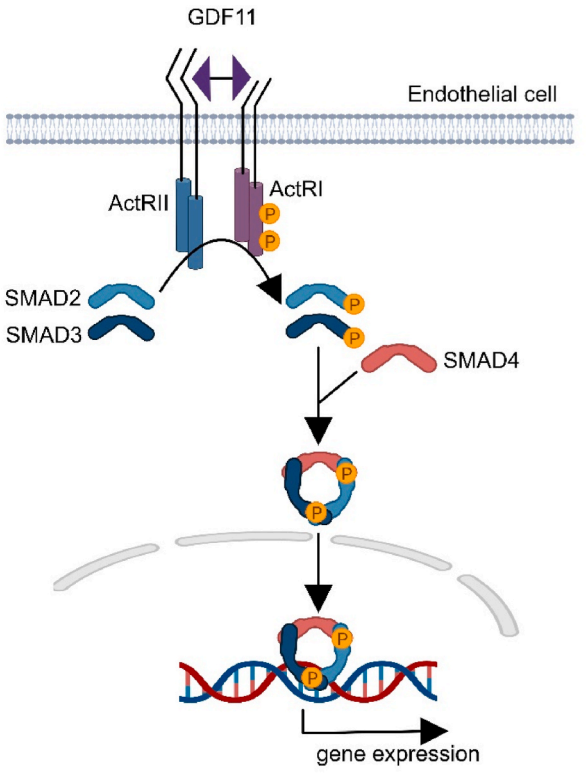


Fig. 5. GDF11 activates TGF-β signaling (Referring to Sheen et al., 2013, PMID: 24244818).

4.5. Growth differentiation factor 11 (GDF11): a comprehensive analysis of its role and significance

4.5.1. Decline in GDF11 expression with age in animal models

GDF11, a protein released into surrounding fluids, has been the subject of various reports. Western blot analysis of mice blood consistently reveals two bands of GDF11, showing an increase of 25 kDa and a decrease of 12.5 kDa with age. There are differing opinions among researchers regarding the identity of these bands. Some suggest that the 25 kDa band corresponds to GDF11 [75,76] while others argue that the 12.5 kDa band represents GDF11 [41]. Poggioli et al., on the other hand, propose that the 25 kDa band is not GDF11, but rather an immunoglobulin light chain. They have found that this immunoglobulin light chain weighs approximately 25 kDa and can also be detected by the Abcam anti-GDF11 antibody [77]. The expression of immunoglobulins in mice has been observed to increase with age [78]. When using blood from mice deficient in IgG, the anti-GDF11 antibody fails to detect the 25 kDa band. However, in both IgG-deficient and wild-type mice, the same antibody reveals a decrease in expression within the 12.5 kDa range as age progresses [77].

4.5.2. Rejuvenating the aging nervous system and chondrocytes

Administration of recombinant GDF11 (rGDF11) increases the levels of GDF11 in older mice to match those found in younger mice. This treatment has been shown to enhance SOX^{2+} neural stem cells (NSCs) in the DG and improve memory significantly [24]. Through the examination of cfos + active neurons, it has been revealed that GDF11 effectively enhances neuronal activity [79]. Furthermore, administering rGDF11 to older mice results in reduced body weight and induces hormonal changes similar to those observed in calorie restriction [80]. Additionally, GDF11 administration stimulates the growth of chondrocytes and promotes the synthesis of cartilage matrix in older mice [38].

4.5.3. Activation of TGF-β signaling to enhance proliferation of endothelial cells

GDF11, a member of the transforming growth factor-beta (TGF-β) superfamily, has been found to play a role in increasing phospho-SMAD2/3 levels (Fig. 5) and inducing the proliferation of primary brain capillary endothelial cells. This effect can be blocked by a TGF-β inhibitor, as demonstrated in an *intra*-study [24].

4.5.4. Reversal of age-related cardiac hypertrophy in females, but not males

In 2013, Loffredo et al. conducted a study in which they discovered that the administration of rGDF11 (0.1 mg/kg) to 23-month-old female mice for a duration of 30 days was able to restore GDF11 levels to that of young mice. This treatment successfully reversed age-related cardiac hypertrophy as measured by the ratio of heart weight to tibia length (HW/TL). Furthermore, it resulted in smaller cardiomyocytes [41]. However, Loffredo et al. also noted that GDF11 did not improve age-related cardiac hypertrophy in male old mice [41]. Similar findings were observed by Smith et al. in their study on aged male mice [75].

4.5.5. Enhancing muscle regeneration in dry ice-induced injury to mitigate age-related impairments

The development of larger regenerating myofibers is a crucial aspect of muscle repair in young mice following dry ice injury. Sinha discovered that the administration of GDF11 has no impact on the size of myofibers in uninjured young or aged muscles. On the other hand, the administration of rGDF11 in aged mice significantly enhances the growth of larger regenerating myofibers after dry ice injury [31]. However, Egerman et al. observed that the administration of rGDF11 to old mice leads to a higher prevalence of smaller fibers in regenerating muscles and no increase in the number of Pax7-positive MuSCs in the cardiotoxin (CTX) injury model [76].

In the experiments conducted by Sinha [31] and Egerman [76], he research subjects and treatment methods were identical, with the only difference being the method of injury induction. Sinha utilized dry ice, while Egerman employed CTX. It was observed that CTX led to the expression of TGF-β [81] and activated the TGF-β signaling pathway. This activation of TGF-β at low levels is essential for facilitating muscle regeneration. However, the simultaneous administration of CTX and GDF11 may result in excessive TGF-β activation, which can cause abnormal deposition of the extracellular matrix. Consequently, this can negatively impact the proliferation and differentiation of MuSCs [82].

4.5.6. The overexpression of GDF11 demonstrates aging-promoting effects on the liver

In their study, Sun et al. [83] demonstrate that upregulating GDF11 in liver cells using adeno-associated viruses hinders autophagy and facilitates liver senescence. Conversely, increased expression of TGF-β can result in metabolic disorders, functional impairments, and promote processes like epithelial-mesenchymal transition (EMT) and excessive extracellular matrix (ECM) deposition [84]. These findings indicate that a low dose of GDF11 could be advantageous in reversing aging, while high doses may worsen tissue burden by excessively activating the TGF-β signaling pathway.

4.5.7. Non-statistically significant reductions in GDF11 levels among elderly patients

A modified aptamer-based proteomic platform was utilized to identify GDF11 in plasma during a clinical trial involving 1899 participants diagnosed with stable coronary heart disease. The findings suggest a tendency for GDF11 levels to decrease with age, although the statistical significance was not demonstrated [85]. In another clinical trial, liquid chromatography-tandem mass spectrometry was employed, revealing no correlation between GDF-11 levels and age among 19 to 100-year-old healthy individuals [86].

4.6. Thrombospondin-4 (THBS4) and SPARC-like protein 1 (SPARCL1) promote synaptic regeneration in vitro in young neurons

Both THBS4 and SPARCL1 are secreted proteins that are released into the extracellular space and play a role in intercellular interactions and signaling. Tandem mass spectrometry analysis has shown that the levels of THBS4 and SPARCL1 in the blood of elderly individuals are significantly lower compared to young individuals [87]. The introduction of THBS4 and SPARCL1 has been found to increase the number of synapses and synaptic responses in human neurons derived from embryonic stem cells, as indicated by the analysis of miniature excitatory postsynaptic currents [87]. However, further in vivo studies are necessary to determine whether they can promote synaptic plasticity.

5. Clinical experiments

A clinical study involving 1899 individuals diagnosed with stable coronary heart disease indicates that higher levels of GDF11 in these individuals are significantly linked to a decrease in cardiovascular events and overall mortality [85].

hUCBP, which stands for human umbilical cord blood plasma, is currently the youngest plasma available. It is enriched with immune regulatory factors and growth factors. In a recent clinical study, a group of 18 older individuals with a mean age of 74 received a weekly intramuscular injection of 1 mL of hUCBP concentrate for a period of 10 weeks. This intervention has been proven to be both safe and effective in improving aging-related indicators, such as creatine levels and the estimated rate of glomerular filtration [8]. Furthermore, the treatment resulted in a reduction of the epigenetic age, as determined by DNA methylation, by an average of 0.82 years [8].

We conducted a search for clinical trials on the official website (clinicaltrials.gov). After excluding withdrawn and terminated experiments, we identified 7 projects related to young plasma (see Supplemental Table 1), 3 projects related to UCBP (see Supplemental Table 2), and 6 projects related to GM-CSF (see Supplemental Table 3). Several completed clinical trials have demonstrated the safety of young blood plasma, hUCBP, and circulating factors in both healthy and unhealthy older individuals. The remaining trials are currently ongoing. Some trials have exceeded their expected completion date for undisclosed reasons, while others have been completed but have not yet published any results.

A study (NCT02968433) has confirmed that young plasma treatment for four weeks improves phonemic fluency ($P = 0.002$) and the stigma subscore of the Parkinson's Disease Questionnaire-39 ($P = 0.013$) after four weeks of maintenance in aged Parkinson's patients [88].

The clinical study (NCT01409915) on the treatment of Alzheimer's disease suggests that GM-CSF therapy is safe, although it does not show significant improvements in cognitive function. Similarly, the clinical study (NCT00901381) on the treatment of acute ischemic stroke indicates that GM-CSF therapy at a dose of 10 mg/kg per day for 5 days is considered safe. However, it does not demonstrate any improvements in neurological damage or the level of disability/dependence at 180 days after the stroke [89].

6. Discussion

Further extensive research is still required to investigate the anti-aging effects of young blood and circulating factors, despite their confirmation in animal models. Additionally, it is important to address ethical concerns and potential adverse reactions.

6.1. Limitation

(1) While the rejuvenating effects of young blood on vital organs and tissues like the heart, brain, liver, bones, and kidneys have been uncovered, further research is needed to investigate its impact on the lungs, vascular hardening, and the immune system.
(2) As a promising alternative therapy, circulating factors have been successful in replicating the rejuvenating effects of young blood on multiple organs and tissues. However, it is worth noting that certain crucial effects attributed to young blood, such as promoting β cell proliferation and reducing oxidative stress in fat cells, have yet to be reported by administering circulating factors.
(3) It is unclear whether there exist other circulating factors that can enhance the aging process. Moreover, do young blood and circulating factors impact senescence-related signaling pathways to a greater extent? Additionally, if one undergoes treatments involving young blood or circulating factors for anti-aging purposes, is lifelong administration necessary? Alternatively, could intermittent treatment be a viable option? If so, what would be the optimal time intervals between each treatment?
(4) Although there have been some clinical reports, the translation from rodents to humans is inevitably uncertain due to physiological and genetic differences. To validate the beneficial effects of young blood and circulating factors, randomized controlled trials with long-term duration should be conducted in clinic. Furthermore, since the aging process is multifaceted, solely relying on young blood or circulating factors may not fully reverse it. Research should also explore the combination of young blood with exercise and calorie restriction to enhance the aging process.

6.2. Ethical concerns and side effects

Technologically, therapeutic plasma exchange (TPE) in current clinical practice can facilitate blood exchange between two individuals [90]. However, the direct transfer of plasma from young individuals to older individuals may lead to accelerated organ aging in the younger group, a phenomenon that has been confirmed in several animal studies related to HPB [91–93].

Even if young people's blood were transfused into the elderly, there would still be ethical concerns regarding the availability of a significant supply of young blood or hUCBP. This could potentially lead to an unfair distribution of plasma. Blood transfusions can also result in various unintended side effects, including immune reactions and hemolysis [90]. Therefore, it is crucial to establish strict criteria for the use of young blood in treatments related to aging.

However, the use of circulating factors does not necessitate consideration of supply or ethical issues as circulating factors can be synthesized using recombinant protein technology. It is important to note that circulating factors may induce certain side effects. In clinical practice, the dose of GM-CSF is strictly controlled to be below 10 μg/kg and administered via subcutaneous injection, resulting in a low incidence of side reactions such as fever and rash among patients [94]. It is worth noting that the utilization of PF4 in elderly individuals with hypercoagulable conditions may increase the risk of thrombosis. On the other hand, the administration of Klotho can effectively reduce the risk of thrombosis as it inhibits ROS/p38MAPK signaling, thereby preventing thrombus formation in animals with chronic kidney disease [95]. Furthermore, the administration of high doses of GDF11 may potentially induce interstitial fibrosis through the activation of TGF-beta signaling. Therefore, it is imperative to conduct thorough safety assessments of circulating factors in preclinical experiments.

7. Conclusion

In conclusion, the rejuvenation of aging organs and regulation of age-related signaling pathways can be achieved through the use of young blood and circulating factors. Their mechanisms are centered around promoting regeneration and reducing inflammation and fibrosis. Promising circulating factors, such as TIMP-2, GM-CSF, MANF, and Klotho, warrant further investigation to assess their potential clinical applications. Moreover, it is important to note that not all aging organs respond positively to a single circulating factor, indicating that the combined utilization of multiple factors may result in a more effective synergistic effect against aging. Furthermore, given the incomplete understanding of aging, interdisciplinary collaboration is crucial, requiring cooperation between biologists, nutritionists, microbiologists, and clinical physicians to integrate information from different levels, in order to comprehensively understand the aging process and accelerate progress in aging research.

Funding

The study was supported by grants from the Office of Science and Technology and Talent Work of Luzhou (2022-JYJ-121).

CRediT authorship contribution statement

Meng-Nan Liu: Writing – original draft, Writing – review & editing. **Qi Lan:** Formal analysis, Project administration, Writing – review & editing. **Hao Wu:** Data curation, Formal analysis, Writing – review & editing. **Cai-Wei Qiu:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

All authors disclosed no relevant relationships.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32652.

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